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

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP),

Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Vittorio Silano (until 21 December 2020[†]), Inger-Lise Steffensen, Christina Tlustos, Laurence Vernis, Holger Zorn, Monika Batke, Margherita Bignami, Emanuela Corsini, Rex FitzGerald, Ursula Gundert-Remy, Thorhallur Halldorsson, Andrew Hart, Evangelia Ntzani, Henri Schroeder, Eugenio Scanziani, Beate Ulbrich, Dina Waalkens-Berendsen, Detlef Woelfle, Zainab Al Harraq, Katleen Baert, Anna F. Castoldi, Maria Carfi, Cristina Croera and Henk Van Loveren

Abstract

In 2015, EFSA established a temporary tolerable daily intake (t-TDI) for BPA of 4 µg/kg bw per day. In 2016, the European Commission (EC) mandated EFSA to re-evaluate the risks to public health from the presence of BPA in foodstuffs and to establish a full tolerable daily intake (TDI). For this re-evaluation, a pre-established protocol which had undergone public consultation was used. The CEP Panel concluded that it is Unlikely to Very Unlikely that BPA presents a genotoxic hazard through a direct mechanism. Therefore, it was concluded that the balance of evidence allows a health-based guidance value (HBGV) to be established. The immune system was identified as the most sensitive health outcome category to BPA exposure. Specifically, an increase of Th17 cells was identified as the critical effect; these cells are pivotal in cellular immune mechanisms and involved in the development of allergic lung inflammation. A reference point (RP) of 0.93 ng/kg bw per day, expressed as human equivalent dose, was identified for the critical effect. The uncertainty analysis indicated that it was around 90% probable that no other endpoint was more sensitive than Th17 cells. Therefore, the CEP Panel concluded that no additional uncertainty factor (UF) was needed and that a HBGV based on the identified RP is justified. Applying an UF of 25 to the RP, a TDI of 0.04 ng BPA/kg bw per day was established. Comparison of this TDI with the dietary exposure estimates from the EFSA 2015 opinion showed that both the mean and the 95th percentile dietary exposures in all age groups exceeded the TDI by two to four orders of magnitude. Even considering the uncertainty in the exposure assessment, since the exceedance was so large, the CEP Panel concluded that there is a health concern from dietary BPA exposure for all age groups.

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Keywords

Bisphenol A, BPA, hazard, toxicity, health risks, TDI, food contact materials

42 Summary

43
44 In 2015, EFSA established a temporary Tolerable Daily Intake (t-TDI) for BPA of 4 µg/kg bw per day
45 (EFSA CEF Panel, 2015). By comparing this t-TDI with the exposure estimates, it was concluded that
46 there was no health concern for any age group from dietary exposure and low health concern from
47 aggregated exposure. However, uncertainty in the outcome was noted and the European Commission
48 (EC) mandated EFSA to re-evaluate the risks to public health from the presence of BPA in foodstuffs
49 and to establish a full TDI on the basis of the new information available. The current version of the
50 draft opinion has been endorsed by the CEP Panel for public consultation.

51
52 The re-evaluation was performed as a systematic approach, including critical appraisal of the studies
53 and weight of evidence (WoE), according to a pre-established protocol which underwent public
54 consultation.

55
56 The new evaluation is based on evidence published from January 1, 2013, until October 15, 2018, not
57 previously considered by EFSA. EFSA also launched a call for data in order to obtain from interested
58 parties and/or stakeholders human and animal hazard data relevant to the re-evaluation of BPA and its
59 t-TDI. An important study foreseen in the new evaluation was the BPA NTP CLARITY study and its
60 associated grantees studies, some of which were published after October 15, 2018, but still included in
61 the evaluation. For the health outcome category (HOC) Genotoxicity the time span of the literature
62 search was extended until 21 July 2021 and also the studies assessed in the 2015 EFSA opinion were
63 re-considered.

64
65 In the 2015 EFSA opinion on BPA (EFSA CEF Panel, 2015), the extrapolation from the reference point
66 (RP) to the t-TDI was performed using an approach by which the toxicokinetic standard sub-factor for
67 the interspecies extrapolation was substituted by a substance (BPA)-specific Human Equivalent Dose
68 Factor (HEDF). The newly available literature data on toxicokinetics of BPA considered in the present
69 opinion comprised two studies in mice, three studies in rats, three studies in pregnant sheep (ewes)
70 and two studies in humans. The studies in mice and rats did not contribute to a better understanding
71 of the toxicokinetic aspects of BPA. The two human studies showed that BPA is absorbed to nearly
72 100% and pre-systemically metabolised to a great extent to glucuronide and sulphate conjugates. The
73 area under the curves (AUCs) adjusted for dose were clearly different in the two studies. The CEP Panel
74 decided to use the median value of the AUCs from both studies for the calculation of the HEDF, because
75 both modes of administration used in those studies are realistic for humans. The median AUC was 15.7
76 nM x h, which is 4-fold higher than the modelled AUC-value used for calculating the HEDF in the 2015
77 EFSA opinion. In order to calculate the HEDF, the AUC data were used from the 2015 EFSA opinion for
78 mice, rats, monkeys and dogs. For ewes, the data reported in the current opinion were used. The
79 following HEDFs were obtained: 0.0115 for mice, 0.165 for rats, 0.095 for monkeys, 0.1395 for dogs,
80 0.1197 for ewes (gavage) and 0.4357 for ewes (diet).

81
82 In the 2015 EFSA opinion on BPA (EFSA CEF Panel, 2015), BPA effects in liver and kidney were judged
83 as Likely based on results from multigeneration studies in mice and rats. Changes in the mean relative
84 kidney weight in a two-generation toxicity study in mice were considered as the most critical effect at
85 low doses and were used to derive the t-TDI of 4 µg/kg bw per day. The newly available literature data
86 indicated that in the health outcome category (HOC) General toxicity, several organs as well as
87 haematological parameters are potential targets of toxicity for BPA. Within this HOC, no human studies
88 were available, while ten clusters with relevant endpoints were identified in animal studies: body weight,
89 liver effects, kidney effects, lung effects, thyroid effects, parathyroid effects, pituitary gland effects,
90 adrenal gland effects, bone marrow effects and effects on haematological parameters. Overall, none of
91 the evaluated clusters of effects in this HOC was considered Very Likely or Likely. In each of the
92 evaluated clusters, effects were noted at least in one exposure period, but there was inconsistency
93 among the available studies and, therefore, these effects were judged as As Likely As Not (ALAN).
94 Mode of action (MoA) studies suggested oxidative stress as a potential pathogenetic mechanism for
95 adverse effects, but other mechanisms may be operational as well.

96

97 In the 2015 EFSA opinion on BPA (EFSA CEF Panel, 2015) it was stated that based on human studies,
98 there were indications that BPA may be linked to immunological outcomes, although a causal link could
99 not be established. In addition, studies in animals lent support to the possibility of effects on the
100 immune system. An effect of concern was, in particular, increased Immunoglobulin E (IgE) in allergic
101 lung inflammation, but like the human studies, the animal studies suffered from shortcomings, for which
102 reason the CEF Panel did not take these effects forward for the risk characterisation. In a later statement
103 in 2016, after evaluating two additional studies of effects of exposure to BPA on the immune system,
104 in which potential allergic conditions were investigated, the CEF Panel confirmed its position that the
105 studies available at that time suggested effects on the immune system, but that the studies were not
106 sufficiently robust to take them forward for risk characterisation (EFSA CEF Panel, 2016). The newly
107 available literature data confirmed that the immune system is a target of toxicity for BPA. Within the
108 HOC Immunotoxicity, one relevant cluster of endpoints was identified in the human studies:
109 asthma/allergy, including data from the exposure periods pregnancy and childhood. In the animal
110 studies, five clusters of relevant endpoints were identified: innate immunity, cellular immunity, humoral
111 immunity, inflammation and allergic lung inflammation. Based on the human data, a positive association
112 between BPA exposure and asthma/allergy was judged as ALAN. Based on the animal data, the clusters
113 allergic lung inflammation, cellular immunity and inflammation showed effects that were judged as
114 Likely. In the other clusters, effects were also noted, but the data were less consistent, and these
115 effects were judged as ALAN. In the cluster allergic lung inflammation, an effect noted was on the
116 production of specific IgE in response to an allergen, which was deemed as adverse as it is a crucial
117 parameter in inducing allergic reactions in the respiratory tract. Other effects in that cluster supported
118 the likelihood of this effect. The likely effect in the cluster cellular immunity was supported by the
119 consistency of the different endpoints within that cluster. The most sensitive parameter affected by
120 BPA was the increased number of Th17 cells. Although Th17 cells are T cells, and hence were put in
121 the cluster cellular immunity, they play a pivotal role in allergic responses, and therefore the effect on
122 Th17 cells is consistent with the effect on the production of specific IgE. *In vivo* evidence was supported
123 by MoA studies. *In vitro* studies indicated the ability of BPA to induce immune deregulation, possibly
124 leading to an increased susceptibility to develop inflammatory diseases.

125
126 In the 2015 EFSA opinion on BPA (EFSA CEF Panel, 2015), in the WoE, a likelihood level of ALAN of
127 metabolic effects of BPA was based on the human and animal evidence. The newly available literature
128 data indicated that BPA may induce adverse metabolic effects. Within the HOC Metabolic effects, five
129 clusters of endpoints were identified in the human studies: obesity, cardiometabolic effects, thyroid
130 effects, type 2 diabetes mellitus (T2DM) and gestational diabetes mellitus, including data from one or
131 more of the exposure periods pregnancy, childhood and adulthood. In the animal studies, eight clusters
132 of relevant endpoints were identified: obesity, fat deposition in the liver, glucose regulation, blood lipids,
133 uric acid, type 1 diabetes mellitus (T1DM), other metabolic hormones and thyroid hormones. Based on
134 the human data, none of the metabolic clusters showed effects that were considered Likely or Very
135 Likely. A positive association between BPA exposure and obesity and T2DM was judged as ALAN, while
136 a positive association between BPA exposure and cardiometabolic effects, thyroid effects and
137 gestational diabetes mellitus was judged as Not Likely. Based on the animal data, no metabolic clusters
138 were considered Very Likely. The cluster uric acid was considered Likely (in the adult exposure period),
139 since increased levels were observed in the liver of mice and in the serum of mice and rats after BPA
140 exposure. The other metabolic endpoints were considered either ALAN (obesity, fat deposition in the
141 liver, glucose regulation, blood lipids and T1DM) or Not Likely (other metabolic hormones and thyroid
142 hormones), in one or more exposure periods. Plausible MoAs of BPA were available for metabolic effects
143 from animal and *in vitro* studies.

144
145 In the 2015 EFSA opinion on BPA (EFSA CEF Panel, 2015), a likelihood level of ALAN was assigned to
146 neurological, neurodevelopmental and neuroendocrine effects of BPA in a WoE approach. The newly
147 available literature data supported that the central nervous system is a target of toxicity for BPA. Within
148 the HOC Neurotoxicity and developmental neurotoxicity, the evaluation of the human data considered
149 endpoints from the cluster neurodevelopment. In the animal studies, three clusters of endpoints were
150 identified: neuromorphology, nervous system functionality and behaviour. Based on the human data,
151 it was concluded that an association between BPA exposure and impaired neurodevelopment was Not
152 Likely. Based on the animal data, all three neurotoxicity clusters showed effects that were judged as
153 Likely. In the neuromorphology cluster, Likely effects were found for the endpoints dendritic spine

154 density of pyramidal cells in hippocampus (CA1 and dentate gyrus areas) after developmental exposure
155 and for the endpoints number of neurons in hippocampus (CA1 and CA3 areas), and dendritic spine
156 density in pyramidal cells in medial part of the prefrontal cortex (PFC) after exposure during the growth
157 phase/young age. In the nervous system functionality cluster, a Likely effect on the endpoint AchE
158 activity during the adult exposure period was identified. In the behaviour cluster, Likely effects were
159 noted for the endpoint anxiety/emotionality during all exposure periods (developmental, growth
160 phase/young age, adult and indirect exposure through the male germline). Furthermore, the endpoint
161 learning/memory showed a Likely influence of BPA from developmental and growth phase/young age
162 exposure, and effects on sensory-motor coordination and salt preference were considered Likely in
163 adults. Several mechanisms of action have been proposed, but the linkage between identified effects
164 of BPA with brain structure, function and development have not been sufficiently explored in the
165 literature to draw conclusions on the MoA.

166
167 In the 2015 EFSA opinion on BPA (EFSA CEF Panel, 2015), the CEF Panel concluded that the evidence
168 was not sufficient to infer a causal link between BPA exposure and reproductive and developmental
169 effects in humans. However, it was re-confirmed that BPA is a reproductive toxicant in experimental
170 animal studies at high doses (above a human equivalent dose (HED) of 3.6 mg/kg bw per day), while
171 a likelihood level of ALAN was given to reproductive and developmental effects of BPA in animals at
172 low doses (below HED 3.6 mg/kg bw per day). The newly available literature data indicated that
173 reproduction is a target of toxicity for BPA. Within the HOC Reproductive and developmental toxicity,
174 five relevant clusters of endpoints were identified in the human studies: fetal and post-natal growth,
175 prematurity, pre-eclampsia, male fertility and female fertility, including data from one or more of the
176 exposure periods pregnancy, childhood and adulthood. In the animal studies, three clusters of relevant
177 endpoints were identified: developmental toxicity, female reproductive toxicity and male reproductive
178 toxicity. The clusters included data from one or more of the exposure periods developmental until
179 weaning, developmental until adulthood, growth phase, adult exposure and indirect (germline)
180 exposure. Based on the human data, none of the clusters showed effects that were judged as Likely or
181 Very Likely. An association between maternal BPA exposure and impaired pre- and post-natal growth,
182 shorter duration of gestation or preterm delivery, reduced male fertility and pubertal development when
183 exposed during childhood, was judged as Not Likely. An association between BPA exposure and reduced
184 female fertility and pre-eclampsia during adulthood and pubertal development when exposed during
185 pregnancy was judged as ALAN. Based on the animal data, both female and male reproductive toxicity
186 clusters showed effects that were judged as Likely. In the female reproductive toxicity cluster, there
187 were Likely effects on ovary weight and histology and uterus histology after developmental exposure,
188 on ovary histology after developmental and adult exposure, on implantation rate after growth
189 phase/young age exposure and on ovary histology (follicle counts) after adult exposure. In the male
190 reproductive toxicity cluster, there were Likely effects on epididymis (exfoliated germ cells and
191 inflammation) after developmental exposure (pre-natal and/or post-natal until adult), on testis histology
192 (decreased seminiferous tubule diameter) after growth phase/young age exposure and on sperm
193 (motility, viability and acrosome reaction) after adult exposure. In the developmental toxicity cluster,
194 effects were also noted, but the results were less consistent, and these effects were judged as ALAN
195 (for the endpoints bone development, mammary gland histology, body weight (in the developmental
196 exposure), mammary gland weight and mammary gland histology (in the developmental and adult
197 exposure) as well as body weight and age at first oestrus (in the growth phase/young age exposure
198 period)). Supporting evidence for plausible MoAs of BPA on reproductive toxicity effects was available.
199 They include oestrogen and androgen receptor (AR) interactions and associated downstream and cross-
200 stream effects, including epigenetic changes.

201
202 In the 2015 EFSA opinion on BPA (EFSA CEF Panel, 2015), the CEF Panel concluded that there were
203 indications that BPA exposure may be associated with cardiovascular effects; these effects were
204 considered to be ALAN. The newly available literature data investigated the cardiovascular system as a
205 target of toxicity for BPA. Within the human HOC Cardiotoxicity, no case-control or cohort studies were
206 available. Thus, the evidence for an association between BPA exposure and cardiotoxicity in human
207 was considered Inadequate. In the animal studies, six clusters of relevant endpoints were identified:
208 absolute and relative heart weight, incidence of cardiac lesions, cardiac structural changes (as measured
209 by echocardiography), effects on cardiac function (as measured by echocardiography), blood pressure
210 and atherosclerotic lesions. Based on the animal studies, the evidence of BPA effects was judged as

211 Not Likely in the majority of the cardiotoxicity clusters, and in few clusters as Inadequate, in one or
212 more exposure periods.

213
214 In the 2015 EFSA opinion on BPA (EFSA CEF Panel, 2015), the CEF Panel judged BPA effects on
215 mammary gland proliferation as Likely based on results from a subchronic rat study with pre-natal
216 exposure to BPA as well as proliferative and related morphological changes in the mammary gland
217 reported in other studies. The evidence for proliferative changes in prostate and other organs (testis,
218 liver) was evaluated as too weak to reach a definite conclusion. Overall, the findings in mammary gland,
219 prostate and other organs were considered 'insufficient to conclude that there is a link to cancer
220 development in later life' and the likelihood level of 'Unlikely to ALAN' was assigned to carcinogenic
221 effects of BPA. The newly available literature data indicated that in the HOC Carcinogenicity and
222 mammary gland proliferative effects, the following organs were targets of BPA-induced toxicity:
223 mammary gland, prostate and uterus. Within the HOC Carcinogenicity and mammary gland proliferative
224 effects, no human studies were available, while five clusters with relevant endpoints were identified in
225 animal studies: mammary gland weight, mammary gland histology, prostate histology, uterus weight
226 and uterus histology. For histology, four sub-clusters were considered: non-neoplastic changes, pre-
227 neoplastic lesions, neoplastic lesions, proliferation and apoptosis as evaluated by quantitative
228 immunohistochemistry. The cluster mammary gland weight was judged Not Likely. The clusters
229 mammary gland histology, prostate histology and uterus weight showed effects that were not
230 consistently reported in the available studies and, therefore, these effects were judged as ALAN. Also,
231 regarding the subclusters linked to lesions in the mammary gland, inconsistencies were noted: in the
232 developmental until weaning exposure period no increase in pre-neoplastic lesions (Not Likely), but a
233 higher incidence in neoplastic lesions (Likely) was observed. In the developmental to adult exposure
234 period an increase in pre-neoplastic lesions (ALAN) was reported but no increase in neoplastic lesions
235 was detected (Not Likely). Therefore, these effects contributed to the overall judgement ALAN in the
236 cluster mammary gland histology. MoA studies in mammary gland addressing epigenetic effects,
237 changes in gene expression and changes in hormone receptor levels suggested various MoAs of BPA.
238 MoA studies on prostate cancer indicated that BPA can enhance the susceptibility to tumorigenesis in
239 rodents co-treated with very high levels of oestradiol (E2) and testosterone, while developmental and
240 chronic exposure to BPA without additional sex hormones did not demonstrate a direct tumorigenic
241 effect. In the cluster uterus histology, the non-neoplastic changes gland cellular anomalies, squamous
242 metaplasia and cystic endometrial hyperplasia were considered adverse and judged as Likely based on
243 studies with developmental exposure (pre-natal and/or post-natal until weaning) to BPA. Whereas MoA
244 studies on uterine cells suggested various MoAs of BPA possibly involved in the induction of proliferative
245 changes, the results of rodent studies did not demonstrate a tumorigenic activity of BPA.

246
247 In the 2015 EFSA opinion on BPA 2015 (EFSA CEF Panel, 2015), the CEF Panel concluded that BPA is
248 not mutagenic (in bacteria or mammalian cells), or clastogenic (micronuclei and chromosomal
249 aberrations). The potential of BPA to produce aneuploidy *in vitro* was not expressed *in vivo*. The positive
250 findings in the postlabelling assays *in vitro* and *in vivo* were judged Unlikely to be of concern, given the
251 lack of mutagenicity and clastogenicity of BPA *in vitro* and *in vivo*. Based on the scientific literature re-
252 considered from the 2015 EFSA opinion and the newly published literature and available data from
253 2013 until 21 July 2021, the CEP Panel concluded that BPA does not induce gene mutations in bacteria,
254 while it induces DNA strand breaks, clastogenic and aneugenic effects in mammalian cells *in vitro*.
255 Oxidative stress related mechanism(s) are possibly implicated in the DNA damaging and clastogenic
256 activity elicited by BPA *in vitro*. There is limited evidence for DNA and chromosomal damaging activities
257 of BPA *in vivo*. The available studies do not provide evidence of aneugenicity of BPA in germ cells *in*
258 *vivo*. In contrast to consistent *in vitro* positive findings, the *in vivo* findings in several studies with either
259 high or with limited reliability were inconsistent. The CEP Panel concluded that there is no evidence
260 supporting an *in vivo* genotoxic hazard posed by BPA through a direct interaction with DNA. The CEP
261 Panel concluded that it is Unlikely to Very Unlikely that BPA presents a genotoxic hazard, the causes of
262 which include a direct mechanism. Therefore, it was concluded that the balance of evidence allows a
263 health-based guidance value (HBGV) to be established.

264
265 Benchmark dose (BMD) analyses for dose-response modelling were performed for the endpoints with
266 an assigned likelihood level of Likely or Very Likely. A cut-off value of 10 for the ratio between the
267 lowest non-zero dose and the BMD lower confidence interval (BMDL) was set. Studies where the ratio

268 was higher or equal to 10 were considered not adequate to be evaluated by the BMD approach, but
269 were considered in the uncertainty analysis. Studies reporting effects that were judged as ALAN were
270 also not subjected to BMD analysis, but were included in the uncertainty analysis. BMD analyses were
271 performed using the BPA administered doses without conversion to HED. HED converted values were
272 used subsequently to compare the different modelling outcomes. Taken all available information into
273 consideration, the CEP Panel identified BPA effects on more than one HOC, and the increased number
274 of Th17 cells in the immune system was considered the critical effect. After conversion of the doses to
275 HED, the CEP Panel identified the lowest BMDL value of 0.93 ng/kg bw per day for the increasing effect
276 of BPA on Th17 cells in mice and used this as RP for the risk assessment of BPA. The CEP Panel did not
277 apply the uncertainty factor (UF) for inter-species variability in toxicokinetics because this was already
278 taken into account by the conversion into HED. The remaining UF of 25 accounting for the inter-species
279 toxicodynamic difference (2.5) and the intra-human variability in toxicokinetics and toxicodynamics (10)
280 was used to derive the TDI.

281
282 The uncertainty analysis indicated that it was around 90% probable that no other endpoint was more
283 sensitive than Th17 cells. The Panel therefore concluded that no additional UF was needed and that a
284 HBGV based on the identified RP is justified. Accordingly, the CEP Panel established a TDI for BPA of
285 0.04 ng/kg bw per day.

286
287 The CEP Panel carried out an assessment of the risk to public health related to the presence of BPA in
288 foodstuffs without performing an updated exposure assessment, in accordance with the terms of
289 reference as provided by the EC. Therefore, the CEP Panel compared the newly derived TDI of 0.04
290 ng/kg bw per day with the dietary exposure estimates for BPA from the 2015 EFSA opinion. The
291 comparison of the dietary exposure estimates with the TDI showed that both the mean and the 95th
292 percentile dietary exposures in all age groups (including all infants and toddler groups) exceeded the
293 TDI by two to four orders of magnitude.

294
295 The CEP Panel noted that a TDI based on the second most sensitive endpoint (ovarian follicle counts)
296 would also be exceeded by the dietary exposure by two to three orders of magnitude.

297
298 The CEP Panel is aware that the exposure assessment presented in the 2015 EFSA opinion may not
299 fully represent the current dietary exposure. Even considering this uncertainty, since the exceedance
300 was so large, the CEP Panel concluded that there is a health concern from dietary BPA exposure for all
301 age groups of the general population.

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DRAFT

382 1. Introduction

383 Bisphenol A (BPA) is used as a monomer in the manufacture of polycarbonates (PC) and epoxy resins
384 and other polymeric materials, as well as for certain paper products (thermal printing). PCs are used in
385 food contact materials such as reusable beverage bottles, infant feeding bottles, tableware (plates and
386 mugs) and storage containers. Epoxy resins are used in protective linings for food and beverage cans
387 and vats.

388 BPA is authorised for use as a monomer in plastic food contact materials, in accordance with
389 Commission Regulation (EU) No 10/2011/EU¹ on plastic materials and articles intended to come into
390 contact with foodstuffs. The former specific migration limit (SML) of 3 mg/kg was reduced to of 0.6
391 mg/kg in 2002 and then further reduced to 0.05 mg/kg following EFSA's 2015 opinion (EFSA CEF Panel,
392 2015). In addition, Commission Implementing Regulations (EU) No 321/2011² and (EU) No 2018/213³
393 place restrictions on the use of BPA, respectively in the manufacture of PC infant feeding bottles and
394 in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No
395 10/2011 as regards the use of that substance in plastic food contact materials

396 1.1. Background and Terms of Reference as provided by the requestor

397 In 2016, EFSA received a mandate from the European Commission on the 'Re-evaluation of the risks
398 to public health related to the presence of BPA in foodstuffs and protocol for the risk assessment
399 strategy'. The mandate states the following:

400 'In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002⁴, the European Commission
401 asks EFSA to:

- 402 • establish a protocol detailing the criteria for new study inclusion and for toxicological evidence
403 appraisal for the re-evaluation of BPA, to ensure an efficient and transparent re-assessment of
404 BPA;
- 405 • re-evaluate the risks to public health related to the presence of BPA in foodstuffs.

406 In particular, the re-evaluation should take into consideration new data available from the results
407 of the US National Toxicology Program (NTP)/Food and Drug Administration (FDA) study due in
408 2017 as well as all other new available information not previously evaluated by EFSA and which
409 fulfil the criteria laid down in an established protocol. This re-evaluation should seek to clarify
410 the remaining uncertainties concerning the toxicological endpoints of BPA, especially those
411 concerning the mammary gland, reproductive, metabolic, neurobehavioural and immune systems
412 and to establish a full tolerable daily intake (TDI on the basis of the new information available.'

413 1.2. Interpretation of the Terms of Reference

414 The first part of the mandate was fulfilled in 2017 with the endorsement by the EFSA Panel on Food
415 Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) and subsequent publication of the
416 BPA hazard assessment protocol (EFSA, 2017a, from this point forwards named as the '2017 protocol').
417 This document states in detail the new methods and/or the criteria that will be used in the BPA re-
418 evaluation for data collection, study inclusion, evidence appraisal and integration. The draft protocol
419 underwent public consultation, was revised according to the comments received and published together
420 with the technical report on the outcome of the public consultation (EFSA, 2017b).

1 Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.1.2011, pp. 1–89.

2 Commission Implementing Regulation (EU) No 321/2011 of 1 April 2011 amending Regulation (EU) No 10/2011 as regards the restriction of use of Bisphenol A in plastic infant feeding bottles. OJ L87, 2.4.2011, pp. 1–2.

3 Commission Regulation (EU) 2018/213 of 12 February 2018 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as regards the use of that substance in plastic food contact materials OJ L 41, 14.2.2018, pp. 6–12.

4 Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L31, 1.2.2002, pp. 1–24.

421 As anticipated both in the protocol and in the public consultation report and further detailed in the EFSA
422 Executive Director's letter of 14 March 2018 to the Commission, the working group (WG) of
423 independent experts in charge of the re-evaluation of BPA safety will test the new methodology on a
424 selection of papers previously appraised in the 2015 BPA opinion (EFSA CEF Panel, 2015) and to
425 compare the outcomes. This testing phase should ensure that the methodology used for the 2015 BPA
426 opinion and 2016 statement on immunotoxicity (EFSA CEF Panel, 2016) is robust, even though not as
427 structured as the new one.

428 The second part of the mandate, the full re-evaluation of the safety of BPA, is aimed to assess whether
429 the new scientific evidence (published from 2013 onwards until 15 October 2019 and not previously
430 evaluated by EFSA) still supports the current temporary tolerable daily intake (t-TDI) for BPA of 4 µg/kg
431 bw per day. As specified in the revised protocol, the evaluation will cover:

- 432 1) the adverse effects in humans associated with the exposure to BPA via any route;
- 433 2) the adverse effects in animals after:
- 434 • oral exposure to BPA at doses equal or below the cut-off of 10 mg/kg bw per day (based on
435 the benchmark dose lower confidence interval (BMDL₁₀) used by the EFSA CEF Panel to set the
436 t-TDI in 2015)
 - 437 • other exposure routes [subcutaneous (s.c.), intraperitoneal (i.p.), intravenous (i.v.), inhalation
438 and intratesticular] at doses equal or below the cut-off of 10 mg/kg bw per day, when converted
439 to oral dose, taking into account the interspecies kinetics differences (see TK Chapter 3.1.1.5
440 of the opinion). No cut-off will be applied for dermal⁵ studies.

441 For point 2, when all the doses in one study converted from other routes to oral will result above the
442 oral cut-off of 10 mg/kg bw per day, the study will be excluded from every step of the assessment.

- 443 3) the human and animal toxicokinetics of BPA.

444 The target population of the hazard assessment is the EU general population, including specific
445 vulnerable groups (embryos, fetuses and infants). The target chemical substance is bisphenol A (BPA;
446 chemical formula C₁₅H₁₆O₂, CAS No 80-05-7 and EC No 201-245-8). BPA derivatives will not be object
447 of the assessment. Any endpoint will be considered as potentially relevant for the assessment and a
448 similar categorisation system of Health Outcome Categories used in the EFSA opinion of 2015 (EFSA
449 CEF Panel, 2015) will be used in the new review as follows: General toxicity (e.g. liver and kidney),
450 Reproductive and developmental, Neurotoxicity and neurodevelopmental toxicity, Immunotoxicity,
451 Metabolic effects, Cardiotoxicity, Carcinogenicity and mammary gland proliferative effects, and
452 Genotoxicity. In addition, toxicokinetic aspects of BPA will be examined. In case newly identified
453 endpoints do not belong to any of the above, a new appropriate category will be added.

454 1.3. Additional information

455 1.3.1. BPA uses

456 BPA is an organic chemical used in the manufacture of PC plastics, epoxy resins and other polymeric
457 materials. PC is used for manufacturing food and liquid containers, such as tableware (plates and
458 mugs), microwave ovenware, cookware and reservoirs for water dispensers, as well as for non-food
459 applications, such as toys and pacifiers with PC shields. BPA-based epoxy phenolic resins are used as
460 protective linings for food and beverage cans and as a coating on residential drinking water storage
461 tanks and supply systems.. BPA is also used in the manufacturing of a number of non-food-related
462 applications, e.g. epoxy resin-based paints, medical devices, surface coatings, printing inks, flame
463 retardants, and also for certain paper products (thermal paper e.g. for cash receipts). BPA is also found
464 in clothes mainly those made from polyester and spandex. It may occur due to its use as an intermediate
465 chemical in the manufacturing of antioxidants for textile finishing and in production of dyes.
466

⁵As there is very little toxicokinetic evidence available on dermal exposure, a conservative approach will be used and no cut-off was established.

467 1.3.2. Previous EFSA assessments

468 EFSA has issued several scientific opinions and statements on BPA in 2007, 2008, 2010, 2011, 2015
469 and in 2016 (EFSA 2007, 2008; EFSA CEF Panel 2010, 2011, 2015, 2016).

470 In 2006 EFSA published a Panel opinion on BPA safety assessment establishing a TDI for BPA of 0.05
471 milligram per kilogram (mg/kg) body weight per day, based on the no adverse effect level of 5 mg/kg
472 body weight per day in multigeneration rodent studies and applying an uncertainty factor (UF) of 100
473 (EFSA, 2007).

474 In 2008 EFSA published a Panel opinion on the toxicokinetics of BPA, reaffirming the TDI established
475 in 2006, concluding that age-dependent toxicokinetics differences of BPA in animals and humans would
476 have no implication for the assessment of BPA previously carried out by EFSA (EFSA, 2008).

477 In 2010 the EFSA CEF Panel carried out a comprehensive evaluation of all the recent toxicological data
478 on BPA and concluded that no new scientific evidence had been published since the EFSA opinions of
479 2006 and 2008 that would call for a revision of the TDI established in 2006 of 50 µg/kg bw per day
480 (EFSA CEF Panel, 2010).

481 In 2011 EFSA published a Panel statement on BPA (EFSA CEF Panel, 2011) in relation to possible
482 divergences between the conclusions of the EFSA Scientific opinion on BPA of September 2010 and
483 those in the reports on BPA published in September 2011 by the French Agency for Food, Environmental
484 and Occupational Health and Safety (ANSES) (ANSES, 2011). EFSA considered that the information in
485 the ANSES report would not trigger a change of the outcome of the 2010 opinion, but that a more in-
486 depth review of additional data in recent literature could be required, including the then ongoing low-
487 dose studies at the National Center for Toxicological Research/FDA and at National Toxicological
488 Program/National Institute of Environmental Health Sciences, which aimed to address, at least in part,
489 the current uncertainties regarding the potential health effects of BPA.

490 In its 2015 opinion, the CEF Panel dealt with the assessment of the risk to public health associated with
491 BPA exposure. EFSA established a t-TDI of 4 µg/kg bw per day due to uncertainties on low-dose effects.
492 By comparing this t-TDI with the exposure estimates, the CEF Panel concluded that there is no health
493 concern for any age group from dietary exposure or from aggregated exposure. The CEF Panel noted
494 considerable uncertainty in the exposure estimates for non-dietary sources, while the uncertainty
495 around dietary estimates was relatively low (EFSA CEF Panel, 2015). In 2016, EFSA released a
496 statement on immunotoxicity of BPA following a request from the Dutch authorities for the re-evaluation
497 of the t-TDI as set by EFSA (EFSA CEF Panel, 2016). The CEF Panel concluded that the results of the
498 immunological studies as referred to in the Dutch report were not sufficient to call for a revision of the
499 t-TDI, but that they would be further considered as the part of the studies to be appraised for the full
500 re-evaluation of the safety of BPA.

501

502 1.3.3. NTP CLARITY-BPA program

503 Part of the mandate received by EFSA from the European Commission for the re-evaluation of the
504 safety of BPA included an evaluation of the data coming from the US NTP research programme, called
505 Consortium Linking Academic and Regulatory Insights on BPA toxicity (CLARITY-BPA). In particular, the
506 re-evaluation should take into consideration new data available from the results of the US NTP/FDA
507 study (The CLARITY-BPA Core Study: A Perinatal and Chronic Extended-Dose-Range Study of Bisphenol
508 A in Rats), initially due in 2017, but actually published in October 2018.

509 The CLARITY-BPA program was developed to study the full range of potential health effects from
510 exposure to BPA. The programme was initiated by National Institute of Environmental Health Science
511 (NIEHS), NTP, and the US FDA.

512 CLARITY-BPA has two components:

513 1) Core Study: A two-year guideline-compliant study of potential BPA toxicity in rats.

514 70) Grantee Studies: Investigational studies conducted by university researchers testing a range of
515 additional endpoints.

516 The Core Study tested potential BPA toxicity in rodents; findings were published in Camacho et al.
 517 (2019), identified, in this opinion, as RefID 11370. It also provided animals and tissues for further study
 518 by project grantees. The Grantee Studies used animals raised in the same conditions and exposed to
 519 the same doses of BPA as the Core Study, and the researchers were blinded to the doses of BPA
 520 administered.

521 The raw data of the Grantees studies were available on the FDA/NTP website⁶ also from
 522 15 October 2018, but the publications of the papers were postponed after this date.

523 The table below lists each grantee, their institution, links to the data for their study focus area, and
 524 links to their published results.

525 **Table 1:** List of Grantees.

Principal investigator	Institution	Study focus area and data link	Publications
Scott Belcher	North Carolina State University	Heart	Gear et al., 2017 [RefID 2229]
Nira Ben-Jonathan	University of Cincinnati	Obesity/Adipose Tissue	Raw data
Kim Boekelheide	Brown University	Testis/Sperm	Dere et al., 2018 [RefID 11815]
Jodi Flaws	University of Illinois at Urbana-Champaign	Ovary	Patel et al., 2017 [RefID 5708]
Nestor Gonzalez-Cadauid	University of California, Los Angeles	Penis	Raw data
Andrew Greenberg	Tufts University	Diabetes/Pancreas/Liver	Raw data
Shuk-Mei Ho	University of Cincinnati	Uterus	Leung et al., 2020 [RefID 13789]
Norbert Kaminski	Michigan State University	Immune/Spleen/Thymus	Li J et al., 2018a [RefID 12460] Li J et al., 2018b [RefID 12461]
Heather Patisaul	North Carolina State University	Behaviour/Brain	Witchey et al., 2019 [RefID 13782] Arambula et al., 2018 [RefID 11050] Arambula et al., 2017 [RefID 232] Arambula et al., 2016 [RefID 231] Rebuli et al., 2015 [RefID 6127]
Gail Prins	University of Illinois at Chicago		Prins et al., 2018 [RefID 13779]
Cheryl Rosenfeld	University of Missouri	Behaviour/Brain	Cheong et al., 2018 [RefID 11739] Johnson et al., 2016 [RefID 3241]
Ana Soto	Tufts University	Mammary Gland	Montévil et al., 2020 [RefID 13788]

⁶ <https://cebs.niehs.nih.gov/cebs/program/CLARITY-BPA>

Frederick vom Saal	University of Missouri	Urogenital System	Uchtmann et al., 2019 [RefID 13784]
Thomas Zoeller	University of Massachusetts Amherst	Thyroid/Brain	Bansal and Zoeller, 2019 [RefID 13783]

526

527 Three grantees studies (from Nira Ben-Jonathan, Nestor Gonzalez-Cadavid and Andrew Greenberg) had
528 not been published in peer-reviewed papers at the time of endorsement of this opinion. Therefore, the
529 materials and methods and the raw data available on the FDA website⁷ on these studies were used for
530 the appraisal assessment (see Annex E). The conclusions from the CLARITY-BPA Core Study stated
531 that:

532 'statistical differences between BPA treatment groups, particularly below 25,000 µg/kg bw per
533 day, and the vehicle control group detected by the low-stringency statistical tests applied to
534 histopathology lesions, were not dose responsive, sometimes occurring in only one low or
535 intermediate dose group, and did not demonstrate a clear pattern of consistent responses within
536 or across organs within the stop-dose and continuous-dose arms and sacrifice times. In contrast,
537 the high oestradiol (E2) dose elicited several oestrogenic effects in females in a clearly
538 interpretable and biologically plausible manner. Several observations at 25,000 µg BPA/kg bw
539 per day may be treatment related, including effects mentioned above in the female reproductive
540 tract (ovary, uterus, and vagina) and in the male pituitary' (Camacho et al., 2019 [RefID 11370]).

541 Both the results from the CLARITY-BPA Core Study and from the Grantees studies were assessed by
542 the CEP Panel and reported in the assessment section of the current opinion.

543 2. Data and Methodologies

544 2.1. Data

545 The evaluation is based on new evidence published from 1 January 2013 until 15 October 2018. The
546 studies published in 2013 and already appraised by EFSA in its 2015 opinion on BPA and in its 2016
547 statement on immunotoxicity of BPA (EFSA CEF Panel, 2015, 2016) have not been re-assessed in the
548 re-evaluation.

549 In the '2017 protocol', the proposed ending date was 30 August 2018 according to the initially planned
550 publication date of the BPA NTP CLARITY study report (NTP. CLARITY-BPA. Chemical Effects in
551 Biological Systems (CEBS). Research Triangle Park, NC (USA): National Toxicology Program (NTP),
552 <https://cebs.niehs.nih.gov/cebs/program/CLARITY-BPA>). However, the BPA NTP CLARITY study report
553 was finally published in September 2018 and the time span for the evidence search was therefore
554 extended until 15 October 2018.

555 For the genotoxicity evidence, the time span of the evidence search was extended until 21 July 2021.

556 The methods that were used for data collection through literature searches and call for data are detailed
557 in Annex A (i.e. Bisphenol A (BPA) hazard assessment protocol).

558 2.2. Literature search

559 The literature search covered the period 1 January 2013 until 15 October 2018. Publications of the
560 Grantees studies, postponed to this date, were also considered (see details in Chapter 1.3.3). For the
561 genotoxicity evidence, the time span of the evidence search was extended until 21 July 2021.

562 Details on the information sources and the search streams used for retrieving the literature to be
563 assessed can be also found in Chapter 3.2 of Annex A and in Appendix A (Outcome of the call for data).

564 **Table 2:** Outcome of the literature searches.

⁷ <https://cebs.niehs.nih.gov/cebs/program/CLARITY-BPA>

Records identified	Through database searching, n = 13,636 Through call for data, n = 7
Title and abstract screening	n = 13,643
Full text screening	n = 3,231
Appraisal	Animal General toxicity, n = 54 Animal Immunotoxicity, n = 42 Animal Carcinogenicity and mammary gland proliferative effects, n = 46 Animal Metabolic effects, n = 82 Animal Neurotoxicity and developmental neurotoxicity, n = 94 Animal Reproductive and developmental toxicity, n = 153 Animal Cardiotoxicity, n = 22 Human Case-control, n = 26 Human Cohort, n = 99
Extraction	Animal studies, n = 298 Human case-control and cohort studies, n = 105
Narrative review	Animal Mode of Action studies, n = 288 <i>In vitro</i> Mode of Action studies, n = 310 Human Mode of Action studies, n = 33 Human Mode of Action studies, n = 33 Human Cross-sectional studies, n = 177

565

566 2.2.1. Call for data

567 The call for data was launched on the EFSA website on 9 March 2018 and interested parties were given
568 the opportunity to submit data until 15 October 2018. The purpose of this call for data was to obtain
569 from interested parties and/or stakeholders human and animal hazard studies/data (published,
570 unpublished or newly generated) relevant to the re-evaluation of BPA and its TDI.

571 The data received by means of this call underwent the same screening for relevance and appraisal
572 procedures planned for studies gathered through bibliographic searches, following the instructions
573 described in the Annex A.

574 All the details on the references submitted and screened are reported in Appendix A.

575 2.2.2. Selection of studies

576 The methods for selecting the studies (screening of titles and abstracts, examining full-test reports for
577 eligibility of studies) are reported in Chapter 4 of Annex A.

578 2.3. Methodologies

579 The methods for collecting the data from the included studies, for appraising and weighting the
580 evidence, for performing the hazard characterisation and for analysing the uncertainties in the
581 assessment are described in Chapters 5, 6, 7, 8, 9 and 10 of Annex A and here below in more detail.

582 2.3.1. BPA Hazard assessment protocol: development and testing

583 The 2017 protocol's methodology (inclusion/exclusion criteria and critical appraisal) (EFSA, 2017a) was
584 first piloted on a set of studies previously appraised by EFSA (e.g. previously concluded to be of high,
585 medium or low reliability). This early pilot phase was performed to ensure that there is not a significant
586 divergence in the outcome of the study appraisals when using the 'old' methodology (applied to the
587 2015 EFSA CEF Panel risk assessment of BPA and 2016 CEF Panel statement on BPA immunotoxicity)
588 vs. the 'new' methodology (according to the EFSA '2017 protocol'). The testing phase, its outcome and
589 the resulting refinement of the appraisal methodology has been described in the EFSA Technical report
590 (EFSA, 2019).

591 The aim of the testing phase was to test the functioning of the internal validity methodology for
592 epidemiological and animal studies, i.e. whether the final tier of internal validity (on a three-level scale,
593 with Tier 1 being the highest quality), automatically assigned on the basis of pre-defined criteria by the
594 program DistillerSR to each study, reflected the internal validity according to expert judgement.

595 For the epidemiological studies, all of them were allocated to Tier 3, in full accordance with expert
596 judgement, mainly due to low confidence in the BPA exposure assessments based on single spot urine
597 sample measurements. A step-wise approach was proposed for refining the appraisal of epidemiological
598 studies: if the question in the protocol on the confidence in exposure was answered negatively, the
599 paper would be directly allocated to Tier 3 and the evaluation would stop.

600 For the animal studies, the 2017 internal validity appraisal tool allocated the studies only to Tier 1 or
601 Tier 3. To improve discrimination of studies into three tiers, the appraisal tool was refined by re-defining
602 the key questions and re-calibrating the rules for the automatic tier allocation. Moreover, it was agreed
603 to allow the experts to re-assign a paper to a different tier of internal validity than that automatically
604 assigned by the tool, if an appropriate justification was given.

605 After revision, the 2019 methodology was re-applied, reaching a percentage of comparability between
606 automatic and expert judgement-based tier allocation equal to 91% when tested on the 40 studies (in
607 43 out of 47 appraisals).

608 The testing phase was also performed to assess the comparability of the study appraisal outcomes (i.e.
609 reliability scores vs. internal validity tiers) by the methodology applied in the EFSA outputs of 2015 and
610 2016 (the '2015 methodology') and the 2019 methodology. It is acknowledged that the 2015 and 2019
611 methodologies present some differences with respect to the elements considered for assessing the
612 study quality (i.e. reliability vs. internal validity). Nonetheless, the key study used to derive BPA's
613 tolerable daily intake in the 2015 opinion was also considered to be of high quality according to the
614 2019 methodology. In addition, the outcome of the appraisal of the papers by the 2019 methodology
615 versus the 2015 methodology was overall comparable or more stringent in 92% of the cases (24 out
616 of 26 appraisals). It follows that despite some intrinsic differences, the 2015 methodology previously
617 used by EFSA to appraise the BPA evidence is considered sufficiently robust, even though not as
618 structured as the 2019 methodology.

619 The amendments to the 2017 protocol and the resulting refined 2019 methodology are fully
620 documented in Annex A.

621 The 2019 methodology has been implemented for the full re-evaluation of the newly obtained BPA
622 evidence as described below.

623 **2.3.2. Definition of Health Outcome Categories and Clusters**

624 Given the large volume and complexity of the emerging evidence and the observed heterogeneity
625 thereof, a structured and detailed definition and organization of the assessed health outcome categories
626 and relevant clusters was deemed necessary. This approach facilitated the process of effectively and
627 successfully appraising the evidence, the proper alignment of human and animal data clusters and,
628 where possible, the integration of human and animal data. This opinion recognizes the following health
629 outcome categories: General toxicity, Immunotoxicity, Metabolic effects, Cardiotoxicity, Neurotoxicity
630 and developmental neurotoxicity, Reproductive and developmental toxicity, Carcinogenicity and
631 mammary gland proliferative effects. Within each health outcome category (HOC), clusters were
632 identified that include several toxicologically relevant endpoints that are physiologically or toxicologically
633 related, and that in concert shed light on the likelihood of an effect of BPA exposure in that cluster. The
634 endpoints are measures of an individual parameter that is adverse in itself, i.e. an apical endpoint, or
635 may be involved in the development of an adverse condition, i.e. an intermediate endpoint.

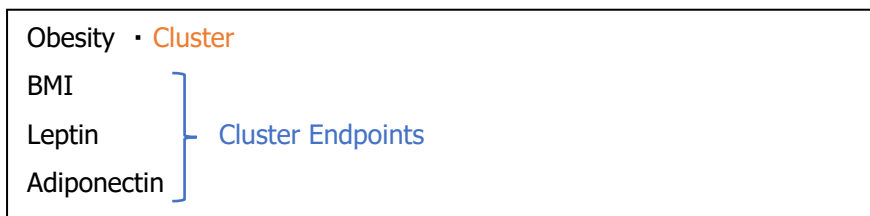
636 The approach used for HOC Genotoxicity is described in Chapter 2.3.5.

637 In epidemiological studies, outcome definition and reporting usually follow the current trends in human
638 morbidity and global burden of disease data. For the group of epidemiological studies related to BPA
639 exposure, an approach for the cluster identification similar to that of the International Classification of
640 Diseases (ICD) system⁸ was used as a starting point. Introduced by WHO, ICD is a hierarchical
641 classification standard for all clinical and research purposes and incorporates an extensive matrix of
642 diseases, disorders and other related health conditions. The following structured process was used:

⁸ <https://www.who.int/standards/classifications/classification-of-diseases>

643 1) Endpoints comprising a distinct disease entity (e.g. obesity) were first identified and included as
 644 a core cluster element.

645 2) Moving downwards in the hierarchy, within each formed cluster all the additionally retrieved
 646 endpoints, representing established clinical or other related research measures or biomarkers of
 647 the core cluster element [e.g. body mass index (BMI), leptin, adiponectin, etc.], were included
 648 as distinct endpoints.



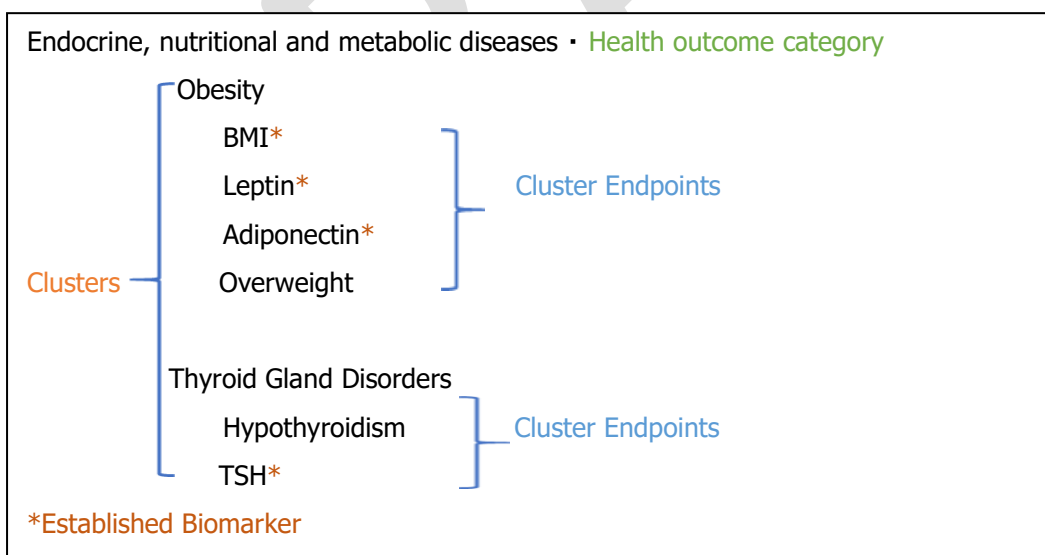
649

650 3) Moving upwards in the hierarchy, the chapter in ICD to which this particular disease belonged
 651 (e.g. Endocrine, nutritional and metabolic diseases) was identified, and a relevant 'Health
 652 outcome category' with the same name was created. These Health Outcome Categories were
 653 linked with a Toxicological Effect Category whenever appropriate. Within this group all the
 654 identified disease entities were included as separate clusters (e.g. Diabetes Mellitus; Thyroid
 655 Gland Disorders; etc.).



656
657

658 4) The Health outcome category tree was then merged.



659
660

661 During this process, it was acknowledged that the approach needed to be customised. First, there are
 662 disease entities that – for the purposes of risk assessment – fit better in another HOC compared with
 663 the one assigned in ICD-10. For example, gestational diabetes is grouped under 'Diseases related to
 664 pregnancy, childbirth and the puerperium' in ICD-10; we chose to include it in the 'Metabolic and related
 665 endocrine effects' category because the toxicological pathway linking exposure and endpoint was
 666 considered closer to metabolic effects. Second, endpoints from different ICD-10 chapters were grouped

667 to create a more coherent HOC group based on their common mechanistic backgrounds. For example,
668 under 'Neurodevelopment', attributes such as cognition, psychomotor development, intelligence, etc.,
669 were grouped, given that all these endpoints share common mode of action (MoA) elements.

670 Based on the above, the **HOC, Clusters (C) and Endpoints (E)** were organised as follows:

671 HOC: General toxicity

672 C: Liver toxicity

673 E: ALT

674 E: AST

675 E: gamma-glutamyl transpeptidase (γ -GTP)

676 C: Kidney toxicity

677 E: Hyperuricemia

678

679 HOC: Effects involving the immune system (Immunotoxicity)

680 C: Asthma/allergy

681 E: Acute bronchiolitis

682 E: Acute otitis media

683 E: Allergic diseases

684 E: Asthma

685 E: Atopic dermatitis

686 E: Atopy

687 E: Bacterial colonisation

688 E: Bronchitis

689 E: Chest infections

690 E: Croup

691 E: Eczema

692 E: Forced respiratory volume in 1s (FEV₁)

693 E: Fraction of exhaled nitric oxide (FENO)

694 E: IgE

695 E: PC20

696 E: Pneumonia

697 E: 'Rashes, eczema or hives'

698 E: Rhinitis (allergic)

699 E: Wheeze

700 C: Infections other than respiratory tract

701 E: Infectious enteritis

702 E: Urinary tract infections

703

704 HOC: Metabolic and related endocrine effects

705 C: Obesity

706 E: Adiponectin

707 E: Birth weight (BW)

708 E: Body Fat

709 E: Body mass index (BMI)

710 E: Fat leg

711 E: Fat mass

712 E: Fat mass index (FMI)

713 E: Fat trunk

714 E: Fat trunk/leg ratio

715 E: Leptin

716 E: Obesity

717 E: Obesity (central)

718 E: Overweight

719 E: Rapid growth

720 E: Skinfold thickness

721 E: Subcutaneous adipose tissue (SAT)

722 E: Visceral adipose tissue (VAT)

723	E: Visceral/Subcutaneous adipose tissue ratio (VAT/SAT)
724	E: Waist circumference
725	E: Weight change
726	E: Weight gain (annual)
727	C: Cardiometabolic effects
728	E: Cholesterol (total)
729	E: High density lipoprotein (HDL)
730	E: Low-density lipoprotein (LDL)
731	E: Triglycerides
732	E: Diastolic blood pressure
733	E: Pulse Pressure
734	E: Systolic blood pressure
735	C: Thyroid
736	E: Free thyroxine (FT ₄)
737	E: Free triiodothyronine (FT ₃)
738	E: Thyroid stimulating hormone (TSH)
739	E: Total thyroxine (TT ₄)
740	E: Total triiodothyronine (TT ₃)
741	C: Type 2 diabetes mellitus (T2DM)
742	E: C-peptide
743	E: C-peptide index (CPI)
744	E: Type 2 diabetes mellitus (T2DM)
745	E: Epidermal growth factor receptor (eGFR)
746	E: Glucose
747	E: Homeostasis model assessment of insulin resistance (HOMA-IR)
748	E: IGF-1
749	E: Insulin
750	E: Leptin
751	C: Gestational diabetes mellitus
752	E: Gestational diabetes mellitus (GDM)
753	E: Glucose
754	E: Impaired glucose tolerance
755	
756	HOC: Mental and neurological effects (Neurotoxicity)
757	C: Neurodevelopment
758	E: Anxiety
759	E: Behaviour
760	E: Behaviour (Behaviour Assessment System for Children–2, BASC-2)
761	E: Behaviour (boys)
762	E: Behaviour (Behaviour Rating Inventory of Executive Function–Preschool, BRIEF-P)
763	E: Behaviour (girls)
764	E: Cognitive development
765	E: Cortisol
766	E: Cortisol reactivity
767	E: Depression
768	E: Executive function
769	E: Intellectual ability
770	E: Psychomotor development
771	E: Social responsiveness scale-2 (SRS-2)
772	E: Sociality
773	E: Sociality – Autistic behaviour (Social Responsiveness Scale)
774	E: Virtual Morris water maze (VMWM)
775	
776	HOC: Reproductive and developmental toxicity
777	C: Fetal and post-natal growth
778	E: Abdominal circumference
779	E: Biparietal diameter

780	E: Birth length
781	E: Birth weight
782	E: Chest circumference
783	E: Femoral length
784	E: Gestational age at birth
785	E: Growth rate
786	E: Head circumference
787	E: Height
788	E: Large for gestational age
789	E: Low birth weight (LBW)
790	E: Length of gestation
791	E: Placental weight
792	E: Ponderal index
793	E: Small for gestational age (SGA)
794	E: Weight
795	E: Weight for length
796	E: Weight for length (birth)
797	E: Weight (fetal)
798	C: Prematurity
799	E: Gestational age at birth
800	E: Length of gestation
801	E: Preterm delivery
802	C: Pre-eclampsia
803	E: Pre-eclampsia (onset, severity)
804	C: Male fertility
805	E: E2
806	E: Estrone
807	E: Fecundability
808	E: Follicle stimulating hormone (FSH)
809	E: FSH:Inhibin B ratio
810	E: Hypo-osmotic swollen
811	E: Inhibin B
812	E: LH:Testosterone ratio
813	E: Live Birth rate
814	E: Luteinising Hormone (LH)
815	E: Sex
816	E: Sperm amplitude head displacement
817	E: Sperm average path velocity
818	E: Sperm beat cross frequency
819	E: Sperm concentration
820	E: Sperm count
821	E: Sperm curvilinear velocity
822	E: Sperm DNA fragmentation index
823	E: Sperm head acrosome area
824	E: Sperm head area
825	E: Sperm head elongation factor
826	E: Sperm head length
827	E: Sperm head perimeter
828	E: Sperm head width
829	E: Sperm high DNA stainability
830	E: Sperm linearity
831	E: Sperm morphology amorphous
832	E: Sperm morphology bicephalic
833	E: Sperm morphology coiled tail
834	E: Sperm morphology cytoplasmatic droplet
835	E: Sperm morphology megalog head
836	E: Sperm morphology micro head

837	E: Sperm morphology neck and midpiece abnormalities
838	E: Sperm morphology other tail abnormalities
839	E: Sperm morphology pyriform
840	E: Sperm morphology round
841	E: Sperm morphology strict criteria
842	E: Sperm morphology taper
843	E: Sperm morphology traditional normal
844	E: Sperm motility
845	E: Sperm straight line velocity
846	E: Sperm straightness
847	E: Sperm straw distance [=motility]
848	E: Sperm volume
849	E: Testicular volume
850	E: Testosterone
851	C: Female fertility
852	E: Aneuploid pregnancy
853	E: Antral follicle count
854	E: Clinical pregnancy
855	E: Corpus luteum rescue
856	E: Day-3 FSH
857	E: Early miscarriage
858	E: Embryo quality
859	E: Endometrial wall thickness
860	E: E2
861	E: E2 trigger level
862	E: Fecundability
863	E: Fertilisation rate
864	E: FSH
865	E: Follicular phase length
866	E: Human chorionic gonadotropin
867	E: Implantation
868	E: Infertility
869	E: Live birth rate
870	E: Luteal phase length
871	E: Luteinising hormone (LH)
872	E: Metaphase II (MII) oocytes
873	E: Miscarriage
874	E: Ovarian volume (OV)
875	E: probability of implantation
876	E: Progesterone
877	E: Sex
878	E: Time to implantation
879	E: Total oocytes
880	C: Pubertal/endocrine
881	E: % Mammary fibroglandular volume (%FGV)
882	E: 2D:4D digit ratio
883	E: Androstenedione
884	E: Anogenital distance anus – penis (AGDap)
885	E: Anogenital distance anus – clitoris (AGDac)
886	E: Anogenital distance anus – fourchette (AGDaf)
887	E: Anogenital distance anus – scrotum (AGDas)
888	E: Breast Development
889	E: Breast volume
890	E: Breastfeeding
891	E: Dehydroepiandrosterone (DHEA)
892	E: Dehydroepiandrosterone Sulfate (DHEA-S)
893	E: E2

894 E: Facial hair growth
 895 E: Free testosterone
 896 E: Genital development
 897 E: Gonadarche
 898 E: Inhibin B
 899 E: Luteinising hormone (LH)
 900 E: Mammary fibroglandular volume (FGV)
 901 E: Menarche onset
 902 E: Perceived insufficient milk supply (PIM)
 903 E: Peripubertal hormone levels
 904 E: Pubarche (boys)
 905 E: Pubarche (girls)
 906 E: Pubertal development scale
 907 E: Pubic Hair
 908 E: Sex hormone-binding globulin (SHBG)
 909 E: Testicular volume
 910 E: Testosterone
 911 E: Thelarche
 912 E: Voice change

913
 914 HOC: Carcinogenicity

915 C: Prostate

916 E: Prostate cancer

917 C: Lymphoid tissues

918 E: Lymphoma

919

920 The following endpoints:

- 921 • myocardial infarction
- 922 • coronary artery stenosis
- 923 • peripheral artery disease.

924 were considered key in the 2015 EFSA Scientific opinion on the risks to public health related to the
 925 presence of bisphenol A (BPA) in foodstuffs (EFSA CEF Panel, 2015) but new information was not found
 926 in the studies considered for the new evaluation.

927 For each of the above-mentioned endpoints, the clusters were formed taking timing of exposure into
 928 consideration. That is, a distinction was made between exposure occurring during pregnancy, childhood
 929 and adulthood, as the aetiology of disease (e.g. neurodevelopment, fertility and other endocrine
 930 abnormalities) differs between stages of development.

931 Considering the problems in exposure measurement found in the literature retrieved, i.e. that no study
 932 measured BPA exposure in a way that was considered appropriate for assessment, and that the
 933 assessment was focused on finding possible adverse associations related to the exposure to BPA, it was
 934 decided to bring forward to WoE analysis only those clusters/exposure periods for which at least two
 935 studies were available and at least one of those studies reported a statistically significant effect for one
 936 of the endpoints measured.

937 In addition, although not meeting the criteria for inclusion, the cluster on Thyroid effects was also
 938 considered in the WoE analysis since maternal and infant thyroid function were considered as relevant
 939 in the 2015 EFSA Scientific opinion on the risks to public health related to the presence of bisphenol A
 940 (BPA) in foodstuffs.

941 The outcome of this approach, i.e. the Clusters (C) and Exposure periods (Exp) brought forward to
 942 weight of evidence (WoE) analysis, are presented for each HOC separately under Chapter 3.1. Hazard
 943 identification.

944 **2.3.3. Consideration of low-dose effects and non-monotonic dose–** 945 **response curves in the risk assessment of BPA**

946 One area of particular interest when reviewing the toxicological profile of BPA is the possible presence
947 of so-called *low-dose effects* (often synonymously referred to as 'doses in the range of human exposure'
948 and/or 'human relevant doses'). Although no clear definition exists, low dose in the case of BPA has
949 been defined as < 5 mg/kg bw per day in EFSA's previous assessment (EFSA CEF Panel, 2015). This
950 value corresponds to the No observed adverse effect level (NOAEL) for changes in body and organ
951 weights identified in two multigeneration reproductive studies in rodents (Tyl et al. 2002, 2006) that
952 formed the basis for the TDI set by EFSA in 2007 (EFSA, 2007) and in 2015.

953 In the scientific debate, the presence of possible low-dose effects and its consequences for risk
954 assessment has been the subject of some controversy and divergent views. This may have been partly
955 fuelled by lack of studies specifically designed to capture such effects. The growing research interest in
956 this area and the increasing number of studies designed to capture possible occurrence of low-dose
957 effects has the potential to shed more light on this issue. This is particularly the case for BPA, and in
958 this assessment a relatively large number of studies covering this dose range (< 5 mg/kg bw per day)
959 was assessed compared with previous opinions. The CLARITY study (NTP Clarity Report, 2018/Camacho
960 et al., 2019 [RefID 11370]) is one example of such studies.

961 Another area of debate in chemical risk assessment is the presence of non monotonic dose response
962 curves (NMDR). Although non-monotonicity is a well-known biological phenomenon (Yordanov and
963 Stelling, 2018) the challenge in chemical risk assessment partly relates to the difficulty of objectively
964 identifying the presence of NMDR in toxicological studies that often include too few dose groups for
965 robust statistical evaluation of non-monotonicity. In addition, in cases where effect sizes are modest,
966 higher statistical power (e.g. more animals) would help but it is seldom achieved.

967 Another important issue that often receives limited attention in the debate is that the presence of non-
968 monotonicity may on its own be of limited importance for risk characterisation if the observed changes
969 are not adverse.

970 Although methodologies to assess NMDR in toxicological studies have been proposed (Beausoleil et al.,
971 2016; Badding et al., 2019), there is currently no consensus on these methods and different approaches
972 of varying robustness, ranging from visual inspection to fitting any non-linear curve though data, have
973 been applied by different researchers. In 2018, EFSA established a WG whose aim was to assess the
974 impact of NMDRs on EFSA's human health risk assessments (EFSA Scientific Committee, 2021b). The
975 reader is referred to that opinion for more comprehensive review on previous activities by EFSA and
976 other international organisations to address NMDR.

977 In line with the recommendations of that opinion, the WG on BPA re-evaluation decided to apply the
978 following criteria to assess if indications for an NMDR were present in individual studies or in the overall
979 body of evidence:

980 Step 1- at single study level:

- 981 • The studies should include at least three doses plus the control.
- 982 • Two adjacent doses should show an effect in the same direction, but these effects would not
983 need to be statistically significant in both cases. Statistical significance (relative to controls) in
984 one the two doses would be sufficient.
- 985 • The biological plausibility of the effect should also be considered.

986 Step 2- at endpoint cluster level:

- 987 • If the effect is not biologically plausible, the effects should be present at the same doses in
988 more than one study (replicability of the results).
- 989 • There should be a time and dose-consistent relationship among related effects/endpoints (e.g.
990 intermediate and apical adverse effects).

991 All studies were evaluated according to these criteria. If a dose-response curve met these criteria, the
992 dose-response would be described as showing 'indications for a NMDR'. If the study authors reported
993 identifying an NMDR in their study evaluated by objective measures that contradicted the WG
994 evaluations (such as curve fitting or other statistical evaluation), more in-depth analyses were
995 performed by the CEP Panel. One study, in particular, prompted detailed assessment for the presence

996 of NMDR, because the endpoint evaluated (mammary gland development) was considered potentially
 997 adverse and the study was appraised as having a low risk of bias (Montévil et al., 2020 [RefID 13788]).
 998 The reader is referred to Appendix B (i.e. the Montévil study: Consideration of low-dose effects and
 999 non-monotonic dose-response reported in that study) for more details on the analyses performed.

1000 In Table 3 a summary of the studies in which the authors indicated a NMDR or the CEP Panel identified
 1001 indications for an NMDR is presented.

1002 **Table 3:** List of studies (RefIDs) with indications for NMDR.

HOC	Indication for NMDR according to study's authors	Indication for NMDR according to WG experts following our criteria
General toxicity		RefIDs 11370, 2614
Metabolic effects	RefIDs 6319, 8375	RefIDs 6319, 8375, 13784, 9247, 12854
Neurotoxicity and developmental neurotoxicity	RefID 9083	RefIDs 9083, 13782, 3462
Cardiotoxicity		RefID 490,
Carcinogenicity and mammary gland proliferative effects	RefIDs 3453, 13788	RefIDs 3453, 3990, 11370, 13788
Reproductive and developmental toxicity	RefIDs 4128, 3453, 13788	RefIDs 4128, 3453, 3990, 13788, 4779, 13099, 1216
Immunotoxicity		RefID 11370

1003

1004 2.3.4. Method for assessing uncertainty

1005 2.3.4.1. Method for uncertainty analysis for hazard characterisation

1006 The purpose of the uncertainty analysis is to assess whether other effects of BPA may potentially occur
 1007 after exposure to lower doses than the endpoint on which the reference point (RP) is based (i.e. the
 1008 effect on Th17 cells) and, if so, inform a decision on what size of additional UF would be suitable to
 1009 take those effects into account. If required, the additional UF together with the UFs for interspecies
 1010 differences for toxicodynamics and intraspecies differences for both toxicokinetics⁹ and toxicodynamics
 1011 would then determine the overall UF to be used when deriving the TDI.

1012 For this purpose, the main part of the uncertainty analysis focused on five HOCs (Immunotoxicity,
 1013 Metabolic effects, Neurotoxicity and developmental neurotoxicity, Reproductive and developmental
 1014 toxicity, and Carcinogenicity and mammary gland proliferative effects) and, within these, on 21 clusters
 1015 of endpoints which were rated ALAN, Likely or Very Likely in the WoE assessment (see Chapter 3.1).
 1016 Consideration of a sixth HOC, General toxicity, was deferred to a later stage (see below). Cardiotoxicity
 1017 was not considered in the uncertainty analysis because the evidence was judged either as Not likely or
 1018 as Inadequate in the WoE assessment. The uncertainty analysis concluded with an assessment of the
 1019 overall uncertainty, where clusters rated less than ALAN and other potentially relevant considerations
 1020 were taken into account (see below).

1021 The uncertainty analysis was conducted in accordance with EFSA's guidance on uncertainty analysis,
 1022 using a combination of methods appropriate to each step of the assessment as described below (a
 1023 'case-specific' uncertainty analysis, EFSA Scientific Committee, 2018a).

⁹ The interspecies differences in toxicokinetics are accounted for by the conversion of the doses into human equivalent doses (HED)

1024 *Overall approach*

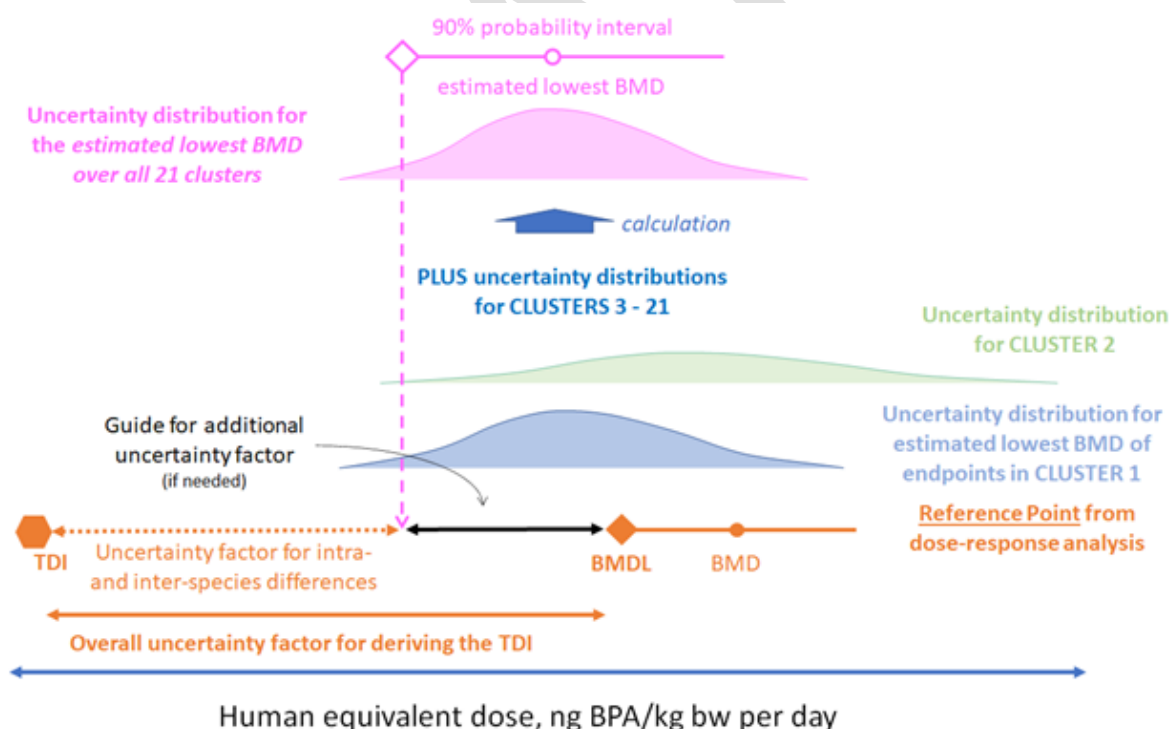
1025 The approach taken for the uncertainty analysis is summarised graphically in Figure 1. The lower part
 1026 of the figure shows the BMDL taken by the CEP Panel as the RP for risk characterisation. An expert
 1027 knowledge elicitation (EKE) was conducted for each of the 21 clusters, providing a distribution
 1028 quantifying uncertainty about the estimated¹⁰ lowest BMD for effects in that cluster that occur in animals
 1029 and are relevant and adverse for humans.

1030 Two examples of such distributions are illustrated graphically in Figure 1.

1031 The distributions for all 21 clusters were then combined by calculation to obtain a distribution
 1032 quantifying uncertainty about the estimated lowest BMD across all 21 clusters. Quantiles of this
 1033 distribution were then used to inform consideration of how large an additional UF (if any) is warranted.
 1034 This is illustrated by the black two-headed arrow Guide for additional UF at the bottom of Figure 1.
 1035 Assessing the TDI with this additional UF would then take the 21 clusters of effects into account. For
 1036 example, taking the fifthth percentile from the combined distribution (as illustrated by the diamond
 1037 symbol at the top of Figure 1) might be considered as equivalent to the normal practice of using the
 1038 lowest BMDL as the RP. However, the final decision on whether and how large an UF is needed also
 1039 took account of other sources of uncertainty affecting the assessment and the level of conservatism
 1040 that is appropriate for setting the TDI required.

1041 The following paragraphs describe the methods used at each stage. Further technical details are
 1042 provided in the annexes referenced below.

1043



1044

1045 **Figure 1:** Graphical overview of the approach taken in the uncertainty analysis.

¹⁰ This is referred to as the 'estimated' lowest BMD to reflect that it was assessed by expert judgement, informed by the studies selected from the weight of evidence assessment, and not by a BMD analysis.

1046 *Assessment of clusters by expert judgement*

1047 Studies to be considered for each cluster were evaluated by two or three experts per cluster, chosen
1048 for their expertise on the endpoints in that cluster. An EKE protocol was devised, adapting EFSA's
1049 guidance for EKE (EFSA, 2014) to elicit judgements from the experts for two questions per cluster. The
1050 short version of Question 1 was 'What is your probability¹¹ that there is at least one endpoint in the
1051 WoE table for this cluster that occurs in animals tested with BPA and is relevant and adverse in
1052 humans?'. Question 2 was 'If one or more endpoints in the WoE table for this cluster occurs in animals
1053 tested with BPA and is both relevant and adverse for humans, what is your prediction for the lowest
1054 BMD of those endpoints, expressed as HED?'. The experts were also provided with a longer version of
1055 each question and a list of supporting definitions (see Annex J) to ensure that each question was well-
1056 defined and interpreted consistently by the experts, as required by EFSA's EKE guidance.

1057 Experts were asked to provide an approximate probability (i.e. a lower and upper probability, EFSA
1058 Scientific Committee, 2018a) in response to Question 1 and a probability distribution for Question 2. It
1059 was anticipated that in some cases a multimodal distribution might be needed to take into account that
1060 the lowest BMD might come from one of several endpoints within the cluster. To allow for this, the
1061 distributions were elicited using the roulette method (EFSA, 2014), in which experts build a histogram
1062 for the distribution representing their uncertainty-

1063 For each cluster, the experts based their assessment on the studies rated as Tier 1 or 2 in the WoE
1064 assessment (see Annex A, Chapters 6-8). Each expert was provided with an Excel template to record
1065 their judgements, together with a summary of the evidence and reasoning they considered for each
1066 question. A copy of the Excel template is provided in Annex J. The experts were introduced to the two
1067 types of probability judgement involved and to the Excel template and a training exercise was conducted
1068 using a relevant example for each type of question. Advice on how to make probability judgements and
1069 operate the template was included during the training and also in the template file.

1070 The experts were advised, when making their judgements, to consider all relevant evidence and
1071 reasoning of which they were aware, and all identifiable sources of uncertainty. For Question 2, experts
1072 were advised to consider the results of BMD analysis when available, as well as no observed adverse
1073 effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs), and to take into account
1074 the magnitude of effects at the LOAEL and the relevant benchmark response (BMR) for each endpoint.
1075 Guidance was provided on how to convert doses reported from animal studies to HEDs and a table of
1076 factors for this purpose was provided (Table 1, in Chapter 3.1.1.4–HED factors). Experts worked
1077 independently on their judgements over a period of 2 to 3 weeks, during which time additional advice
1078 and support was available when needed.

1079 *Review and revision of cluster assessments*

1080 The experts' judgements and reasoning were reviewed and discussed in a series of half-day web
1081 meetings, one for each HOC, where each cluster and question was considered in turn. Each meeting
1082 was attended by the experts who made the judgements being reviewed, plus a facilitator and two
1083 rapporteurs. For each question, the experts were invited to discuss their judgements and reasoning,
1084 which were displayed in a summary file on screen. When discussing Question 2, key studies influencing
1085 their judgements were identified and NOAELs, LOAELs and BMDLs/BMDs/BMDUs from those studies
1086 were added to a graph displaying the experts' distributions for Question 2, to assist the experts in their
1087 discussion. At the end of the discussion of each question, the experts were invited to review their
1088 personal judgements and reasoning and to revise them if they wished, in the light of the discussion.
1089 The discussion was conducted in a similar manner to that used in the 'Sheffield' EKE method (EFSA,
1090 2014), except that no attempt was made to reach a consensus between experts, partly due to time

¹¹ The question asks the experts for 'your probability' expressed as a percentage, because these are subjective probabilities which represent the judgement of each expert (EFSA Scientific Committee, 2018a).

1091 limitations and partly because it was considered useful to take account of differences between experts
1092 later in the process (see below).

1093 *Calculations to derive a distribution for the estimated lowest BMD over the 21 clusters*

1094 The experts' revised judgements were combined using the R software (R Core Team, 2021), in a series
1095 of steps, described below. The R code that was written for this purpose was reviewed independently
1096 and is available at the link provided in Annex K.

1097 In the first step, parametric distributions were fitted to the judgements for Question 2 for each expert
1098 in each cluster, using the fitting methods and criteria described in Annex K. The set of parametric
1099 distributions comprised normal, t, beta, skewed normal and skewed t plus mixtures of non-skewed
1100 normal or t-distributions to provide an appropriate fit for clusters where the experts' roulette histograms
1101 were flat or bimodal.

1102 Second, for each cluster and expert, the probability from Question 1 was combined with the distribution
1103 from Question 2 by multiplication. The former is the expert's probability that there is at least one
1104 endpoint in the cluster that occurs in animals and is relevant and adverse for humans; the latter is their
1105 distribution for the estimated lowest BMD in that cluster if there is at least one such endpoint (i.e.,
1106 conditional on Question 1). Multiplying these provides a cumulative probability function (Cpf) for the
1107 estimated lowest BMD of effects that occur in animals and are relevant and adverse for humans. This
1108 was repeated for each expert in each cluster, resulting in 2 or 3 cpfs per cluster. As their probability for
1109 Question 1 was expressed as a range, each cpf had a lower and upper bound on its curve. The lower
1110 and upper bound of the cpf resulted from multiplying the distribution for Question 2 by, respectively,
1111 the lower and upper bound of the probability for Question 1.

1112 The third step combined the cpfs for all 21 clusters by a probability calculation to produce the cpf for
1113 the estimated lowest BMD across all selected clusters for endpoints that occur in animals and are
1114 relevant and adverse for humans, assuming that the judgements for different clusters are independent.
1115 The calculation used the same principles that apply when calculating the probability of obtaining 'heads'
1116 at least once when tossing a coin two times, and is described in text and equations in Annex K. Quantiles
1117 from the cpf for the estimated lowest BMD across clusters are the output required to inform
1118 consideration of the need for an additional UF for deriving the TDI (as illustrated by the pink diamond
1119 in Figure 1).

1120 As mentioned above, the second step produced 2-3 cpfs per cluster, each with a lower and upper
1121 bound. These reflect imprecision in the experts' judgements (the ranges of probabilities provided for
1122 Question 1) and differences between experts assessing the same cluster, which are both part of the
1123 overall uncertainty. To take account of this, the lower and upper bounds for the 2 or 3 cpfs for each
1124 cluster were combined by enveloping, i.e., taking the minimum and maximum cumulative probability
1125 at each dose. This reduces the 2-3 cpfs to a single cpf for each cluster, with lower and upper bounds
1126 reflecting the imprecision of the Question 1 probabilities and the differences between experts. The
1127 calculation for step 3 was then performed twice: once with the lower bound of the cpf for each cluster,
1128 producing a lower bound for the cpf for the estimated lowest BMD across clusters; and once with the
1129 upper bound of the cpf for each cluster, producing an upper bound for the cpf for the estimated lowest
1130 BMD across clusters.

1131 The multiplication in step 1 and probability calculations in step 3 above are based on assuming
1132 independence between the cpfs for different clusters. The potential impact of deviations from
1133 independence were explored by sensitivity analysis (below).

1134 *Sensitivity analysis*

1135 A sensitivity analysis was conducted to identify which clusters had most influence on the cpf for the
1136 estimated lowest BMD across all clusters, so that these clusters could be subjected to further review
1137 (see below). A second sensitivity analysis was conducted to compare the cpf obtained when the

1138 parametric distributions were fitted to the experts' judgements for Question 2 with the cpf obtained
1139 when non-parametric distributions were fitted by linear interpolation between judgements for each
1140 expert. These two sensitivity analyses were repeated twice later in the assessment, to check whether
1141 the results changed when the experts' judgements were revised. A third sensitivity analysis was
1142 conducted later in the assessment, to examine the potential impact of deviations from independence
1143 of judgements between selected clusters. The sensitivity analyses were performed in R, using code
1144 included in Annex KJ, and the results were used to inform the review and discussion of the main
1145 calculation results and assessment of overall uncertainty (see below).

1146 *Expert review and revision of calculation results*

1147 The results of the calculations and sensitivity analysis were reviewed and discussed in a web meeting
1148 attended by all the experts who conducted the cluster assessments, plus other members of the WG
1149 conducting the BPA assessment and the same facilitator and two rapporteurs as before. This was
1150 followed by a facilitated discussion of the cluster with most influence on the cpf for the estimated lowest
1151 BMD across all clusters. The two experts who had assessed this cluster described the reasoning for
1152 their judgements on Questions 1 and 2. This was followed by an extensive and detailed discussion by
1153 the whole group, after which the same two experts (who had specialist expertise on this cluster) revised
1154 their judgements in the light of the discussion. Their revised judgements were then used to repeat the
1155 calculations and sensitivity analysis and produce revised outputs.

1156 In the same meeting, the WG considered the HOC General toxicity. Comparing the summarised data
1157 for General toxicity to the elicited distributions from the other health categories, the experts agreed
1158 that it was not necessary to elicit judgements for the General toxicity clusters.

1159 *Elicitation with additional experts for the most influential cluster*

1160 In the light of the range of opinions expressed when discussing the most influential cluster, it was
1161 agreed to elicit judgements on Question 2 for this cluster from all the experts in the WG. This was done
1162 at the next meeting. To inform those judgements, the meeting started with a presentation on the
1163 biology, mechanisms and health consequences for this cluster of endpoints, discussion of the choice of
1164 BMR for the key endpoint in this cluster, review of the dose-response studies available for this cluster
1165 and discussion of uncertainties affecting those studies. This was followed by a repetition of the EKE
1166 training previously provided, for the benefit of those who had not participated in earlier judgements.
1167 The experts were then asked to consider their plausible limits for Question 2 and a brief discussion was
1168 held to agree on consensus lower and upper limits that covered the range of their individual limits. The
1169 experts were next asked to work separately to make their judgements on the distribution within the
1170 consensus limits, using the roulette method and the Excel template that was described above. The
1171 judgements were collected and displayed in a table for discussion, together with parametric and
1172 empirical (histogram) distributions fitted to each individual's judgements. The reasons for the range of
1173 opinion were then explored, by a structured discussion and listing of the evidence and reasoning for
1174 the lower and upper ends of the range covered by the experts' individual distributions. No attempt was
1175 made to elicit a consensus distribution, because the differences between experts were large and it was
1176 important to examine their impact on calculation of the cpf for the estimated lowest BMD across clusters
1177 (see below). Instead, the experts were invited to review and revise their individual distributions in the
1178 light of the discussion, if they wished. The calculations described earlier were then repeated, using each
1179 expert's fitted distribution in turn, to show how the different individual judgements for the most
1180 influential cluster affected the cpf for the estimated lowest BMD across all clusters.

1181 *Consideration of additional uncertainties and dependencies*

1182 The final step of an uncertainty analysis should be assessment of overall uncertainty, combining the
1183 results of earlier steps with any additional uncertainties that are not yet quantified (EFSA Scientific
1184 Committee, 2018a). For this purpose, the results of sensitivity analysis for selected dependencies
1185 between clusters were presented, and a structured discussion was held to elicit a list of additional
1186 sources of uncertainty. Considering these, the WG judged that the dependencies and additional

1187 uncertainties would not alter the assessment of overall uncertainty provided already by the range of
1188 distributions resulting from calculations using the judgements of different experts for the most
1189 influential cluster. The WG therefore agreed to use that range of distributions as the basis for assessing
1190 overall uncertainty about the estimated lowest BMD across all clusters. It was also agreed to explore
1191 options for integrating or averaging the range of judgements to assist the WG in developing consensus
1192 conclusions on the overall uncertainty. The outcome of this was presented and discussed at the next
1193 meeting.

1194 *Averaging cpfs of different experts for the same cluster*

1195 Revised calculations were conducted to explore the effect of aggregating the cpfs of different experts
1196 for each cluster by averaging before calculating the cpf of the estimated lowest BMD across clusters.
1197 As there is no basis for reducing the approximate probability (range of probabilities) for Question 1 to
1198 a precise probability (e.g. the midpoint), the averaging was repeated with the lower and upper bounds
1199 of the approximate probability for Question 1.

1200 Within each cluster, the cpfs of different experts for the same cluster were aggregated by taking the
1201 unweighted linear pool, which gives equal weight to each expert. The average cpfs for all the clusters
1202 were then combined in the same way as in previous calculations (see above). This produced a cpf for
1203 the estimated lowest BMD across all clusters, which again had a lower and upper bound reflecting the
1204 combined effect of the differences between lower and upper bounds of the approximate probabilities
1205 for Question 1 for all clusters. Sensitivity analysis was conducted to show how the lower and upper
1206 bounds of the cpfs would change if the WG were to agree on a precise consensus probability for
1207 Question 1 for the most influential cluster.

1208 The WG members were asked whether they would agree on a consensus range or precise probability
1209 for Question 1 for the most influential cluster. To inform discussion of this, the reasoning of the two
1210 experts with specialist knowledge of this cluster were displayed. Next, the WG experts were asked
1211 whether they accepted the results of averaging across experts in each cluster as their consensus
1212 assessment for the combined cpf for all clusters, or whether they preferred to report multiple cpfs
1213 reflecting their differing individual distributions for Question 2 for the most influential cluster. The WG
1214 experts' responses were then used in a final repetition of the calculations, producing a lower and upper
1215 bound for the consensus cpf for the estimated lowest BMD across all clusters.

1216 *Sensitivity analysis for consensus distribution*

1217 The sensitivity analyses were repeated using the consensus probability of 66% for Question 1 on allergic
1218 lung inflammation and the averaged individual distributions for each cluster. The purpose of this was
1219 to check whether the final consensus cpf for the estimated lowest BMD across all clusters was more
1220 sensitive to the method of distribution fitting and possible dependencies between clusters than was the
1221 case in earlier sensitivity analysis.

1222 *Additional UF for deriving the TDI*

1223 In the final step in the uncertainty analysis, the WG reviewed the results of the preceding steps and a
1224 consensus was sought on whether, when deriving the TDI, an additional UF is needed to allow for
1225 uncertainty about whether effects on other endpoints may potentially occur after exposure to lower
1226 doses than the endpoint on which the RP was based. To inform this discussion, tabulated percentiles
1227 of the cpf for the estimated lowest BMD across all clusters were displayed, based on the lower and
1228 upper consensus bounds of the cpf that resulted from the decisions made by the WG in the preceding
1229 step. This was accompanied by a parallel table, showing the ratio of the RP (BMDL) to each percentile
1230 of the consensus lower and upper bounds of the cpf. The WG was also reminded of the wider range of
1231 cpfs that results when individual judgements were not averaged. A final discussion was then conducted
1232 to seek consensus on whether an additional UF was needed and, if so, how large it should be.

1233 2.3.4.2. Method for uncertainty analysis for genotoxicity

1234 The purpose of the uncertainty analysis for genotoxicity was to assess the degree of certainty for the
1235 conclusion on whether BPA presents a genotoxic hazard by a direct mechanism (direct interaction with
1236 DNA), taking into account the available evidence and also the associated uncertainties. This overall
1237 question was divided into two sub-questions, which were assessed by three WG members with specialist
1238 expertise in genotoxicity assessment. The three experts were asked to express their judgement about
1239 each question in the form of an approximate probability (i.e., a lower and upper probability), and to
1240 summarise the evidence and reasoning on which their judgement was based.

1241 The experts' judgements were elicited by a structured procedure based on EFSA's Guidance on expert
1242 knowledge elicitation (EFSA, 2014), adapted to the context of the genotoxicity assessment. The specific
1243 questions to be addressed by the three experts were defined as follows:

- 1244 • Sub-question 1: What is your probability (%)¹² that there is a genotoxic hazard in humans from
1245 BPA?
- 1246 • Sub-question 2: If there would be a genotoxic hazard in humans from BPA, what is your
1247 probability that its causes include a direct mechanism?

1248 The word 'include' in sub-question 2 was introduced to accommodate the possibility that both direct
1249 and indirect mechanisms could operate together.

1250 The experts were provided with guidance on how to assess and express their probability judgements
1251 for the two questions. They were asked to consider all the data they had reviewed for the genotoxicity
1252 assessment, including results from *in vitro* studies and animal models, taking into account their
1253 relevance to humans; the available human data were considered not relevant (see Annex L).

1254 The three experts first worked on the questions independently, based on the evidence they had already
1255 reviewed and evaluated for the opinion, and recorded their probabilities and the reasoning for their
1256 judgements in an excel template similar to that which was used for Question 1 in the uncertainty
1257 analysis for non-genotoxic endpoints (see preceding section and Annex M). This was followed by a
1258 facilitated meeting, where the three experts presented their judgements and reasoning and discussed
1259 them together with the WG Chair. After the meeting, the three experts were invited to review and, if
1260 they wished, revise their judgements and reasoning in the light of the discussion.

1261 Each expert's revised probabilities for the two sub-questions were multiplied to provide a probability
1262 for the overall question. This is appropriate because the second question is conditional on the first. The
1263 first sub-question provides a probability for BPA presenting a genotoxic hazard; the second question
1264 provides a conditional probability that, *if* BPA presents a genotoxic hazard, there is a direct mechanism.
1265 So the product of these is a probability that both are true: that BPA does present a genotoxic hazard
1266 and that there is a direct mechanism. Because the experts' probabilities were approximate (ranges),
1267 the calculation is done by interval arithmetic (see Annex B.7 in EFSA Scientific Committee, 2018b) and
1268 the resulting probabilities are also approximate.

1269 The three experts presented and discussed their revised judgements and reasoning in a facilitated
1270 meeting with the full WG. The WG discussed the results of the calculations combining the experts'
1271 probabilities for the two questions and expressed the conclusion of the WG both as a probability range
1272 and using verbal likelihood terms from the approximate probability scale, which is recommended by
1273 EFSA (Table 2 in EFSA Scientific Committee, 2018a) for harmonised use in EFSA assessments. Finally,
1274 the WG discussed the implications of their conclusion for whether a TDI could be set for BPA or whether
1275 a Margin of Exposure approach was required.

¹² The question asks the experts for 'your probability' expressed as a percentage, because these are subjective probabilities which represent the judgement of each expert (EFSA Scientific Committee, 2018a).

1276 2.3.5. Method for assessing genotoxicity

1277 The evaluation of data quality for hazard/risk assessment includes the evaluation of reliability and
1278 relevance (Klimisch et al., 1997; OECD, 2005; ECHA, 2011; EFSA Scientific Committee, 2017c; EFSA
1279 Scientific Committee, 2021a).

1280
1281 In the assessment of genotoxicity studies, the data quality has been evaluated based on reliability and
1282 relevance. Reliability has been assessed using a scoring system based on criteria published by Klimisch
1283 et al. (1997).

1284 In a second step, the relevance (high, limited, low) of the study results was assessed based on reliability
1285 of the study and other aspects, e.g. genetic endpoint, purity of test substance, route of
1286 administration and status of validation of the assay.

1287
1288 Genotoxicity studies evaluated as of high or limited relevance have been considered in a WoE
1289 approach. Genotoxicity studies evaluated as of low relevance have not been further considered in the
1290 assessment.

1291
1292 The different steps of the evaluation are described in the following sections.

1293 2.3.5.1. Evaluation of reliability of results of genotoxicity studies – general 1294 considerations

1295 Reliability is defined as “evaluating the inherent quality of a test report or publication relating to
1296 preferably standardized methodology and the way that the experimental procedure and results are
1297 described to give evidence of the clarity and plausibility of the findings” (Klimisch et al., 1997).

1298 In assigning the reliability score, the compliance with the Organization for European Economic
1299 Cooperation and Development (OECD) Test Guidelines (TGs) or standardized methodology and the
1300 completeness of the reporting as detailed below were considered.

1301 The reliability scores were:

- 1302 1) reliable without restriction
- 1303 2) reliable with restrictions
- 1304 3) insufficient reliability
- 1305 4) reliability cannot be evaluated
- 1306 5) reliability not evaluated, since the study is not relevant and/or not required for the risk
1307 assessment (in case the study is reported for reasons of transparency only).

1308 These reliability scores were defined as follows (Klimisch et al., 1997):

- 1309 1) Reliable without restriction

1310 “This includes studies or data from the literature or reports which were carried out or
1311 generated according to generally valid and/or internationally accepted testing guidelines
1312 (preferably performed according to Good Laboratory Practice (GLP)) or in which the test
1313 parameters documented are based on a specific (national) testing guideline (preferably
1314 performed according to GLP) or in which all parameters described are closely
1315 related/comparable to a guideline method.”

- 1316
1317 2) Reliable with restrictions

1318 “This includes studies or data from the literature or reports (mostly not performed according
1319 to GLP), in which the test parameters documented do not totally comply with the specific

1320 testing guideline, but are sufficient to accept the data or in which investigations are described
1321 which cannot be subsumed under a testing guideline, but which are nevertheless well
1322 documented and scientifically acceptable.”

1323
1324 3) Insufficient reliability¹³

1325 “This includes studies or data from the literature/reports in which there are interferences between the
1326 measuring system and the test substance or in which organisms/test systems were used which are
1327 not relevant in relation to the exposure (...) or which were carried out or generated according to a
1328 method which is not acceptable, the documentation of which is not sufficient for an assessment and
1329 which is not convincing for an expert judgment.”

1330 4) Reliability cannot be evaluated¹⁴

1331 “This includes studies or data from the literature, that do not give sufficient experimental details and
1332 that are only listed in short abstracts or secondary literature (books, reviews, etc).”

1333 In case a study is reported for reasons of transparency only, a further score (5) may be required.

1334 5) Reliability not evaluated

1335 The study is not relevant and/or not useful for the risk assessment. The following references
1336 (Klimisch et al., 1997; OECD, 2005; ECHA, 2011; EFSA Scientific Committee, 2011, EFSA
1337 Scientific Committee, 2017c; EFSA Scientific Committee, 2021a) may be consulted for more
1338 details and examples.

1339 Each reliability box in the summary tables (see Annex L) started with the reliability score, followed by
1340 comments justifying the score. This is equally applicable for *in vitro* as *in vivo* studies.

1341 **2.3.5.2. Evaluation of relevance of results of genotoxicity studies – general** 1342 **considerations**

1343 The relevance of the study (high, limited or low) is based both on its reliability and on the relevance of
1344 the test results.

1345 The relevance of the test results was mainly, but not exclusively, based on:

- 1346 • Genetic endpoint (high relevance for gene mutations, structural and numerical chromosomal
1347 alterations as well as results obtained in an *in vivo* comet assay, which belongs to the assays
1348 recommended by the EFSA Scientific Committee (2011) for the follow-up of a positive *in vitro*
1349 result; lower relevance for other genotoxic effects). Other test systems although potentially
1350 considered of limited or low relevance may provide useful supporting information.
- 1351 • Route of administration (e.g. oral vs. intravenous, intraperitoneal injection, subcutaneous
1352 injection, inhalation exposure) in case of *in vivo* studies.
- 1353 • Status of validation (e.g. for which an OECD TG exists or is in the course of development,
1354 internationally recommended protocol, validation at national level only, no validation).
- 1355 • Reliability and relevance of the test system/test design irrespectively of whether a study has
1356 been conducted in compliance with GLP or not.

¹³ Klimisch et al. (1997) used the term “Not reliable”, however, “Insufficient reliability” was considered more appropriate.

¹⁴ Klimisch et al. (1997) used the term “Not assignable”, however, “Reliability cannot be evaluated” was considered more appropriate.

- 1357 • Information on BPA purity grade and/or the supplier. If only the supplier was available, the
1358 company's website was consulted to retrieve the purity grade, or the authors were contacted
1359 to ask for it. If none of the two information were reported or obtained, the relevance was
1360 considered low and the study was excluded from the WoE assessment.

1361 Studies for which the relevance of the result was judged to be low were not considered further.

1362 **2.3.5.3. Presentation of the study evaluations**

1363 All evaluated studies were summarised, either in a narrative form (Appendix E) or in tables (reported
1364 in Annex L), to structure the outcome of the evaluations in a transparent way and to provide a possibility
1365 to consider the relevance of study results in a weight-of-evidence approach. Remarks were included in
1366 the column "Reliability" and assigned relevance to the test results to justify the judgments. Minor and/or
1367 major deviations from OECD TGs were reported in column "Reliability" (e.g. lack of positive control,
1368 inappropriate exposure conditions, limited reporting etc.).

1369 In these tables, the studies were grouped based on genetic endpoints or test systems and
1370 chronologically within these groups. The results were evaluated and presented as positive, negative,
1371 equivocal or inconclusive. If considered relevant for the interpretation of the genotoxicity endpoints,
1372 non-genotoxicity endpoints (e.g. reactive oxygen species (ROS) production) were reported in a
1373 narrative way only, but the results were not classified as "positive" or "negative".

1374 **2.3.5.4. WoE approach**

1375 The WoE approach applied to the evaluation of genotoxicity data is based on EFSA Scientific Committee
1376 recommendations (EFSA Scientific Committee, 2011, 2017b,c). As recommended by the EFSA Scientific
1377 Committee (EFSA Scientific Committee, 2011, 2017b), a documented WoE approach for the evaluation
1378 and interpretation of genotoxicity data' has been applied, taking into account not only the quality and
1379 availability of the data on genotoxicity itself, but also all other relevant data that may be available.
1380 These include data on MoA and on toxicokinetics when available. The main steps of the WoE approach
1381 applied in the genotoxicity assessment of BPA are described below.

1382 *Assembling of the evidence into lines of evidence of similar type*

1383 In a first step, the CEP Panel evaluated all available *in vitro* and *in vivo* studies addressing the three
1384 main endpoints of genotoxicity: gene mutations, structural and numerical chromosomal aberrations
1385 (CA) in addition to DNA damage endpoint (evaluated by Comet assay). The study results addressing
1386 each of these endpoints were grouped into lines of evidence. Only the studies of high and limited
1387 relevance were included.

1388 Studies investigating the BPA MoA were considered, e.g. DNA oxidation, ROS (when genotoxicity was
1389 also investigated in the same study), DNA binding, interference with proteins involved in chromosome
1390 segregation during cell division, modulation of expression of genes involved in DNA repair or in
1391 chromosome segregation and markers of DNA double strand breaks (DSBs) (e.g. γ H2AX). Evidence
1392 from the mechanistic studies may support the lines of evidence for the genotoxicity endpoints.

1393 *Weighting of the evidence*

1394 A quantitative method to weight the evidence was not considered appropriate due to the quantity and
1395 heterogeneity of the evidence to be integrated. A qualitative method based on expert judgment was
1396 applied. All studies evaluated for reliability and relevance (as described above) were listed in tables
1397 (Annex L). The evaluation of the studies of high and limited relevance was described in the opinion,
1398 including the conclusion for each line of evidence. The consistency of the evidence was assessed and
1399 presented in the opinion.

1400 *Integrating all the evidence*

1401 The lines of evidence of the above genotoxicity endpoints were assessed separately. To elucidate the
1402 MoA of BPA, mechanistic studies were considered.

1403 Integrating evidence from the MoA with lines of evidence from genotoxicity endpoints allows a reduction
1404 in the uncertainty on the potential genotoxicity. In case genotoxic effects were observed, evidence from
1405 the MoA may allow clarification if the genotoxicity is due to a direct or indirect mechanism.

1406 **3. Assessment**1407 **3.1. Hazard identification**1408 **3.1.1. Toxicokinetics and metabolism**1409 **3.1.1.1. Outcomes of the 2015 BPA opinion**

1410 Species-stage and life-stage dependent differences in the toxicokinetic profile of BPA must be
1411 considered when comparing toxicokinetic data from different species.

1412 BPA-glucuronidation is the major metabolic pathway of BPA in humans, non-human primates and
1413 rodents. Glucuronidated BPA is a biologically inactive form of BPA at the oestrogen receptors (ERs);
1414 however, it cannot be excluded that the glucuronidated form may have effects at ER-independent sites.
1415 BPA can also be conjugated via sulfation to a lesser extent. In addition to the conjugation pathways, *in*
1416 *vivo* and *in vitro* studies suggest that in the rat, BPA may be subject to oxidation to bisphenol *O*-quinone
1417 by cytochrome P450 and to 5-hydroxy-BPA (EU-RAR, 2003, 2008) to a small extent. EFSA (2007)
1418 reported oxidative BPA metabolites to also occur in mice.

1419 The oral systemic bioavailability of unconjugated BPA in adults is 2.8% in rats, 0.45% in mice and 0.9%
1420 in monkeys, based on oral versus intravenous (i.v.) toxicokinetic data. Lower concentrations of
1421 unconjugated BPA and BPA conjugates were measured in the amniotic fluid of rats and of monkeys in
1422 comparison with the serum levels. In early pregnancy, exposure of the fetus at the same dose (in µg/kg
1423 body weight of the pregnant animal) might be higher than in later pregnancy because of the
1424 development of metabolising enzymes in the liver of the fetus after i.v. exposure to BPA. BPA is present
1425 in rat milk from BPA-treated dams in both unconjugated and conjugated forms. In rat milk, BPA-
1426 glucuronide (BPA-G) comprises approximately 80% of the total BPA concentration. Pup exposure via
1427 lactation is low, i.e. approximately 1/300 of the maternal dose. Unconjugated BPA has also been
1428 reported in human milk. BPA-conjugating enzymes UDP-glucuronyl-transferases (UGT) and
1429 sulfotransferases (SULT) are polymorphically expressed in humans.

1430 The default intraspecies UFs used to derive a health-based guidance value (HBGV) are considered
1431 sufficient to account for possible differences in rates of metabolism of BPA between human individuals.
1432 Data from toxicokinetic studies in various laboratory animal species provide the basis for internal dose
1433 conversion metrics for neonatal-to-adult stages and for different routes of exposure. Moreover,
1434 physiologically-based pharmacokinetic (PBPK) models have been developed to predict the internal
1435 exposures in laboratory animals and humans in a route-specific manner.

1436 Overall, this body of information permits the application of the human equivalent dose (HED) concept
1437 for providing HEDs for points of departure derived from critical animal data. This was achieved by
1438 estimating human equivalent dose factors (HEDF) from the ratio of the area under the curve (AUC) for
1439 the test species and simulated AUCs for humans. Available experimental evidence indicates a 24-h
1440 percutaneous penetration of BPA across human skin of 2.3–8.6%. For exposure scenarios with dermal
1441 contact to thermal paper, the CEF Panel used a conservative value of 10% dermal absorption.

1442 The CEF Panel did not consider skin metabolism, as the conjugation enzymes involved in the metabolism
1443 of BPA are expressed at a very low level. For scenarios with aggregated oral and dermal exposures,
1444 PBPK modelling was used to estimate the internal dose metrics (AUCs) for unconjugated BPA, with
1445 which equivalent oral exposures were subsequently calculated.

1446 **3.1.1.2. New data on BPA toxicokinetics**

1447 Publications on BPA toxicokinetics (published from 2013 to 2018, and not considered in the 2015
1448 opinion) were assessed in a narrative way and are reported here.

1449 *Animal data*1450 **Mice**

1451 The group of deCatanzaro (Pollock et al., 2014 [RefID 5870], 2017a [RefID 5869], 2017b [RefID 5871];
1452 Borman et al., 2017 [RefID 655]; Pollock et al., 2018) performed a series of studies in which they
1453 investigated the effect of triclosan, diethylhexyl phthalate, butyl paraben, propylparaben and
1454 tetrabromobisphenol A, with and without concurrent triclosan, as well as a mixture of triclosan,
1455 tetrabromobisphenol A, butyl paraben, propylparaben and diethylhexyl phthalate on the serum and
1456 tissue concentrations of ¹⁴C-BPA, which were given concomitantly, in female and male mice. The co-
1457 administration of the substances influenced the tissue distribution of ¹⁴C-BPA by increasing the
1458 radioactivity of the one single measured serum sample, taken 1 h after administration, and of some
1459 tissue levels.

1460 As no investigation was performed to elucidate the underlying mechanism for the higher radioactivity,
1461 the data are of limited interest for this risk assessment of BPA.

1462 Draganov et al. (2015) [RefID 1689] investigated the kinetics of BPA in neonatal mice on post-natal
1463 day (PND) three following single-dose oral or subcutaneous (s.c.) administration. The study was divided
1464 in three parts: (i) a mass-balance part to confirm the mode of administration, (ii) a pharmacokinetic
1465 part, in which total radioactivity was measured in female PND3 rats, and (iii) a metabolic profiling part,
1466 in which two groups of rats received ³H-BPA either orally or subcutaneously. Blood from five rats per
1467 time point was collected and their plasma was pooled for analysis of total radioactivity and analysis of
1468 BPA, BPA-G and BPA-sulfate (BPA-S), after high-performance liquid chromatography (HPLC) separation
1469 and fractionated measurements. The method was validated by liquid chromatography-tandem mass
1470 spectrometry (LC-MS/MS) confirmation of the radioactive peaks. The authors evaluated several
1471 techniques for administration and found a technique with a recovery of 92.3 ± 3.36% (oral) and
1472 88.2 ± 2.9% (by s.c. administration) being sufficiently precise for performing part (ii) and (iii) of the
1473 study. The AUC of plasma concentration–time profile of the total radioactivity was 1879 ng/g × h
1474 following oral and 1576 ng/g × h following s.c. administration of 400 µg/kg bw ³H-BPA. The AUC for
1475 BPA, BPA-G and BPA-S was 15 ng/g × h, 1065 ng/g × h and 33.8 ng/g × h after oral administration
1476 and 49.8 ng/g × h, 913 ng/g × h, 31.2 ng/g × h after s.c. injection, indicating first pass metabolism
1477 by the oral route of administration. The AUC of non-conjugated BPA in this study of 15 ng/g × h (or
1478 65.8 nM × h) with a dose of 400 µg/kg bw compared well with the AUC of 26 nM × h reported by
1479 Doerge et al. (2011) for a dose of 100 µg/kg bw given to neonatal mice on PND3.

1480 Pollock and deCatanzaro (2014) [RefID 5867], investigated the distribution of BPA into several organs
1481 in mice with special focus on the uterus. In one experiment, performed in 4-month-old female mice,
1482 doses of 0.5 µg/kg, 5 µg/kg or 50 µg/kg ¹⁴C-BPA were given by the oral route and the distribution of
1483 the radioactivity was measured. In another experiment, 50 µg/kg ¹⁴C-BPA was administered by the oral
1484 route to 4-month-old female mice either once, or dosing for 7 or 28 days, and the distribution of
1485 radioactivity in several organs was measured. In all the experiments, ¹⁴C-BPA measurements were done
1486 1 h after the dosing. Radioactivity was measurable in the organs investigated and also in the serum
1487 following all doses with the exception of 5 µg/kg bw. In this dose group, no radioactivity could be
1488 measured in the olfactory bulb, cerebellum, frontal cortex and hypothalamus. Following repeated dosing
1489 (50 µg/kg ¹⁴C-BPA), the concentration in the uterus was higher than in heart, lung, muscle and adipose
1490 tissue after 28 but not after 7 days treatment; in this experiment concentration in the ovary was also
1491 higher than in heart, lung, muscle and adipose tissue following 28 days treatment. This study does not
1492 contribute to an interspecies extrapolation of the kinetics.

1493 **Rat**

1494 Pollock and deCatanzaro (2014) [RefID 5867] also investigated the distribution of BPA into several
1495 organs in rats with special focus on the uterus. In the first experiment, the distribution of ¹⁴C-BPA (dose
1496 50 µg/kg bw by the oral route) was studied in cycling female rats either without pre-treatment or with
1497 pre-treatment with ICI 182.780, an oestrogen antagonist, or E2. In the second experiment, the
1498 distribution of ¹⁴C-BPA (dose 50 µg/kg bw by the oral route) was studied in inseminated female rats.

1499 In the third experiment, the concentration of the parent compound (aglycone) versus the total BPA in
1500 the rat uterus was the focus of the study following 50 µg/kg BPA on the oral route. In all the
1501 experiments, ¹⁴C-BPA measurements were done 1 h after the dosing. Radioactivity was measurable in
1502 the organs investigated, in the serum, following all doses with the exception of 5 µg BPA /kg bw. In
1503 this dose group, no radioactivity could be measured in the olfactory bulb, cerebellum, frontal cortex
1504 and hypothalamus. In oestrus cycling rats, in inseminated rats (for every of the three doses), the
1505 concentration of radioactivity in the uterus was higher compared with that in heart, lung, muscle,
1506 adipose tissue and ovary. Following repeated dosing in mice (50 µg/kg bw of ¹⁴C-BPA), concentration
1507 in the uterus was higher than in heart, lung, muscle and adipose tissue after 28 but not after 7 days
1508 treatment; in this experiment concentration in the ovary was also higher than in heart, lung, muscle
1509 and adipose tissue following 28 days treatment. In uterine samples from cycling rats, the concentration
1510 of the aglycone was 71.9 ± 3.4% of the total BPA concentration. As it is known that more than 90%
1511 of the total BPA in the plasma of rats is conjugated BPA (e.g. Churchwell et al., 2014), it is most
1512 probable that the higher concentration of aglycone found in this study is due to deconjugation of the
1513 BPA conjugates during sample preparation. This study does not contribute to an interspecies
1514 extrapolation of the kinetics.

1515 The study of Kazemi et al. (2016a) [RefID 11132] was performed in three groups of male rats that
1516 received 5, 25 and 125 µg/kg bw per day of BPA, by gavage, for 35 consecutive days. The number of
1517 rats per group was not given. Three other groups of rats received nonylphenol (doses 5, 25, and 125
1518 µg/kg bw per day also for 35 days). The results of this part of the study will not be reported here. At
1519 the end of the study, 2 mL blood samples were taken and BPA was measured in the serum. Specimens
1520 of the testes were also obtained and after processing the concentration of BPA was measured. The
1521 analytical method used HPLC separation and fluorescence detection. Neither limit of detection (LOD)
1522 nor limit of quantification (LOQ) for the method are given. The authors report concentrations of 0.03
1523 µg/mL, 0.3 µg/mL and 1.13 µg/mL BPA in serum and 0.3 µg/mL, 0.82 µg/mL and 3.59 µg/mL BPA in
1524 the testes with doses of 5, 25, 125 µg/kg bw per day. The concentrations are several orders of
1525 magnitude higher than those reported from other authors when using specific methods (use of labelled
1526 BPA and LC/MS/MS) (Churchwell et al., 2014). The analytical method used does not give a BPA-specific
1527 signal and therefore, the results of this study do not contribute to the knowledge on BPA kinetics.

1528 In the course of a study, aimed at investigating the effect of increasing doses of BPA (0.5, 5, 50 and
1529 250 mg/kg bw per day) on the development of bile duct proliferation by Jeong et al. (2017) [RefID
1530 3133], toxicokinetic studies were carried out. Plasma samples were taken on day 1 (PND6) and day 91
1531 of the study at 0.25, 0.5, 1, 2, 4, 8 and 24 h after dosing by gavage and BPA was determined using a
1532 specific and sensitive method. AUC and C_{max} were determined. On day 91, the plasma levels following
1533 0.5 mg/kg bw and 5 mg/kg bw were below the level of quantification (1.68 ppb= 1.68 µg/L = 7.4 nM).
1534 The AUCs were 26 times (50 mg/kg bw) and 125 times (250 mg/kg bw) higher on day 1 (PND6) than
1535 on day 91, consistent with an early life (PND6) lower expression of conjugation enzymes of BPA. A
1536 marked supra-linear increase of AUC with dose indicated saturation of metabolism in PND6 animals at
1537 such high doses.

1538 **Other animals**

1539 The study of Collet et al. (2015) [RefID 1268] aimed to predict the clearance in humans by allometric
1540 scaling based on concentration–time data after intravenous administration of 5 mg/kg bw BPA to mice,
1541 rats, dogs, piglets, ewes and horses. From the data, plasma clearance was calculated and resulted in
1542 0.00078 (mice), 0.019 (rats), 0.31 (dogs), 1.3 (piglets), 1.6 (ewes) and 6.2 (horses) L/min, values close
1543 to the hepatic blood flow in the respective species. The human clearance was estimated to be 1.79
1544 (95th prediction interval 0.36–8.83) L/min, which exceeded the human liver blood flow indicating a
1545 possible clearance in the gut wall.

1546 In the study of Gauderat et al. (2016) [RefID 2219], fetal sheep at the end of pregnancy were exposed
1547 to an intravenous (iv) administration of both BPA and BPA-G at the same molar dose (21.9 µmol/kg;
1548 corresponding to 5 mg/kg bw BPA and to 8.86 g/kg bw BPA-G). After BPA administration, BPA
1549 disappeared from the plasma with a half-life of 0.31 h and BPA-G and BPA-S were found in the plasma,
1550 BPA-G being the main metabolite. Both BPA-G and BPA-S had a half-life longer than the parent
1551 compound. After BPA-G administration, its half-life was 28 h and not only BPA-G was detected in the
1552 plasma of the fetal sheep but also BPA, however, in very low concentrations (200-fold to 8000-fold less
1553 than BPA-G), indicating that BPA-G could be de-glucuronidated in the fetal sheep. In the maternal

1554 plasma BPA, BPA-G and BPA-S were found following i.v. administration of BPA to the fetal sheep,
1555 whereas BPA-G was the only molecule determined in the maternal plasma after BPA-G administration
1556 to the fetal sheep. This indicates that BPA, BPA-G and BPA-S are transported from the fetal blood via
1557 the placenta into the maternal blood. The ratios between fetal and maternal plasma concentrations
1558 indicate that BPA and BPA-S are better transported than BPA-G.

1559 BPA kinetics was studied in the adult sheep, 2 weeks after termination of the pregnancy. After BPA
1560 administration, BPA disappeared from the plasma with a half-life of 1.6 h; BPA-G was the main
1561 metabolite with a half-life of 0.84 h. BPA-S was also formed and disappeared with a half-life similar to
1562 that of BPA.

1563 Repeated dosing of BPA-G (five doses) produced similar low BPA levels than after single dosing.
1564 Similarly, low BPA levels could also be measured in the amniotic fluid after five doses of BPA-G to
1565 pregnant sheep.

1566 For the extrapolation to the human situation, it should be considered that the study was performed in
1567 sheep at the end of pregnancy. In the human fetus, extremely low expression of glucuronidation
1568 enzymes has been reported up to 20 weeks; until this time enzyme protein levels were not detectable
1569 (Divakaran et al., 2014 [RefID 1631]; Coughtrie, 2015). At birth, the levels were 10% of the levels
1570 expressed in older infants and in adults. In the human fetus, BPA can only be glucuronidated to a very
1571 small extent; the process described in the fetal sheep of releasing a small extent of BPA from BPA-G
1572 may also take place in humans but the resulting BPA concentration would be low.

1573 In the study of Guignard et al. (2016) [RefID 2450], four ewes received a dose of 100 mg/kg bw BPA
1574 by nasogastric gavage and 13 days apart of 10 mg/kg bw BPA through food pellets. Blood was taken
1575 from the jugular vein in short intervals in the first 2 h and then every second hour until 10 h post dosing
1576 as well as 24 h after dosing. The first blood sample was taken 1.8 min after ingesting the pellets and
1577 4.8 min after nasogastric feeding. BPA and BPA-G in serum was estimated by LC-MS with a low inter-
1578 and intraday variation (<15%) and a LOQ of 1 ng/mL for BPA and 5 ng/mL for BPA-G. It is remarkable
1579 that in the first 2 h the serum concentration following pellet ingestion was equal or even higher than
1580 following nasogastric feeding despite the fact that the dose was 10-fold lower. The AUC-BPA/dose was
1581 2-fold to 4.5-fold lower following nasogastric feeding than following pellet ingestion. This finding
1582 together with the higher ratio of BPA-G/BPA following nasogastric feeding indicate high first pass
1583 metabolism on this mode of administration, whereas administration by food pellets with long-time
1584 contact to the buccal membranes indicate absorption of BPA directly into the systemic circulation.
1585 Therefore, the systemic availability of BPA administered by food pellets in ewes was higher than by
1586 nasogastric feeding [relative bioavailability of nasogastric feeding vs food pellets was $31 \pm 5\%$;
1587 mean \pm standard error mean (SEM)]. The authors compared the AUCs after i.v. administration to
1588 calculate absolute bioavailability with the AUCs following nasogastric tubing and following
1589 administration by food pellets. The absolute bioavailability was calculated to amount to $0.8 \pm 0.2\%$ and
1590 $2.7 \pm 0.3\%$ (mean \pm SEM) by nasogastric feeding and by food pellets, respectively.

1591 In the study of Guignard et al. (2017) [RefID 2451], the authors compared the relative systemic
1592 availability following oral (by pellets) versus s.c. administration of BPA at a dose of 10 mg/kg bw and 5
1593 mg/kg bw, respectively, in four ewes (50–70 kg). The serum concentrations of BPA, BPA-G and BPA-S
1594 were measured by a reliable method [LC-MS with a low interday and intraday variation (<15%) and a
1595 LOQ of 1 ng/mL for BPA and 5 ng/mL for BPA-G]. When comparing the AUCs, the relative systemic
1596 availability of the oral dose was $3.3 \pm 0.3\%$. The AUCs of the BPA-G were similar after adjustment for
1597 dose. The ratio of the AUC-BPA-G/AUC-BPA was 25 higher following oral compared with s.c.
1598 administration due to the high first pass effect. In the second part of the study, doses of 0.5 μ g/kg bw,
1599 50 μ g/kg bw and 5000 μ g/kg bw by s.c. administration were continuously administered throughout the
1600 pregnancy. The serum BPA profile on gestation day (GD) 62 was in accordance with the predicted
1601 values following a dose of 5000 μ g/kg bw using a bi-compartmental model parametrised with
1602 parameters from the first part of the study with 5 mg/kg bw s.c. BPA. Two weeks before the expected
1603 term a caesarean section was performed. Maternal and fetal serum from the jugular vein, cord blood
1604 and amniotic fluid were obtained at delivery. Concentrations could not be measured for the 0.5 μ g/kg
1605 bw dose and not in all animals in the medium dose of 50 μ g/kg bw. In cord blood, fetal blood and
1606 amniotic fluid, the BPA-G and BPA-S concentrations were much higher than BPA ones (100- to 1000-
1607 fold and 2- to 50-fold, respectively). The BPA concentration in the fetal serum was 2–5-fold lower than

1608 the concentration in cord blood and in the amniotic fluid. In the maternal blood, the concentration of
1609 BPA was 7.5-fold lower than the concentration of BPA-G and 4-fold higher than the concentration of
1610 BPA-S, the findings being compatible with escaping first pass metabolism by the s.c. administration in
1611 the ewe, whereas by delivery to the fetal circulation via cord vessel BPA undergoes first pass. BPA in
1612 the amniotic fluid is due to renal elimination of BPA-G and de-glucuronidation as was demonstrated in
1613 the earlier study of Gauderat et al. (2016) [RefID 2219].

1614 *Human data*

1615 Ten male adult volunteers were exposed to 30 µg/kg bw deuterated BPA (d6-BPA) dissolved in tomato
1616 soup, via the oral route (Teeguarden et al., 2015b [RefID 7155]). Blood samples and urine samples
1617 were collected at frequent intervals up to 24 h. BPA, total BPA, BPA-G and BPA-S were determined in
1618 serum and in urine using a specific and sensitive LC-ES/MS/MS analytical method. The plasma BPA half-
1619 life for distribution and terminal elimination were 0.87 ± 0.3 h (mean \pm SEM) and 5.5 ± 0.5 h
1620 (mean \pm SEM), respectively. C_{max} of aglycone was 0.43 ± 0.14 nM (SEM). AUC of aglycone was
1621 2.5 ± 0.4 nM \times h (SEM). The administered dose was excreted to 104 ± 2.6 % (SEM) as total BPA in
1622 the urine within 24 h. Concerning the conjugates, further metabolites, such as a mixed BPA-
1623 glucuronide/sulfate bis-conjugate and BPA-bis-sulfate, have been identified.

1624 In the study of Thayer et al. (2015) [RefID 7183], 14 adults (six men and eight women) received 100
1625 µg/kg bw deuterated BPA (d6-BPA), applied to a cookie, by the oral route. Blood and urine samples
1626 were taken at frequent intervals and aglycone and total BPA were determined in serum and in urine
1627 using a specific and sensitive LC-ES/MS/MS analytical method, BPA-G and BPA-S conjugates were
1628 determined in urine. The plasma BPA half-life for distribution and terminal elimination were 1.2 ± 0.32
1629 h and 5.6 ± 1.2 h, respectively. No significant difference was found between the terminal half-life of
1630 total BPA (6.4 ± 2.0 h) and that of BPA. C_{max} of the aglycone was 6.5 ± 3.2 nM, $0.39 \pm 0.17\%$ of the
1631 total BPA concentration of 1711 ± 495 nM. AUC of aglycone accounted to be 23 ± 6.2 nM \times h,
1632 $0.56 \pm 0.16\%$ of the AUC of total BPA. Within 48 h, $95 \pm 7.1\%$ of the administered dose was excreted
1633 as total BPA in the urine and $0.11 \pm 0.19\%$ of the total BPA was BPA aglycone. Concerning the
1634 conjugates, BPA-G was the main metabolite with $87 \pm 6.9\%$ in the urine, whereas BPA-monosulfate
1635 accounted for $3 \pm 2.3\%$. There was some indication that further conjugates occur in humans, such as
1636 a mixed BPA-glucuronide/sulfate bis-conjugate and BPA-bis-sulfate.

1637 *PBPK models*

1638 A previously developed PBPK model for BPA in adult rhesus monkeys (Fisher et al., 2011) was modified
1639 to characterise the pharmacokinetics of BPA and its phase II conjugates in adult humans following oral
1640 ingestion (Yang X et al., 2015). By the oral route, metabolism is taking place in the enterocytes lining
1641 the gastrointestinal tract and in the liver. Therefore, data from *in vitro* studies on BPA metabolism in
1642 the liver and in the small intestine were used and the PBPK model was parameterised using oral
1643 pharmacokinetic data with deuterated BPA (d6-BPA) delivered in cookies to adult humans after
1644 overnight fasting. The availability of the serum concentration–time course of unconjugated d6-BPA
1645 offered direct empirical evidence for the calibration of BPA model parameters.

1646 The recalibrated PBPK adult human model for BPA was then evaluated against published human
1647 pharmacokinetic studies with BPA (Teeguarden et al., 2015b [RefID 7155]; Thayer et al., 2015 [RefID
1648 7183]). A hypothesis of decreased oral uptake was needed to account for the reduced peak levels
1649 observed in adult humans, where d6-BPA was delivered in soup and food was provided before BPA
1650 ingestion, suggesting the potential impact of dosing vehicles and/or fasting on BPA disposition. With
1651 the incorporation of Monte Carlo analysis, the recalibrated adult human model was used to address the
1652 interindividual variability in the internal dose metrics of BPA for the US general population. Model-
1653 predicted peak BPA serum levels were in the range of pM, with 95% of human variability falling within
1654 an order of magnitude.

1655 This recalibrated PBPK model for BPA in adult humans provides a scientific basis for assessing human
1656 exposure to BPA that can serve to minimise uncertainties incurred during extrapolations across doses
1657 and species.

1658 In the Teeguarden et al. (2015b) [RefID 7155] study, the Yang et al. (2015b) model was used to
1659 address the question whether buccal absorption has taken place in this study in which serum

1660 concentrations of BPA were measured following intake of deuterated BPA in tomato soup (see
1661 description of the Teeguarden et al., 2015b [RefID 7155] study above). The authors were of the opinion
1662 that the results of the pharmacokinetic model simulations together with a faster appearance half-life of
1663 the metabolite d6-BPA-G compared with d6-BPA (0.29 h vs 0.45 h) were evidence against a meaningful
1664 absorption of BPA in humans through any non-metabolising tissue following BPA administration in
1665 tomato soup on the oral route.

1666 Karrer et al. (2018) [RefID 12289] adjusted the model developed by Yang et al. (2015b) by modifying
1667 the maximal velocity of the glucuronidation in the small intestine. This was scaled up to the body weight
1668 for comparing the pharmacokinetic behaviour of BPA with that of bisphenol S (BPS), bisphenol F (BPF)
1669 and bisphenol AF (BPAF). The model was extended by including dermal exposure and the Thayer et al.
1670 (2015) [RefID 7183] data were used to calibrate the model. In the context of this assessment, it is
1671 noted that the predicted AUC 0–24 h for a dose of 30 µg/kg bw (the dose used by Teeguarden et al.,
1672 2015b [RefID 7155]), was 4.15 (2.91–5.15) nM × h, whereas the experimental value, measured by
1673 Teeguarden et al. (2015b) [RefID 7155], was 2.5 (1.4–5.7) nM × h.

1674 **3.1.1.3. Summary of the new toxicokinetic outcomes**

1675 Several studies in animals and in humans were published after the closing date for literature review in
1676 the previous EFSA opinion on BPA (EFSA CEF Panel, 2015).

1677 Most studies in mice and rats do not report new findings that may contribute to a better understanding
1678 of the kinetics of BPA, since total radioactivity was measured without separation of parent compound
1679 and metabolites. Two studies (Draganov et al. (2015) [RefID 1689] and Jeong et al. (2017) [RefID 3133])
1680 confirmed earlier findings in mice (Doerge et al., 2011) and rats on the immature metabolism of
1681 neonatal mice and rats (Doerge et al., 2010).

1682 Collet et al. (2015) [RefID 1268] investigated the concentration–time data and the derived clearance
1683 after intravenous administration to several species (mouse, rat, dog, piglet, ewe, horse). As the data
1684 have been obtained after intravenous administration, they may be used to estimate the absolute
1685 bioavailability of BPA but are not suitable for deriving the HEDFs in the context of this assessment in
1686 which only effects observed after oral administration are considered.

1687 In the Gauderat et al. (2016) [RefID 2219] study, fetal sheep at the end of pregnancy received an
1688 intravenous administration of BPA and also BPA-G. After BPA-G administration, not only BPA-G was
1689 detected in the plasma of the fetal sheep, but also BPA, though in very low concentrations (200-fold to
1690 8000-fold less than BPA-G), indicating that BPA-G could be partially de-glucuronidated in the fetal
1691 sheep. It was also shown that glucuronidation takes place in the fetal sheep with only a small fraction
1692 being transported through the placenta to the ewe. For the extrapolation to the human situation it
1693 should be considered that in the human fetus extremely low expression of glucuronidation enzymes
1694 was reported (Divakaran et al., 2014 [RefID 1631]; Coughtrie, 2015). At birth, the levels were 10% of
1695 the levels expressed in infants with a higher age and in adults. Hence, in the human fetus, BPA could
1696 be glucuronidated to only a very small extent; the process described in the fetal sheep of producing
1697 BPA from BPA-G may take place, but the resulting BPA concentrations would be very small.

1698 Guignard et al. (2016) [RefID 2450] described different systemic availability of BPA when given by
1699 nasogastric tubing or as pellets to four ewes. They calculated the absolute bioavailability as $0.8 \pm 0.2\%$
1700 and $2.7 \pm 0.3\%$ (mean \pm SEM) by nasogastric tubing and by food pellets, respectively, by comparing
1701 the results of this study with those following intravenous administration. The difference in bioavailability
1702 suggests that BPA, when administered as pellets and masticated by the ewes, can be absorbed through
1703 the buccal mucosa, taken up in the systemic circulation and therefore escape pre-systemic elimination.
1704 This leads to a higher systemic bioavailability compared with direct administration in the gastrointestinal
1705 tract.

1706 Two studies in human volunteers (Teeguarden et al., 2015b [RefID 7155] and Thayer et al., 2015
1707 [RefID 7183]) were available after the publication of the 2015 EFSA opinion. In the study of Thayer et
1708 al., BPA was applied to a cookie and after drying chewed down (no time frame given), whereas in the

1709 study of Teeguarden et al. (2015b) [RefID 7155], BPA was added to tomato soup which was eaten
1710 within 11 min. In both studies a highly sensitive and specific method was used for the measurements
1711 of BPA and its conjugated metabolites. It is noted that the dose-corrected AUC of BPA (aglucon) is 2.8
1712 fold higher in the study of Thayer et al. (2015) [RefID 7183] compared with the dose-corrected AUC of
1713 BPA (aglucon) in the study of Teeguarden et al., 2015b [RefID 7155].

1714 **3.1.1.4. Human equivalent dose (HED)**

1715 In the previous EFSA opinion on BPA (EFSA CEF Panel, 2015), the extrapolation from the RP to the TDI
1716 was performed using an approach by which the toxicokinetic standard subfactor for the interspecies
1717 extrapolation was substituted by a BPA-specific HEDF. The HEDF is calculated by dividing the AUCs of
1718 unmetabolized (parent) BPA of animals (e.g. mice or rats) by the AUCs of humans (AUC animal/AUC
1719 human), both AUC values being corrected for the dose (AUC [corrected for dose] animal/AUC [corrected
1720 for dose] human).

1721 The concentrations used for the calculation of the AUCs are those in the blood of the systemic circulation
1722 that is the relevant metric for the exposure of organs/tissues others than the liver. They are measured
1723 after orally administered BPA is absorbed (100%, Völkel et al., 2002) and pre-systemically metabolised
1724 in the enterocytes of the gut wall and the liver cells. Because of the high metabolic capacity of the
1725 enterocytes and the liver cells, most of the BPA is metabolised. The amount of unmetabolized BPA
1726 released into the circulation, the systemic availability, is roughly between 0.45% (mice) and 6% (rats)
1727 of the oral dose, leading to concentrations in the systemic circulation of below 1 nM after a dose of 100
1728 µg/kg bw. Human data on i.v. administration, which are needed to calculate the systemic availability,
1729 are lacking, because toxicokinetic data are available only following oral administration.

1730 Concerning the liver, the situation is different from other organs/tissues. Before reaching the liver, BPA
1731 is metabolised only by gut enterocytes. Therefore, the concentration entering the liver is higher than
1732 that leaving the liver into the systemic circulation. However, for effects in the liver, the liver
1733 concentration and the related AUC is the relevant metric. No data were available on the proportion of
1734 the pre-systemic elimination of BPA from enterocytes in the gut wall and from the liver cells. This would
1735 be needed for parameterising a PBPK model allowing the prediction of the concentration in the liver
1736 and the related AUC. In the absence of such data, the AUC in the peripheral blood was used also for
1737 the liver to substitute the toxicokinetic part of the UF by an HEDF. For all species this is a conservative
1738 assumption, because the concentration in the liver and the related AUC are higher than in the systemic
1739 circulation, i.e. when using the AUCs in the systemic circulation, effects are assumed to occur at lower
1740 concentrations than in reality.

1741 The uncertainty related to this procedure is due to differences in the extent of pre-systemic enterocyte
1742 metabolism between species, the liver blood flow and the liver weight, whereas it can be assumed that
1743 the partitioning from blood to liver tissue does not differ between species to a marked extent. The CEP
1744 Panel considered that uncertainty might be in the range of a factor of 0.1 to 2.

1745 At the time the 2015 EFSA opinion (EFSA CEF Panel, 2015) was written, results from the human studies
1746 performed by Thayer et al. (2015) [RefID 7183] and Teeguarden et al. (2015a [RefID 9938], 2015b
1747 [RefID 7155]) were not available and, therefore, the AUC for humans was estimated using a PBPK
1748 model (Yang X et al., 2013). The AUC predicted using this model for human adults was 3.6 nM × h for
1749 a dose of 100 µg/kg bw or 0.036 nM × h/1 µg/kg bw, which is 6.4 fold and 2.3 fold lower than the
1750 mean values of the experimental results by Thayer et al. (2015) [RefID 7183] and by Teeguarden et
1751 al. (2015b) [RefID 7155], respectively. The AUC estimated using the model of Yang et al. (2013) for
1752 the human infant (>3 months up to 12 months) was predicted to be 3.0 nM × h for a dose of 100
1753 µg/kg bw or 0.03 nM × h/1 µg/kg bw. No experimental data are available for this age group.

1754 The CEP Panel noted the difference by a factor of 2.8 in the means of the dose-corrected AUC between
1755 the study of Thayer et al. (2015) [RefID 7183] and that of Teeguarden et al. (2015b) [RefID 7155],
1756 which might be explained by three hypotheses:

- 1757 1) The findings represent the variability in the population, as the AUC-range predicted by Karrer
1758 et al. (2018) [RefID 12289] using the corrected Yang et al. (2015) model, calibrated by the
1759 data of Thayer et al. (2015) [RefID 7183], overlaps with the range of AUC in the study of
1760 Teeguarden et al. (2015b) [RefID 7155].

1761 2) The findings are due to saturation of the metabolism in the enterocytes, as the intracellular
 1762 concentrations in the upper part of the small intestine might exceed the K_m for glucuronidation
 1763 when the dose is 100 $\mu\text{g}/\text{kg}$ bw, whereas for 30 $\mu\text{g}/\text{kg}$ bw the concentration is below the K_m
 1764 for glucuronidation.

1765 3) The findings are due to the difference in the application of BPA with only a small contact time
 1766 to the mucosa of the mouth when eating the tomato soup and a longer contact time and,
 1767 therefore, higher absorption through the mucosa of the mouth, whereby the absorbed BPA
 1768 escapes first pass. This hypothesis is supported by the findings in ewes of Guignard et al.
 1769 (2016) [RefID 2450], who described different systemic availability of BPA when given by
 1770 nasogastric tubing (no contact to buccal mucosa) or as pellets (extensive contact to buccal
 1771 mucosa). It is supported by observations in humans for nitroglycerol with a systemic availability
 1772 of <1% when a solution was administered orally and swallowed *versus* approximately 30%
 1773 when applied sublingual (Noonan and Benet, 1985, 1986).

1774 Hypothesis (1) was rejected because the data of the two studies are statistically significantly different
 1775 ($p < 0.05$; Mann–Whitney U -test). In addition, in the study of Thayer et al., the participants had
 1776 different genotypes for glucuronidation and, hence, the difference could not be explained by an
 1777 involuntary selection of participating subjects all having the slow metabolising genotypes.

1778 Hypothesis (2) was also discarded, because a linear relationship between doses (between 0.5 and 100
 1779 $\mu\text{g}/\text{kg}$ bw) and simulated AUCs was demonstrated using the PBPK model described by Karrer et al.
 1780 (2018) [RefID 12289] (see Appendix B).

1781 Concerning hypothesis (3), the finding in the study on ewes (Guignard et al., 2016 [RefID 2450])
 1782 indicates that some absorption in the mouth can occur, escaping the first pass metabolism and leading
 1783 to a higher AUC. This might be an explanation for the higher AUC in the Thayer et al. (2015) [RefID
 1784 7183] study with respect to the Teeguarden et al. (2015b) [RefID 7155] study. It should be noted that
 1785 in the former opinion on BPA (EFSA CEF Panel, 2015), the sublingual absorption was discussed and this
 1786 exposure route was considered negligible in the context of a chronic exposure by food, where the BPA
 1787 concentration is <0.1 mg/kg food, while in the animal experiment (Gayrard et al., 2013 [RefID 2223]),
 1788 the BPA concentration in solution was three orders of magnitude higher. In the current hypothesis, the
 1789 difference of the AUCs between the Thayer et al. (2015) [RefID 7183] and the Teeguarden et al.
 1790 (2015b) [RefID 7155] studies is less than one order of magnitude (the factor is 2.8) and the CEP Panel
 1791 considers that the BPA absorption in the mouth is plausible and the most probable explanation for the
 1792 difference in the concentration and AUCs found in the two studies.

1793 The CEP Panel considered that both study designs, [Teeguarden et al. (2015b) [RefID 7155], Thayer
 1794 et al. (2015) [RefID 7183]] represent realistic behaviour when humans are eating food. To take this
 1795 consideration into account when selecting the AUC for calculating the HEDF, the CEP Panel decided to
 1796 select a value representing the central tendency of the data (e.g. mean, median etc.) combining the
 1797 data of both studies. Because of the small number of participants in the two studies, the median value
 1798 was chosen. The CEP Panel noted the difference of the resulting AUC-value of 15.7 nM x h per 100
 1799 $\mu\text{g}/\text{kg}$ bw compared to the AUC-value of 3.6 nM x h per 100 $\mu\text{g}/\text{kg}$ bw used in the former evaluation
 1800 (EFSA CEF Panel, 2015). This difference is due to the fact that the human AUC used in 2015 was the
 1801 result of PBPK modelling, whereas repeated measures after a single dose in humans were used to
 1802 estimate the AUC in the current assessment. The mean observed AUC per 100 $\mu\text{g}/\text{kg}$ bw in the
 1803 experimental study of Teeguarden et al. (2015b) [RefID 7155] was close to the result of PBPK
 1804 modelling. However, the AUC of 15.7 nM x h per 100 $\mu\text{g}/\text{kg}$ bw is the median value of two studies in
 1805 which the observed lowest and the highest AUC, both dose-corrected, differ by a factor of 7.

1806 The CEP Panel also considered that the kinetic metric to be selected is the AUC. Therefore, HEDFs were
 1807 calculated using the median AUCs from animal studies. The resulting HEDF-values are 0.0155 for mice
 1808 and 0.1656 for rat (see Table 4).

1809 **Table 4:** Human equivalent dose factor (HEDF), comparing AUCs for doses of 100 $\mu\text{g}/\text{kg}$ bw.

Species (oral route)	AUC (nM x h)	HEDF (AUC animal/AUC human)
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Human (Thayer et al., 2015 [RefID 7183] and Teeguarden et al., 2015b [RefID 7155]) (median)	15.7	
Mouse(n=1) ^(*) (Doerge et al., 2011)	0.244	0.0155
Rat (mean) (Doerge et al., 2010)	2.6	0.1656
Ewe – gavage (mean) (Guignard et al., 2016 [RefID 2450])	1.88	0.1197
Ewe – diet (mean) (Guignard et al., 2016 [RefID 2450])	6.84	0.4357
Rhesus monkey (mean) (Doerge et al., 2010)	1.5	0.095
Dog (mean) (Gayrard et al., 2013 [RefID 2223])	2.19	0.1395

1810 ^(*) Every time point concentration measurement was done from pooled blood of n= 12 mice and one AUC was derived.

1811 3.1.1.5. Conversion factors from BPA in feed/drinking water to mg/kg bw per day 1812 during gestation/lactation period

1813 The doses reported in the animal studies in drinking water (mg/L) or feed (mg /kg) were converted to
1814 mg/kg bw per day. The factors used to convert the BPA doses given in feed or drinking water to mg/kg
1815 bw per day were retrieved from the EFSA guidance on selected default values to be used by the EFSA
1816 Scientific Committee, Scientific Panels and Units in the absence of actual measured data (EFSA Scientific
1817 Committee, 2012).

1818 When a dose is given before gestation as well as during gestation (e.g. exposure started 2 weeks before
1819 mating until PND21), the chronic conversion factor was always used for pre-mating, mating, gestation
1820 and lactation periods on dams.

1821 According to the EFSA Scientific Committee (2012), the conversion factors from drinking water to body
1822 weight for female mice were 0.191 for subacute (4 weeks) and 0.164 for subchronic (13 weeks) studies.
1823 In the study by Pacchierotti et al. (2008, reported in the Chapter 3.1.9 on Genotoxicity HI) female mice
1824 were treated for 7 weeks. By linear interpolation for 7 weeks a conversion factor of 0.182 (rounded to
1825 0.18) could be used.

1826 The conversion factors used are reported in Table 5.

1827 **Table 5:** Conversion factors from BPA in feed/drinking water to mg/kg bw per day during
1828 gestation/lactation period.

Species	Conversion factor from drinking water			Conversion factor from feed
	Chronic	Subchronic	Subacute	Chronic
Mice	0.09	0.15	0.18	0.15
Rat	0.05	Not applicable	Not applicable	0.05
Hamster	0.05	Not applicable	Not applicable	0.05
Rabbit	0.03	Not applicable	Not applicable	0.03
Rhesus monkey	Not applicable			Not applicable
Marmoset monkey	Not applicable			Not applicable

1829

1830 3.1.1.6. Converting factors from doses given by intravenous, intraperitoneal, 1831 subcutaneous, inhalation or intratesticular route to doses given by oral route

1832 The exposure routes other than oral (i.e. intravenous, intraperitoneal, subcutaneous, inhalation,
1833 intratesticular) were converted to oral doses taking into account the toxicokinetic species-specific
1834 factors reported in Table 6.

1835 Dosing by gavage (intra-gastric) is similar to oral dosing and no conversion is needed. For rabbits,
 1836 hamsters and gerbils data were not available; therefore, the doses eliciting adverse effects when given
 1837 by a non-oral route could not be converted to an oral route and could not be used as the basis for a
 1838 TDI, although the information could support findings in other species.

1839 **Table 6:** Converting factors from doses given by intravenous, intraperitoneal, subcutaneous or
 1840 inhalation route to doses given by oral route

Species	Systemic availability (bioavailability) (%)			Multiplication factor (converting factor) from i.v./s.c./inhalation internal dose to oral dose	Multiplication factor (converting factor) from i.p. internal dose to oral dose
	oral	i.v./s.c.	i.p. (50% of the dose to be calculated as if given by the oral route+ 50% of the dose if given by the i.v./s.c route)***		
Rat	2.8	100	51.4	35.7	18.35
Mouse	0.45	100	50.22	222.2	111.6
Sheep	0.8*– 2.7**	100	50.4–51.35	125–37	63–19.1
Monkey	0.9	100	50.45	111.1	56.1

1841 Administration by * nasogastric tubing; ** pellets; ***See Lukas et al. (1971) for details of calculation.

1841
1842
1843

1844 In the study of Chen L et al. (2018) [RefID 11712] BPA was given by intratesticular injection. Hence,
 1845 the calculations were performed to derive the oral dose which would have resulted in the same
 1846 concentration in the testes, as resulting from the injection into the testes. The following physiological
 1847 and distributional data were considered: weight of a testis, equal distribution in the testis, partition
 1848 coefficient (PC) (predicted from the algorithm of Schmitt, 2008).

1849 **Table 7:** Calculations of the oral doses from the intratesticular doses (i.e. for Chen L et al., 2018
 1850 [RefID 11712]), considering the data reported in Ariyaratne et al. (2000), Doerge et al. (2010) and
 1851 Pilari et al. (2017).

Dose (pmol)	Weight of a testis (g)	Conc. in a testis (nM)	Conc. in blood (nM)		Blood (nM)	Dose (µg/kg bw)	PC testis/blood*	Conc in blood (nM) for test. conc.	Dose (µg/kg bw)	Dose in mg/kg bw
100	1.6 ± 0.02	1.6	62.5 (=100/1.6)	0.38 ± 0.19	0.38	100	3	20.83 (=62.5/3)	5481.6 (=100/0.38 *20.83)	5.5
1000			625 (=1000/1.6)					208.3 (=625/3)		

1852 * Ratio concentration in testis/concentration in blood (partition coefficient, PC) = ~3 (Yoo et al., 2000)

1853 The oral dose needed would be 5.5 mg/kg bw or 54.8 mg/kg bw to produce a concentration in the
 1854 testes after injection of 100 pmol or 1000 pmol.

1855

1856 3.1.2. General toxicity

1857 3.1.2.1. Epidemiological studies

1858 Two human studies were available addressing effects of BPA on General toxicity: Lee S et al. (2018)
 1859 [RefID 12428] on liver toxicity and Hu et al. (2019) [RefID 12151] on kidney toxicity.

1860 However, the criteria to bring forward to (WoE analysis the clusters/exposure periods were not met,
1861 i.e. there were no clusters/exposure periods for which at least two studies were available with at least
1862 one reporting a statistically significant adverse association for one of the endpoints measured.

1863 **3.1.2.2. Animal studies**

1864 For the HOC General toxicity, a total of 54 studies was appraised by the CEP Panel. The details of the
1865 appraisals (internal and external validity) are reported in Annex E.

1866 The endpoints for each study identified as relevant are reported in Annex F. BPA effects in liver and
1867 kidney (liver and kidney weight) were already key in the 2015 EFSA opinion (EFSA CEF Panel, 2015,
1868 Section 3.2.5). For more details see Annex A, Section 2.5.

1869 *Identification of clusters of relevant endpoints*

1870 From the newly available BPA literature, data on body weight, liver and kidney toxicity were retrieved
1871 along with effects in lung, thyroid, parathyroid, pituitary gland, adrenal gland and bone marrow.
1872 Changes in organ weights, histology or clinical chemistry potentially associated with toxicity in these
1873 organs were considered as relevant endpoints under the respective clusters, i.e. liver effects, kidney
1874 effects, lung effects, thyroid effects, parathyroid effects, pituitary gland effects, adrenal gland effects
1875 and bone marrow effects. An additional cluster encompasses haematological effects.

1876 **Body weight effects**

1877 Based on the newly evaluated studies, body weight was identified as a relevant endpoint. To evaluate
1878 compound-related effects on the growth of rodents, body weight data are commonly collected in
1879 toxicology studies (Hoffman et al., 2002). For this endpoint, a substantial decrease (typically $\geq 10\%$)
1880 or a substantial increase (typically $\geq 20\%$) in body weight or body weight gain were considered adverse.
1881 In this evaluation, body weight was regarded as a transversal endpoints and changes in this endpoint
1882 were assessed in the HOC Metabolic effects.

1883

1884 **Liver effects**

1885 Based on the newly evaluated studies the following relevant endpoints were grouped into the cluster
1886 Liver effects:

- 1887
- 1888 • absolute and relative liver weight;
 - 1889 • histological changes including cystic and other hepatic degeneration, congestion, inflammation,
necrosis, steatosis, angiectasis, hepato-diaphragmatic nodule, and apoptosis;
 - 1890 • clinical chemistry parameters, i.e. liver enzymes [alkaline phosphatase (ALP), alanine
1891 transferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH)], total
1892 protein, total bile acids, bilirubin, activated partial thromboplastin time (aPTT), albumin,
1893 globulin, albumin/globulin (ALB/GLO) ratio.

1894 Increases in liver to body weight ratio were considered more relevant than absolute weight changes
1895 for the evaluation of the WoE (Bailey et al., 2004). Among various causes for liver weight increases,
1896 hepatocellular hypertrophy is a common response following xenobiotics administration in toxicity
1897 studies and may be linked to an adaptive enzyme induction in the absence of adverse effects in
1898 histopathology or clinical chemistry parameters related to liver toxicity (Hall et al., 2012). Several
1899 xenobiotics are known as enzyme inducers and lead to liver enlargement associated with hypertrophy
1900 and/or transient hyperplasia (Maronpot et al., 2010). Other histological changes characterised by
1901 glycogen or lipid accumulation (fatty change or steatosis) may be induced by physiological or
1902 pathological conditions that can influence metabolic processes (Thoolen et al., 2010).
1903 Hepatodiaphragmatic nodule is a congenital lesion, usually interpreted as 'background lesion'. However,
1904 a treatment related effect cannot be excluded in the progeny of animals exposed during pregnancy
1905 (Greaves, 2007). Angiectasis is an age-related lesion but it can also be caused by chemicals. Cystic
1906 degeneration is another age-related lesion, whose pathogenesis is not fully understood. Fatty change
1907 or steatosis may be linked to single cell necrosis. Cell degeneration and apoptosis may be triggered by
1908 either physiological or pathological processes induced by xenobiotics or several diseases. Necrosis may
1909 also be associated with inflammatory responses in the liver (Greaves, 2007). It is also well known that

1910 changes in albumin and increases in serum levels of liver enzymes such as ALT, AST and LDH are
1911 indicators of liver damage (Hall et al., 2012). Other clinical chemistry changes in fat metabolism, e.g.
1912 triglyceride and cholesterol levels, were attributed to the HOC Metabolic effects.

1913 **Kidney effects**

1914 The following relevant endpoints were retrieved for the cluster Kidney effects:

- 1915 • absolute and relative kidney weight
- 1916 • histological changes such as mineralisation, hyperplasia, renal tubule cysts, nephropathy
- 1917 • clinical chemistry parameters, e.g. urinary volume, blood urea nitrogen (BUN), urea and
1918 creatinine in serum.

1919 For kidney weight changes, absolute and preferentially relative weights were considered, however it
1920 was noted that the relationship to body weight (or brain weight) is not strictly proportional (Bailey et
1921 al., 2004). Increases in kidney weight may be associated with either adaptive or pathological processes
1922 particularly in the presence of relevant histological alterations (Greaves, 2007). Mineralisation is
1923 encountered frequently in rodent toxicity studies. Simple tubule hyperplasia can be chemically induced
1924 and may be a consequence of single cell degeneration with compensatory regeneration (Frazier et al.,
1925 2012).

1926 Xenobiotics may induce inflammatory reactions and lead to a damage of glomerular epithelial cells.
1927 Functional changes associated with glomerular lesions include loss of plasma albumin, urea and
1928 creatinine (Greaves, 2007).

1929 **Lung effects**

1930 BPA effects in lungs were only retrieved from the new literature not evaluated in the former EFSA
1931 opinions (EFSA 2007; EFSA CEF Panel, 2010, 2015). The following relevant endpoint was identified:

- 1932 • absolute and relative weight.

1933 Changes in lung weight can be associated to several pathologic conditions. Increased lung weight can
1934 be due to the pathological accumulation of fluids within the airways (e.g. haemorrhage, oedema,
1935 catarrhal exudate) or to the thickening of the interstitial tissue due to infiltration of inflammatory (e.g.
1936 lymphocytes, macrophages, eosinophils) or reactive cells (e.g. fibroblasts). Other lesions such as
1937 granuloma and tumours can also induce weight gain of the lung. The efficacy of lung weight as an
1938 indicator of lung injury in rat inhalation studies has been assessed by Wahlström et al. (2013), in which
1939 study the weight change in lungs as biomarker for predicting lung histopathology was over 80%.

1940 **Thyroid effects**

1941 BPA effects on thyroid gland were taken from the NTP Clarity Report (2018)/Camacho et al. (2019)
1942 [RefID 11370] and the following histological changes were identified as relevant endpoints:

- 1943 • hyperplasia (C-cell, follicular cells, focal);
- 1944 • ultimobranchial cysts.

1945 The thyroid gland of rodents (and especially of male rats) is highly sensitive to derangement by
1946 xenobiotics and physiologic perturbations, predisposing to a higher incidence of proliferative lesions
1947 (e.g. hyperplasia and adenomas of follicular cells) in response to chronic TSH stimulation than in the
1948 human thyroid (Brändli-Baiocco et al., 2018).

1949 **Parathyroid effects, pituitary gland effects, adrenal gland effects**

1950 BPA effects on these organs were taken from the NTP Clarity Report (2018)/Camacho et al. (2019)
1951 [RefID 11370] and the following histological changes were identified as relevant endpoints:

- 1952 • in the parathyroid: hyperplasia;
- 1953 • in the pituitary gland: pars distalis hyperplasia, pars distalis cysts;
- 1954 • in the adrenal gland: cortex hypertrophy, cytoplasmic vacuolisation, medulla hyperplasia.

1955 Endocrine organs are often affected in toxicological studies in which proliferative changes are observed.
1956 Endocrine organ hypertrophy and hyperplasia occur in humans and experimental animals such as
1957 rodents either spontaneously during ageing or following long-term treatment, e.g. with hormonally
1958 active substances. Proliferative changes can be reversible or may eventually develop into neoplastic
1959 lesions (Greaves, 2007).

1960 **Bone marrow effects**

1961 BPA effects on bone marrow were taken from the NTP Clarity Report (2018)/Camacho et al. (2019)
1962 [RefID 11370] and the following histological changes were identified as relevant endpoints:

- 1963 • hypocellularity;
- 1964 • myeloid cell hyperplasia.

1965 As the bone marrow is a site of intense cell multiplication and maturation, it can be influenced by
1966 chemicals that affect specific haematopoietic cell types or cellular proliferation/differentiation (Reagan
1967 et al., 2011). Bone marrow should be examined in context with toxicology studies, as the
1968 histomorphology may reflect changes resulting from moribund condition, body weight loss, or
1969 decreased body weight gain, which could be mistakenly concluded to be test article related (Reagan et
1970 al., 2011). Concerning the method of investigation, it should be noted that histopathological assessment
1971 of the bone marrow tissue is qualitative, whereas cytology is quantitative and is required for a correct
1972 assessment of haematopoietic cell differentiation and maturation (Willard-Mack et al., 2019).

1973 **Haematological effects**

- 1974 • The following relevant endpoints were retrieved: haemoglobin, mean corpuscular haemoglobin
1975 (MCH), eosinophils, platelets, packed cell volume.

1976 The interaction of a toxic compound or its metabolites with cellular constituents may lead to changes
1977 in haematological parameters. Such changes in experimental animals have a high predictive value for
1978 human toxicity (Arika et al., 2016). Considering the natural variations of haematological parameters in
1979 animals, the adversity of any changes related to BPA treatment have to be evaluated against the
1980 background of historical control data (de Kort et al., 2020).

1981

1982 *WoE of the clusters of relevant endpoints*

1983 The main information extracted from the studies addressing relevant endpoints in the HOC General
1984 toxicity are summarised in Annex G. The outcome of the weight of the evidence is described in the text
1985 below and presented in a tabulated format in Annex H.

1986 The clusters of the effects of BPA on General toxicity considered for this assessment were the following:

- 1987 • body weight effects;
- 1988 • liver effects;
- 1989 • kidney effects;
- 1990 • lung effects;
- 1991 • thyroid gland effects;
- 1992 • parathyroid gland effects;
- 1993 • pituitary gland effects;
- 1994 • adrenal gland effects;
- 1995 • bone marrow effects;
- 1996 • effects on haematological parameters.

1997

1998 **Body weight effect**

1999 The assessment of body weight for each exposure period is described in detail in the Metabolic effects'
2000 category (Chapter 3.1.4.2).

2001 **Kidney effects**

2002 Developmental exposure (pre-natal and/or post-natal until weaning)

2003 For this exposure period, one Tier 1 study in rats (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
2004 11370]), one Tier 2 (Esplugas et al., 2018 [RefID 11900]) and one Tier 3 study in mice (Patel et al.,
2005 2013 [RefID 5697]) were identified.

2006 In the rat study there was no change in kidney weights after 1 year of age in male and female rats
2007 treated at oral doses of 2.5, 25, 250, 2500 and 25000 µg/kg bw per day (NTP Clarity Report,
2008 2018/Camacho et al., 2019 [RefID 11370]). In a mouse study (Patel et al., 2013 [RefID 5697]) there
2009 was no change in kidney weights. Therefore, an effect of BPA on kidney weight during developmental
2010 exposure was judged as Not Likely.

2011 In the rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) several histological
2012 effects in kidney were reported. An increase (trend, Poly-3 test) in transitional epithelium hyperplasia
2013 was observed in males at 2 years but not in females. In females a significantly increased incidence of
2014 renal tubule cysts was found at 2.5 µg/kg bw per day (Poly-3 test) along with a non-statistically
2015 significant increase at an adjacent dose of 25 µg/kg bw per day after 2 years (=NMDR). At the same
2016 time (2 years) the incidence of nephropathy increased in females at the highest dose of 25000 µg/kg
2017 bw per day (only in secondary test) but not in males; no such effect was seen at 1 year in both sexes.
2018 No effect on mineralisation was reported in kidneys of both sexes or time points (1 or 2 years).
2019 Regarding the clinical chemistry effects (i.e. on the levels of creatinine and BUN) in the mouse study
2020 (Esplugas et al., 2018 [RefID 11900]) no effects were found on creatinine, but an increase of urea
2021 levels was observed in males at a single subcutaneous dose of 25 µg/kg bw per day (which is equivalent
2022 to an oral dose of 5555 µg/kg bw per day); females were not tested in this study. These data on clinical
2023 chemistry parameters were considered as limited evidence of kidney effects and therefore graded as
2024 ALAN.

2025 Considering the absence of effects on kidney weight (Not Likely) and on clinical chemistry parameters
2026 only observed in mice (ALAN), the CEP Panel assigned a likelihood level of ALAN to the kidney effects
2027 of BPA in the developmental exposure period, based on the histological findings in the rat study.
2028 Therefore, none of the endpoints was taken forward for BMD analysis. However, they were considered
2029 in the uncertainty analysis (see Appendix D).

2030 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

2031 For this exposure period the following studies were identified: two Tier 1 studies, i.e. one in rats (NTP
2032 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) and one in mice (Chatsantiprapa et al., 2016
2033 [RefID 9822]) and two Tier 3 studies, one in rats (Jeong et al., 2017 [RefID 3133]) and one in mice
2034 (Patel et al., 2013 [RefID 5697]).

2035 In the Tier 1 studies there was no change in kidney weights in rats after 1 year of treatment at oral
2036 doses from 2.5 to 25000 µg/kg bw per day (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
2037 11370]) and in mice at 9 and 13 weeks of age in male and females treated daily for 9 weeks,
2038 respectively (Chatsantiprapa et al., 2016 [RefID 9822]). In contrast, in the Tier 3 rat study there was
2039 a dose-related increase in relative weight in males (but not in females) at the high oral dose, i.e. 250000
2040 µg/kg bw per day, treated from PND6 for 90 days (Jeong et al., 2017 [RefID 3133]). On the contrary,
2041 a decrease in kidney weights was observed in the Tier 3 mouse study in males treated with 5 µg/kg bw
2042 per day from GD11.5 up to 4 months of age (Patel et al., 2013 [RefID 5697]). The effect of BPA on
2043 kidney weight during this exposure period was judged as Not Likely.

2044 In the absence of weight changes several histological findings were observed in the Tier 1 rat study
2045 (NTP). In females an increased dose trend of mineralisation was observed along with increased
2046 incidence of renal tubule cysts at the lowest dose of 2.5 µg/kg bw per day and nephropathy at the
2047 doses of 25 and 2500 µg/kg bw per day after 1 year of treatment. After 2 years a significantly increased
2048 incidence and severity of nephropathy was reported in females treated with the lowest dose of 2.5
2049 µg/kg bw per day and a significantly increased incidence of this lesion was also observed at the highest
2050 dose of 25000 µg/kg bw per day. In males of the same study hyperplasia of the transitional epithelium

2051 was increased at the dose of 25 µg/kg bw per day and renal tubule cysts were increased at doses of
2052 250 and 2500 µg/kg bw per day (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370],
2053 Summary Table) after 2 years. Based on these findings, the effect of BPA on kidney histological changes
2054 during this exposure period was judged as ALAN.

2055 No effects were observed on the levels creatinine and BUN in this rat study while a dose-dependent
2056 decreases in creatinine was reported in the Tier 3 study in rats (Jeong et al., 2017 [RefID 3133]) at
2057 500, 5000, 50000 and 250000 µg/kg bw per day in males (but not in females) without a change in BUN
2058 levels. Therefore, the effect of BPA on these parameters during this exposure period was judged as Not
2059 Likely.

2060 Considering the absence of BPA effects on kidney weight in mice and rats (except for an increase in
2061 male rats at very high doses) and limited effects on clinical chemistry parameters in rats, the CEP Panel
2062 assigned a likelihood level of ALAN to the kidney effects of BPA in the developmental and adult exposure
2063 period, based on the histological findings in a Tier 1 study in rats. Therefore, none of the endpoints
2064 was taken forward for BMD analysis. However, they were considered in the uncertainty analysis (see
2065 Appendix D).

2066 Growth phase/young age exposure

2067 No studies were available for this exposure period.

2068 Adult exposure (after puberty)

2069 For this exposure period the following studies were identified: three Tier 2 studies, i.e. one in mice
2070 (Dong et al., 2013 [RefID 1676]) and two in rats (Poormoosavi et al., 2018 [RefID 12913]; Ola-Davies
2071 et al., 2018 [RefID 12837]) and three Tier 3 studies, i.e. one in mice (Liang et al., 2018 [RefID 12508]),
2072 one in rats (Elobeid et al., 2015 [RefID 1803]) and one in monkeys (Vijaykumar et al., 2017 [RefID
2073 7477]).

2074 No changes in relative kidney weight were found in the two mouse studies, neither in the Tier 2 study
2075 in females (Dong et al., 2013 [RefID 1676]) at doses of 1.8 or 0.18 µg/kg bw per day nor in the Tier 3
2076 study in gonadectomised males (Liang et al., 2018 [RefID 12508]) treated s.c. with testosterone
2077 propionate and BPA at 40 to 4000 µg/kg bw per day (converted to oral doses of 8888 to 888800 µg/kg
2078 bw per day). No changes in kidney weight were found also in the Tier 2 rat study (Ola-Davies et al.,
2079 2018 [RefID 12837]). However, in the other Tier 2 rat study (Poormoosavi et al., 2018 [RefID 12913])
2080 there was an increase of absolute kidney weight along with an increase in serum creatinine and urea
2081 levels in males (females not tested) treated with a high single dose of 10,000 µg/kg bw per day for 8
2082 weeks. Based on these findings, the effect of BPA on kidney weight was judged as ALAN.

2083 Similarly, an increase of creatinine and BUN were also observed in the Tier 2 rat study (Ola-Davies et
2084 al., 2018 [RefID 12837]) in males at 10000 µg/kg bw per day and in the Tier 3 rat study (Elobeid et
2085 al., 2015 [RefID 1803]) in males (females not tested) at the two highest doses of 10000 and 50000
2086 µg/kg bw per day but not at lower doses of 100 and 1000 µg/kg bw per day. In a Tier 3 study
2087 (Vijaykumar et al., 2017 [RefID 7477]) with male monkeys (females not tested) there were no changes
2088 in the creatinine and serum urea levels after treatment with 2.5, 12.5 and 25 µg/kg bw per day for 70
2089 days. The effect of BPA on these parameters during this exposure period was judged as ALAN.

2090 Overall, dose-related effects on kidney weight and clinical parameters during adult exposure were
2091 observed in male rats only at BPA doses at or above 10000 µg/kg bw per day, however not in
2092 gonadectomised male mice treated with testosterone.

2093 The CEP Panel assigned a likelihood level of ALAN to the kidney effects of BPA in the adult exposure
2094 period, mainly based on the effects on kidney weight and clinical chemistry parameters in male rats at
2095 high doses. No histological data were available for adult exposure. Therefore, none of the endpoints
2096 was taken forward for BMD analysis. However, they were considered in the uncertainty analysis (see
2097 Appendix D).

2098 Indirect (germline) exposure

2099 No studies were available for this exposure period.

2100

2101 Overall cluster selection of the endpoints/studies for BMD analysis for kidney effects

2102 Overall, the CEP Panel assigned a likelihood level of ALAN to kidney effects following BPA treatment at
2103 different life stages from *in utero* to adulthood.

2104 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
2105 Likely effect of BPA in any exposure period, therefore none of the endpoints was taken forward for
2106 BMD analysis.

2107 **Liver effects**

2108 Developmental exposure (pre-natal and/or post-natal until weaning)

2109 For this exposure period the following studies were identified: seven Tier 1 studies, i.e. one in mice
2110 (van Esterik et al., 2014 [RefID 7393]) and six studies in rats (Jiang et al., 2014 [RefID 3190]; Lejonklou
2111 et al., 2017 [RefID 3975]; Xia et al., 2014 [RefID 8103]; NTP Clarity Report, 2018/Camacho et al., 2019
2112 [RefID 11370]; Dunder et al., 2018 [RefID 11866]; Greenberg, 2018 (NTP Grantee study) [RefID
2113 13785]); four Tier 2 studies, i.e. three in mice (Eckstrum et al., 2018 [RefID 11874]; Esplugas et al.,
2114 2018 [RefID 11900]; Meng Z et al., 2018 [RefID 12708]) and one in rats (Quan et al., 2017 [RefID
2115 6025]); one Tier 3 study in rats (Sadowski et al., 2014a [RefID 6361]).

2116 No changes in liver weight were reported in three studies in mice up to doses of 3000 µg/kg bw per
2117 day (van Esterik et al., 2014 [RefID 7393]; Eckstrum et al., 2018 [RefID 11874]; Meng Z et al., 2018
2118 [RefID 12708]) and in six studies in rats up to doses of 50000 µg/kg bw per day (Jiang et al., 2014
2119 [RefID 3190]; Lejonklou et al., 2017 [RefID 3975]; Sadowski et al., 2014a [RefID 6361]; Xia et al.,
2120 2014 [RefID 8103]; Dunder et al., 2018 [RefID 11866] and Greenberg (2018) (NTP Grantee study)
2121 [RefID 13785]). In one rat study, liver weight decreased in males (females not tested) at 1000 and
2122 100000 µg/kg bw per day but without dose–response (Quan et al., 2016 [RefID 6024]) and there was
2123 a trend indicating an increase of liver weight in male rats treated with doses between 2.5 and 25000
2124 µg/kg bw per day but not in female rats (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]).
2125 Considering that the liver weight was not statistically increased at any dose compared with controls in
2126 the latter study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]), an inverse effect was
2127 reported in the study by Quan et al. (2016) [RefID 6024] and in most studies in rats and mice the
2128 relative liver weight was unchanged, the CEP Panel judged the effect as Not Likely.

2129 Several histological findings in liver were reported in rat studies. An increase in steatosis was only
2130 observed in one study in male rats (females not tested) treated with a single dose of 40 µg/kg bw per
2131 day at weeks 15 and 26 but not at week 3 (semiquantitative data; Jiang et al., 2014 [RefID 3190]).
2132 Three other rat studies reported no steatosis at doses up to 50000 µg/kg bw per day (Lejonklou et al.,
2133 2017 [RefID 3975]; in adult females and males (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
2134 11370]; Dunder et al., 2018 [RefID 11866]). In addition, in the NTP study increases in cystic
2135 degeneration (at 2500 and 25000 µg/kg bw per day) and mononuclear cell infiltration (symptom of
2136 inflammation) in liver (only at 2.5 and 25000 µg/kg bw per day) were observed in female rats but not
2137 in males. An increase of focal inflammation was also observed in a Tier 3 study (Joeng et al., 2017
2138 [RefID 3133]). In the NTP study there was also an increase of angiectasis in male but not in female
2139 rats at 25 µg/kg bw per day but without dose–response. An increase in hepatocyte apoptosis was
2140 reported in male rats (females not tested) at a single dose of 50 µg/kg bw per day after 15 and 21
2141 weeks but not at 3 weeks (Xia et al., 2014 [RefID 8103]). Considering the histological observations in
2142 female or male rat liver which were not always consistent among the reported studies, e.g. on steatosis,
2143 the CEP Panel judged these effects as ALAN.

2144 Clinical chemistry parameters potentially associated with liver toxicity were observed in some studies.
2145 Two rat studies reported moderate increases in ALT (Jiang et al., 2014 [RefID 3190]; Xia et al., 2014
2146 [RefID 8103]; both from the same laboratory) for which a likelihood of ALAN was given. However,
2147 neither this effect nor other increases of liver enzyme activities (i.e. AST or ALP) were observed in the
2148 NTP study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). A moderate increase in ALP
2149 was observed in males at a single subcutaneous dose of 25 µg/kg bw per day (which is equivalent to
2150 an oral dose of 5555 µg/kg bw per day); females were not tested in this study (Esplugas et al., 2018
2151 [RefID 11900]). A decrease in the levels of total bile acid and total protein in male but not in female
2152 rats was reported in the NTP study only at one dose of 25 µg/kg bw per day (NTP Clarity Report,
2153 2018/Camacho et al., 2019 [RefID 11370]).

2154 The CEP Panel assigned a likelihood level of ALAN to the liver effects of BPA in the developmental
2155 exposure period, based on several histological findings and changes in some clinical parameters.
2156 Therefore, none of the endpoints was taken forward for BMD analysis. However, they were considered
2157 in the uncertainty analysis (see Appendix D).

2158 Developmental and adult (pre-natal and post-natal in pups until adulthood)

2159 For this exposure period the following studies were identified: three Tier 1 studies, i.e. one in mice
2160 (Chatsantiprapa et al., 2016 [RefID 9822]) and two studies in rats (NTP Clarity Report, 2018/Camacho
2161 et al., 2019 [RefID 11370]; Greenberg, 2018 (NTP Grantee study) [RefID 13785]); one Tier 2 study in
2162 mice (Ke et al., 2016 [RefID 3447]) and two Tier 3 studies, i.e. one in mice (Biasiotto et al., 2016 [RefID
2163 575]) and one in rats (Jeong et al., 2017 [RefID 3133]).

2164 No changes in liver weight were reported in one study in female mice (Chatsantiprapa et al., 2016
2165 [RefID 9822]) and three studies in male mice treated either pre-natally plus post-natally with BPA doses
2166 of 0.05 up to 500 µg/kg bw per day until week 9 (Chatsantiprapa et al., 2016 [RefID 9822]) and week
2167 23 (Biasiotto et al., 2016 [RefID 575]) or post-natally with a single dose of 0.5 µg/kg bw per day until
2168 8 weeks (Ke et al., 2016 [RefID 3447]). However, there was an increase in absolute and relative liver
2169 weight in the latter study in older males treated for 10 months. In juvenile female and male rats treated
2170 post-natally for 90 days with 500 to 250000 µg/kg bw per day, no effect on relative liver weight was
2171 reported (Jeong et al., 2017 [RefID 3133]). No effect on absolute liver weight was also reported in
2172 female rats in the Tier 1 study (Greenberg, 2018 (NTP Grantee study) [RefID 13785]). In contrast, in
2173 a rat study with pre-natal and post-natal exposure until 1 year to 2.5 up to 25000 µg/kg bw per day
2174 females showed a trend of increased liver weight while males showed a significant decrease only at the
2175 lowest dose (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). Considering that the liver
2176 weights in the subacute/subchronic mice and rat studies were unchanged and only effects without a
2177 clear dose–response (trend in female rats, only lowest dose in male rats or in a single doses study in
2178 male mice) were observed after pre-natal and/or post-natal exposure up to 1 year, the CEP Panel
2179 judged the effect as ALAN.

2180 Several histological changes in liver were observed in one rat study after pre-natal and post-natal
2181 exposure (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). Angiectasis was reported for
2182 females at the highest dose (25000 µg/kg bw per day) and for males at the lowest dose (2.5 µg/kg bw
2183 per day) after 2 years (Camacho, but not in NTP final, [RefID 11370]). No effect on cystic degeneration
2184 was found after one or 2 years in rats at any dose (NTP Clarity Report, 2018/Camacho et al., 2019
2185 [RefID 11370]). In males an increase in the incidence of hepatodiaphragmatic nodules was observed
2186 at 2500 µg/kg bw per day after 1 year (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370])
2187 but is difficult to explain why this effect was not present after 2 years considering that the lesion is
2188 congenital and irreversible. In addition, mononuclear cell infiltration at all doses except for 25 µg/kg
2189 bw per day and steatosis (fatty change) was reported at 25 µg/kg bw per day in males after 1 year.
2190 However, no effect on steatosis was reported in female rats in the Tier 1 study (Greenberg, 2018, NTP
2191 Grantee study [RefID 13785]). The CEP Panel noted that the histological changes in liver were only
2192 observed in males and/or only at one dose (angiectasis, hepatodiaphragmatic nodules, steatosis).
2193 Therefore, the effects were judged as ALAN.

2194 Changes in clinical chemistry parameters potentially associated with liver toxicity were observed in rat
2195 studies. A decrease in the ALB/GLO ratio was observed at high doses in juvenile female and male rats
2196 after 90 days of treatment while the albumin level was not changed (Jeong et al., 2017 [RefID 3133],
2197 Tier 3). In a rat study with pre-natal and post-natal treatment up to 1 year (NTP Clarity Report,
2198 2018/Camacho et al., 2019 [RefID 11370], Tier 1), there was a trend for slightly lower albumin levels
2199 in males but not in females; there was no effect on total protein levels in both sexes. A small increase
2200 in ALP levels in females was observed in the rat studies at intermediate doses after 1 year (statistically
2201 significant only at 250 µg/kg bw per day) (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
2202 11370]) and after 90 days of treatment at high dose of 250000 µg/kg bw per day (Jeong et al., 2017
2203 [RefID 3133]). In both studies no changes were found in ALP levels in males and in ALT levels for
2204 females and males. A decrease in AST levels reported only in males in the subchronic study (Jeong et
2205 al., 2017 [RefID 3133]) was not considered of toxicological relevance and was not observed in a Tier 1
2206 rat study at 1 year (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). No effects on liver
2207 enzyme levels (ALT, AST) were observed in male mice (Ke et al., 2016 [RefID 3447]). In addition, there

2208 were increased bilirubin levels in the subchronic rat study in both sexes mainly at high doses up to
2209 250000 µg/kg bw per day (Jeong et al., 2017 [RefID 3133]) and a trend for lower total bile acid levels
2210 in males in the rat study with pre-natal and post-natal treatment up to 1 year but not in females (NTP
2211 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). Based on the inconsistent findings related
2212 to liver enzymes and bilirubin levels after pre- and post-natal exposure the CEP Panel judged clinical
2213 chemistry effects in liver as ALAN.

2214 The CEP Panel assigned a likelihood level of ALAN to the liver effects of BPA in the development and
2215 adult exposure period, mainly based on several histological findings and some clinical chemistry
2216 changes. Therefore, none of the endpoints was taken forward for BMD analysis. However, they were
2217 considered in the uncertainty analysis (see Appendix D).

2218 Growth phase/young age exposure

2219 No studies were available for this exposure period.

2220 Adult exposure (after puberty)

2221 For this exposure period the following studies were identified: Two Tier 1 studies in rats (Ding et al.,
2222 2016 [RefID 1621]; Vahdati Hassani et al., 2017 [RefID 2614]), eight Tier 2 studies, i.e. four in rats
2223 (Amraoui et al., 2018 [RefID 11503]; Mahmoudi et al., 2018 [RefID 12656]; Özyaydin et al., 2018b
2224 [RefID 12854]; Poormoosavi et al., 2018 [RefID 12913]) and four in mice (Dong et al., 2013 [RefID
2225 1676]; Lin Y et al., 2018 [RefID 4338]; Lv Q et al., 2017 [RefID 4697]; Marmugi et al., 2014 [RefID
2226 4884]) and three Tier 3 studies, i.e. one in rat (Kazemi et al., 2017 [RefID 3441]), one in mice (Liang
2227 et al., 2018 [RefID 12508]) and one in monkeys (Vijaykumar et al., 2017 [RefID 7477]). All these
2228 studies were performed in male animals, except for one study in female mice (Dong et al., 2013 [RefID
2229 1676]).

2230 Increases in liver weight were reported in three Tier 2 studies with rats at a single high dose of 10000
2231 µg/kg bw per day (Amraoui et al., 2018 [RefID 11503]; Mahmoudi et al., 2018 [RefID 12656];
2232 Poormoosavi et al., 2018 [RefID 12913]) and one Tier 2 study in mice at 50 µg/kg bw per day (Lin Y
2233 et al., 2018 [RefID 4338]). In contrast, liver weights were unchanged in rat studies at doses between
2234 50–50000 µg/kg bw per day, i.e. in two Tier 1 studies (at 50 µg/kg bw per day after 35 weeks (Ding
2235 et al., 2016 [RefID 1621]) or up to 50000 µg/kg bw per day after 30 day (Vahdati Hassani et al., 2017
2236 [RefID 2614]), one Tier 2 study (up to 500 µg/kg bw per day; Özyaydin et al., 2018b [RefID 12854])
2237 and in mouse studies at doses between 50–5000 µg/kg bw per day, i.e. three Tier 2 studies (one in
2238 females (Dong et al., 2013 [RefID 1676]) and two in males (Lv Q et al., 2017 [RefID 4697]; Marmugi
2239 et al., 2014 [RefID 4884]) and one Tier 3 mouse study with subcutaneous treatment in mice (Liang et
2240 al., 2018 [RefID 12508]). Considering that the results in these studies were not consistent and observed
2241 with different BPA doses and exposure periods the CEP Panel judged increases in liver weight ALAN.

2242 Histological changes in liver were observed in two Tier 2 rat studies at 10000 µg/kg bw per day, i.e.
2243 congestion, degeneration, inflammation, necrosis (Mahmoudi et al., 2018 [RefID 12656]) and steatosis
2244 (Özyaydin et al., 2018b [RefID 12854]). In a Tier 2 rat study with doses up to 500 µg/kg bw per day no
2245 histological changes were reported (Özyaydin et al., 2018b [RefID 12854]). Based on the findings in two
2246 single-dose studies the CEP Panel judged the histological changes in liver as ALAN.

2247 Changes in clinical chemistry parameters potentially associated with liver toxicity were observed in
2248 several rat studies. A decrease in the albumin level was reported in a Tier 2 rat study at 10000 µg/kg
2249 bw per day (Amraoui et al., 2018 [RefID 11503]), while no change in albumin, globulin or ALP was
2250 observed in a Tier 3 monkey study up to 25 µg/kg bw per day (Vijaykumar et al., 2017 [RefID 7477]).
2251 Opposite effects on ALP levels were reported in two rat studies, i.e. an increase in a Tier 2 study at
2252 10000 µg/kg bw per day (Poormoosavi et al., 2018 [RefID 12913]) and a decrease in a Tier 3 study at
2253 not clear doses (inconsistent reporting in the publication) (Kazemi et al., 2017 [RefID 3441]). The
2254 likelihood of effect given for ALP was judged as ALAN.

2255 Similarly, ALT levels increased moderately in three rat studies (two Tier 2 studies, i.e. Mahmoudi et al.,
2256 2018 [RefID 12656], Amraoui et al., 2018 [RefID 11503] and one Tier 1 study, Vahdati Hassani et al.,
2257 2017 [RefID 2614]) and markedly in one study (Poormoosavi et al., 2018 [RefID 12913]) while no
2258 change in ALT were reported in a Tier 3 rat study at not clear doses (inconsistent reporting in the

2259 publication) (Kazemi et al., 2017 [RefID 3441]). In a Tier 2 mouse study no changes in ALT were found
2260 at a single dose of 5000 µg/kg bw per day (Marmugi et al., 2014 [RefID 4884]).

2261 AST levels increased moderately in three rat studies (two Tier 2 studies, i.e. Mahmoudi et al., 2018
2262 [RefID 12656], Amraoui et al., 2018 [RefID 11503] and one Tier 1 study, Vahdati Hassani et al., 2017
2263 [RefID 2614]) and markedly in one study (Poormoosavi et al., 2018 [RefID 12913]) while a decrease
2264 in AST was reported in a Tier 3 rat study at not clear doses (inconsistent reporting in the publication)
2265 (Kazemi et al., 2017 [RefID 3441]). In a Tier 2 mouse study no changes in AST were found at a single
2266 dose of 5000 µg/kg bw per day (Marmugi et al., 2014 [RefID 4884]).

2267 An increase in LDH levels were observed in two rat studies, one with a single dose of 10000 µg/kg bw
2268 per day (Mahmoudi et al., 2018 [RefID 12656], Tier 2) and one at 500 µg/kg bw per day but not at
2269 higher doses (Vahdati Hassani et al., 2017 [RefID 2614], Tier 1).

2270 Also, bilirubin levels increased in two Tier 2 rat studies at 10000 µg/kg bw per day (Amraoui et al.,
2271 2018 [RefID 11503] and Poormoosavi et al., 2018 [RefID 12913]) while no change was observed in
2272 Tier 3 a monkey study up to a dose of 25 µg/kg bw per day (Vijaykumar et al., 2017 [RefID 7477]).
2273 The CEP Panel judged the changes in liver enzymes and bilirubin in male rats as ALAN.

2274 The CEP Panel assigned a likelihood level of ALAN to the Liver effects of BPA in the adult exposure
2275 period, based on several histological findings and changes in some clinical parameters. Therefore, none
2276 of the endpoints was taken forward for BMD analysis. However, they were considered in the uncertainty
2277 analysis (see Appendix D).

2278 Indirect (germline) exposure

2279 No studies were available for this exposure period.

2280 Overall cluster selection of the endpoints/studies for BMD analysis for liver effects

2281 Overall, dose-related effects on liver weight and clinical parameters during adult exposure were
2282 observed in male rats mainly at BPA doses at or above 10000 µg/kg bw per day. An increased liver
2283 weight was also reported in females after developmental and adult exposure, but not in male rats and
2284 in females or males exposed only during developmental exposure.

2285 In summary, the liver effects following BPA treatment at different life stages from *in utero* to adulthood
2286 were judged as ALAN based on histological findings in female and/or male rats and increased liver
2287 weight and changes in liver enzymes after adult exposure in male rats. The overall likelihood across all
2288 exposure periods, i.e. the highest likelihood given in the cluster liver effects, was ALAN.

2289 The CEP Panel considered that the information from the studies available did not show a Likely or Very
2290 Likely effect of BPA exposure on the liver and did not take the findings forward for BMD analysis.

2291

2292 **Lung effects**

2293 Developmental exposure (pre-natal and/or post-natal until weaning)

2294 For this exposure period only one Tier 2 study in rats (Quan et al., 2017 [RefID 6025]) was identified.
2295 In this study an increase in relative lung weight was observed in male offspring (females not tested)
2296 following pre-natal exposure to a very high dose, i.e. 100000 µg/kg bw per day but not at lower doses
2297 (at or below 10000 µg/kg bw per day).

2298 The CEP Panel assigned a likelihood level of ALAN to the Lung effects of BPA in the developmental
2299 exposure period. Therefore, this endpoint (lung weight) was not taken forward for BMD analysis.
2300 However, it was considered in the uncertainty analysis (see Appendix D).

2301 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

2302 For this exposure period three studies were identified: One Tier 3 study in rats (Jeong et al., 2017
2303 [RefID 3133]) and one Tier 1 study (Patel et al., 2015b [RefID 5698]) and one Tier 2 single-dose study
2304 in mice (Kasneji et al., 2017 [RefID 3399]). The increase of relative lung weight in the rat study at a
2305 very high dose of 250000 µg/kg bw per day in males but not in females was not considered as sufficient

2306 evidence for a lung effect in the absence of supporting data from the mouse studies at a single low
2307 dose.

2308 Therefore, the CEP Panel assigned a likelihood level of Not Likely to the Lung effects of BPA in the
2309 developmental and adult exposure period. Therefore, this endpoint was not taken forward for BMD
2310 analysis.

2311 Growth phase/young age exposure

2312 No studies were available for this exposure period.

2313 Adult exposure (after puberty)

2314 No studies were available for this exposure period.

2315 Indirect (germline) exposure

2316 No studies were available for this exposure period.

2317 Overall cluster selection of the endpoints/studies for BMD analysis for lung effects

2318 Overall, in the absence of data on lung weight following only adult exposure, the CEP Panel assigned a
2319 likelihood level of ALAN (based on BPA effects at high doses in male rats) and Not Likely to the Lung
2320 effects of BPA in the exposure periods developmental and developmental and adult, respectively. The
2321 overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster lung effects,
2322 was ALAN.

2323 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
2324 Likely effect of BPA in any exposure period, therefore none of the endpoints was taken forward for
2325 BMD analysis.

2326

2327 **Thyroid gland effects**

2328 Developmental exposure (pre-natal and/or post-natal until weaning)

2329 For effects on thyroid gland histology following developmental exposure, only one Tier 1 study in
2330 Sprague–Dawley rats was identified (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370])

2331 In this study, C-cell hyperplasia was observed in females at 2.5 µg/kg bw per day on PND365, but not
2332 at higher doses or at PND730 or in males. No effect on follicular-cell hyperplasia was seen. An increase
2333 of ultimobranchial cysts was observed in females at 250 µg and 2500 µg/kg bw per day (NMDR) on
2334 PND730 but not on PND365 or in males.

2335 Based on these results, the CEP Panel considered the non-neoplastic changes in thyroid gland in the
2336 developmental exposure period ALAN. Therefore, none of the endpoints was taken forward for BMD
2337 analysis. However, they were considered in the uncertainty analysis (see Appendix D).

2338 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

2339 For effects on thyroid gland histology following developmental and adult exposure, only one Tier 1
2340 study in Sprague–Dawley rats was identified (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
2341 11370]).

2342 In this study, C-cell hyperplasia was seen in males on PND730 (dose trend and significant at 2500 µg/kg
2343 bw per day), but not on PND365 or in females. Follicular-cell hyperplasia was seen in females at 2.5 on
2344 PND730 and in males at 25 µg/kg bw/day and non-significant at 250 µg/kg bw per day on PND730. No
2345 effect on ultimobranchial cysts was observed.

2346 Based on these results, the CEP Panel considered the non-neoplastic changes in thyroid gland in the
2347 developmental and adult exposure period ALAN. Therefore, none of the endpoints was taken forward
2348 for BMD analysis. However, they were considered in the uncertainty analysis (see Appendix D).

2349 Growth phase/young age exposure

2350 No studies were available for this exposure period

2351 Adult exposure (after puberty)

2352 For effects on thyroid gland histology following adult exposure, only one Tier 1 study in rats was
2353 identified (Zhang J et al., 2017 [RefID 8770]).

2354 In this study, no effect on hyperplasia (focal) was observed.

2355 Based on these results, the CEP Panel considered the non-neoplastic changes in thyroid gland in the
2356 adult exposure period as Not Likely. Therefore, none of the endpoints was taken forward for BMD
2357 analysis.

2358 Indirect (germline) exposure

2359 No studies were available for this exposure period.

2360 Overall cluster selection of the endpoints/studies for BMD analysis for thyroid gland effects

2361 Overall, the CEP Panel assigned a likelihood level of ALAN to the thyroid gland effects of BPA in the
2362 developmental and developmental and adult exposure periods, based on an inconsistent increase in C-
2363 cell and follicular-cell hyperplastic changes in rats, and Not Likely for the adult exposure period. The
2364 overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster thyroid
2365 gland effects, was ALAN.

2366 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
2367 Likely effect of BPA in any exposure period, therefore none of the endpoints was taken forward for
2368 BMD analysis.

2369

2370 **Parathyroid effects**

2371 Developmental exposure (pre-natal and/or post-natal until weaning)

2372 For this exposure period only one Tier 1 study in rats was identified (NTP Clarity Report, 2018/Camacho
2373 et al., 2019 [RefID 11370]). The histological examination showed only a trend for an increase of
2374 hyperplasia in males but not in females. The CEP Panel assigned a likelihood level of ALAN to the
2375 Parathyroid effects of BPA in the developmental exposure period. Therefore, this endpoint was not
2376 taken forward for BMD analysis. However, it was considered in the uncertainty analysis (see Appendix
2377 D).

2378 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

2379 For this exposure period only one Tier 1 study in rats was identified (NTP Clarity Report, 2018/Camacho
2380 et al., 2019 [RefID 11370]). The histological examination showed only a trend for an increase of
2381 hyperplasia in males but not in females. The CEP Panel assigned a likelihood level of ALAN to the
2382 Parathyroid effects of BPA in the developmental and adult exposure period. Therefore, this endpoint
2383 was not taken forward for BMD analysis. However, it was considered in the uncertainty analysis (see
2384 Appendix D).

2385 Growth phase/young age exposure

2386 No studies were available for this exposure period.

2387 Adult exposure (after puberty)

2388 No studies were available for this exposure period.

2389 Indirect (germline) exposure

2390 No studies were available for this exposure period.

2391 Overall cluster selection of the endpoints/studies for BMD analysis for parathyroid effects

2392 Overall, considering for both exposure periods a hyperplastic change in male (but not in female) rats,
2393 the CEP Panel assigned an overall likelihood level of ALAN to the Parathyroid effects of BPA.

2394 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
2395 Likely effect of BPA in any exposure period, therefore none of the endpoints was taken forward for
2396 BMD analysis.

2397

2398 **Pituitary gland effects**

2399 Developmental exposure (pre-natal and/or post-natal until weaning)

2400 For this exposure period two Tier 1 studies in rats were identified (NTP Clarity Report, 2018/Camacho
2401 et al., 2019 [RefID 11370]; Mandrup et al., 2016 [RefID 4831]).

2402 The histological examination showed an increase of hyperplasia in the pars distalis in females at doses
2403 of 2.5 and 25 µg/kg bw per day and in males at a 250 µg/kg bw per day in one study (NTP Clarity
2404 Report, 2018/Camacho et al., 2019 [RefID 11370]), but no effects for this endpoint were detected in
2405 females in the other study (Mandrup et al., 2016 [RefID 4831]).

2406 The CEP Panel assigned a likelihood level of ALAN to the Pituitary gland effect of BPA (hyperplasia) in
2407 the developmental exposure period. Therefore, this endpoint was not taken forward for BMD analysis.
2408 However, it was considered in the uncertainty analysis (see Appendix D).

2409 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

2410 For this exposure period only one Tier 1 study in rats was identified (NTP Clarity Report, 2018/Camacho
2411 et al., 2019 [RefID 11370]). The histological examination showed only a trend for an increase of
2412 hyperplasia in the pars distalis in males but not in females. There was no change in the incidences of
2413 cysts in males and females.

2414 The CEP Panel assigned a likelihood level of ALAN to the Pituitary gland effects of BPA in the
2415 developmental and adult exposure period. Therefore, none of the endpoints was taken forward for BMD
2416 analysis. However, they were considered in the uncertainty analysis (see Appendix D).

2417 Growth phase/young age exposure

2418 No studies were available for this exposure period.

2419 Adult exposure (after puberty)

2420 No studies were available for this exposure period.

2421 Indirect (germline) exposure

2422 No studies were available for this exposure period.

2423 Overall cluster selection of the endpoints/studies for BMD analysis for pituitary gland effects

2424 Overall, in the absence of data following only adult exposure, the CEP Panel assigned an overall
2425 likelihood level of ALAN to the Pituitary gland effects of BPA.

2426 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
2427 Likely effect of BPA in any exposure period, therefore none of the endpoints was taken forward for
2428 BMD analysis.

2429

2430 **Adrenal gland effects**

2431 Developmental exposure (pre-natal and/or post-natal until weaning)

2432 For this exposure period only one Tier 1 study in rats was identified (NTP Clarity Report, 2018/Camacho
2433 et al., 2019 [RefID 11370]). In females a cortex hypertrophy was observed at the highest dose only
2434 (i.e. 25,000 µg/kg bw per day). In males a trend for medulla hyperplasia was reported. No change in
2435 cytoplasmic vacuolisation was found in males or females.

2436 The CEP Panel assigned a likelihood level of ALAN to the Adrenal gland effects of BPA in the
2437 developmental exposure period. Therefore, none of the endpoints was taken forward for BMD analysis.
2438 However, they were considered in the uncertainty analysis (see Appendix D).

- 2439 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)
- 2440 For this exposure period only one Tier 1 study in rats was identified (NTP Clarity Report, 2018/Camacho
2441 et al., 2019 [RefID 11370]). No effects on the adrenal gland were observed in this exposure period.
- 2442 The CEP Panel assigned a likelihood level of Not Likely to the Adrenal gland effects of BPA in the
2443 developmental and adult exposure period. Therefore, none of the endpoints was taken forward for BMD
2444 analysis.
- 2445 Growth phase/young age exposure
- 2446 No studies were available for this exposure period.
- 2447 Adult exposure (after puberty)
- 2448 No studies were available for this exposure period.
- 2449 Indirect (germline) exposure
- 2450 No studies were available for this exposure period.
- 2451 Overall cluster selection of the endpoints/studies for BMD analysis for adrenal gland effects
- 2452 Overall, in the absence of data following only adult exposure and considering the inconsistent effects
2453 reported following developmental exposure, the CEP Panel assigned a likelihood level of ALAN and Not
2454 Likely to the Adrenal gland effects of BPA in the developmental and developmental and adult exposure
2455 periods, respectively. The overall likelihood across all exposure periods, i.e. the highest likelihood given
2456 in the cluster adrenal gland effects, was ALAN.
- 2457 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
2458 Likely effect of BPA in any exposure period, therefore none of the endpoints was taken forward for
2459 BMD analysis.
- 2460
- 2461 **Bone marrow effects**
- 2462 Developmental exposure (pre-natal and/or post-natal until weaning)
- 2463 For this exposure period only one Tier 1 study in rats was identified (NTP Clarity Report, 2018/Camacho
2464 et al., 2019 [RefID 11370]). In this study no effects were reported for females and only effects at single
2465 doses (without dose–response) were observed in males for hypocellularity and myeloid hyperplasia.
- 2466 The CEP Panel assigned a likelihood level of Not Likely to the Bone marrow effects of BPA in the
2467 developmental exposure period. Therefore, none of the endpoints was taken forward for BMD analysis.
- 2468 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)
- 2469 For this exposure period only one Tier 1 study in rats was identified (NTP Clarity Report, 2018/Camacho
2470 et al., 2019 [RefID 11370]). No effects on bone marrow were observed in this exposure period except
2471 for an increase in hypocellularity in females at the highest dose.
- 2472 The CEP Panel assigned a likelihood level of ALAN to the Bone marrow effects of BPA in the
2473 developmental and adult exposure period. Therefore, none of the endpoints was taken forward for BMD
2474 analysis. However, they were considered in the uncertainty analysis (see Appendix D).
- 2475 Growth phase/young age exposure
- 2476 No studies were available for this exposure period.
- 2477 Adult exposure (after puberty)
- 2478 No studies were available for this exposure period.
- 2479 Indirect (germline) exposure
- 2480 No studies were available for this exposure period.

- 2481 Overall cluster selection of the endpoints/studies for BMD analysis for bone marrow effects
- 2482 Overall, considering the scattered findings in the two available exposure periods, the CEP Panel
2483 assigned a likelihood level of Not Likely and ALAN to the bone marrow effects of BPA in the
2484 developmental and developmental and adult exposure periods, respectively. The overall likelihood
2485 across all exposure periods, i.e. the highest likelihood given in the cluster bone marrow effects, was
2486 ALAN.
- 2487 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
2488 Likely effect of BPA in any exposure period, therefore none of the endpoints was taken forward for
2489 BMD analysis.
- 2490
- 2491 **Haematological effects**
- 2492 Developmental exposure (pre-natal and/or post-natal until weaning)
- 2493 For this exposure period only one Tier 1 study in rats was identified (NTP Clarity Report, 2018/Camacho
2494 et al., 2019 [RefID 11370]). In this study no effects were reported for females and for males except
2495 for a trend for an increase of MCH in females but not in males. Therefore, the CEP Panel assigned a
2496 likelihood level of Not Likely to the haematological effects of BPA in the developmental exposure period,
2497 and none of the endpoints was taken forward for BMD analysis.
- 2498 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)
- 2499 For this exposure period one Tier 1 study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
2500 11370]) and one Tier 3 study (Jeong et al., 2017 [RefID 3133]) in rats were identified.
- 2501 In the NTP study [RefID 11370], for eosinophils some decreases in males (%) and in females were
2502 reported without dose–response. Regarding haemoglobin trends for an increase were observed in males
2503 and females and the increase in males was also significant at the highest dose. A trend for an increase
2504 in packed cell volume was reported only for males. Opposite trends in females (increase, also significant
2505 at the highest dose) and males (decrease) were found for platelets.
- 2506 Based mainly on the findings in the Tier 1 study the CEP Panel assigned a likelihood level of ALAN to
2507 the Haematological effects of BPA in the developmental and adult exposure period. Therefore, none of
2508 the endpoints was taken forward for BMD analysis. However, they were considered in the uncertainty
2509 analysis (see Appendix D).
- 2510 Growth phase/young age exposure
- 2511 No studies were available for this exposure period.
- 2512 Adult exposure (after puberty)
- 2513 For this exposure period only one Tier 3 study in monkeys was identified (Vijaykumar et al., 2017 [RefID
2514 7477]).
- 2515 No changes in haematological parameters were observed in this study at doses up to 25 µg/kg bw per
2516 day.
- 2517 The CEP Panel considered that the evidence was Inadequate to judge the likelihood level of the
2518 Haematological effects of BPA in the adult exposure period, so none of the endpoints was taken forward
2519 for BMD analysis.
- 2520 Indirect (germline) exposure
- 2521 No studies were available for this exposure period.
- 2522
- 2523 Overall cluster selection of the endpoints/studies for BMD analysis for haematological effects
- 2524 Overall, the CEP Panel assigned an overall likelihood level of ALAN across all three exposure periods,
2525 i.e. the highest likelihood given in the cluster haematological effects.

2526 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
 2527 Likely effect of BPA in any exposure period, therefore none of the endpoints was taken forward for
 2528 BMD analysis.

2529 **3.1.2.3. Integration of likelihoods from human and animal studies**

2530 There was no human evidence available for this category. Thus, the overall likelihood of effects of BPA
 2531 was based on the animal evidence.

2532 Table 8 presents the likelihoods of effect for each cluster and the overall likelihood in the animal stream
 2533 in General toxicity.

2534 **Table 8:** Overall likelihood from the animal studies for General toxicity.

Human stream	Animal stream	Integrated likelihood
Cluster: Body weight	Cluster: Body weight	
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) ALAN	
	Growth phase/young age exposure ALAN	
	Adult exposure (after puberty) Not Likely	
	Indirect (germline) exposure Not Likely	
	<i>Overall likelihood</i> ALAN	ALAN
Cluster: Kidney effects	Cluster: Kidney effects	
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) ALAN	
	Adult exposure (after puberty) ALAN	
	<i>Overall likelihood</i> ALAN	
	ALAN	ALAN
Cluster: Liver effects	Cluster: Liver effects	
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) ALAN	
	Adult exposure (after puberty) ALAN	
	<i>Overall likelihood</i> ALAN	
	ALAN	ALAN
Cluster: Lung effects	Cluster: Lung effects	
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	
	<i>Overall likelihood</i> ALAN	
	ALAN	ALAN
Cluster: Thyroid gland effects	Cluster: Thyroid gland effects	

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)	ALAN	
	Adult exposure (after puberty)	Not Likely	
	<i>Overall likelihood</i>	<i>ALAN</i>	<i>ALAN</i>
Cluster: Parathyroid effects		Cluster: Parathyroid effects	
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)	ALAN	
	<i>Overall likelihood</i>	<i>ALAN</i>	<i>ALAN</i>
Cluster: Pituitary gland effects		Cluster: Pituitary gland effects	
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)	ALAN	
	<i>Overall likelihood</i>	<i>ALAN</i>	<i>ALAN</i>
Cluster: Adrenal gland effects		Cluster: Adrenal gland effects	
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	
	<i>Overall likelihood</i>	<i>ALAN</i>	<i>ALAN</i>
Cluster: Bone marrow effects		Cluster: Bone marrow effects	
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)	ALAN	
	<i>Overall likelihood</i>	<i>ALAN</i>	<i>ALAN</i>
Cluster: Haematological parameters		Cluster: Haematological parameters	
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)	ALAN	
	Adult exposure (after puberty)	Inadequate evidence	
	<i>Overall likelihood</i>	<i>ALAN</i>	<i>ALAN</i>

2535

2536 **3.1.2.4. In vitro and mechanistic studies**

2537 Regarding scoring of likelihood of effects in the WoE for the HOC General toxicity, no relevant clusters
 2538 were available from the human studies, and no cluster was scored Very Likely or Likely for any exposure
 2539 periods in the animals. Most clusters in the animal studies were scored ALAN, some Not Likely and one
 2540 Inadequate evidence. In the following paragraphs, MoA studies for the ALAN clusters among the HOC

2541 General toxicity are considered. The MoA studies on body weight are described in Chapter 3.1.4.4,
2542 under the HOC Metabolic effects.

2543 **Kidney effects**

2544 Among the HOC General toxicity, the cluster Kidney effects was considered. Based on animal studies,
2545 the CEP Panel assigned a likelihood level of ALAN to the kidney effects of BPA in the developmental
2546 and developmental and adult exposure periods, mainly based on the histological findings (transitional
2547 epithelium hyperplasia, renal tubule cysts, nephropathy) in a rat study; and in the adult exposure period
2548 mainly based on the effects on kidney weight and clinical chemistry parameters in male rats at high
2549 doses.

2550 No evidence was available regarding the MoA of BPA for this effect in the human studies. Three MoA
2551 studies were considered, two within the mammalian stream and one in the *in vitro* stream. These
2552 studies focused on oxidative stress. Elobeid et al. (2015) [RefID 1803] demonstrated that BPA exposure
2553 induced nephrotoxicity through oxidative stress by altering the apoptotic pathway involved. Esplugas
2554 et al. (2018) [RefID 11900] showed that gene expression of CYP1A2 was downregulated in mice
2555 exposed to BPA. The authors hypothesised that CYP-mediated modifications of BPA may give rise to
2556 the formation of unstable reactive intermediates, as well as radical fragments leading to oxidative
2557 damage.

2558 In conclusion, data obtained from the few MoA studies indicated oxidative stress as a potential
2559 pathogenic mechanism for the weak evidence of kidney damage observed in animal studies.

2560 **Liver effects**

2561 Among the HOC General toxicity, the cluster Liver effects was considered. Based on animal studies, the
2562 CEP Panel assigned a likelihood level of ALAN to the liver effects of BPA in the developmental,
2563 developmental and adult and adult exposure periods, mainly based on liver weight and several
2564 histological observations and changes in some clinical chemistry parameters.

2565 Epigenetic effects via hepatic DNA methylation were studied in several rodent studies with peri-/post-
2566 natal exposure to BPA. Using liver tissue samples from Agouti mouse offspring exposed via their
2567 mothers (fed diets with 50 µg/kg or 50000 µg/kg equivalent to 7.5 or 7500 µg/kg bw per day) during
2568 gestation and lactation, non-monotonic changes in DNA methylation were observed in genome-wide
2569 analyses (Kim JH et al., 2014 [RefID 3521]). Epigenetically dysregulated pathways related to
2570 metabolism and stimulus response were identified by gene set enrichment testing.

2571 Several studies report a link between DNA hypomethylation of lipogenic genes and hepatic lipid
2572 accumulation (steatosis). Anderson et al. (2017) [RefID 187] studied four genes in livers of adult female
2573 mouse offspring from dams treated with 0.05, 50 or 50000 µg BPA/kg diet or control dams and
2574 concluded that the DNA methylation in these genes had an impact on pathways related to body weight,
2575 body fat phenotypes and energy expenditure. Long-term exposure to BPA (0.5 µg/kg bw per day; via
2576 drinking water) was studied in male mice treated from birth up to 10 months resulting in hepatic
2577 accumulation of triglycerides and cholesterol (Ke et al., 2016 [RefID 3447]). These outcomes were
2578 associated with increased expression levels of key genes involved in lipid synthesis along with altered
2579 levels of DNA methylation and expression of genes for critical transcription factors (Srebf1 and Srebf2).
2580 The *in vivo* results were supported by knockdown experiments in the murine Hepa1-6 cell line (Ke et
2581 al., 2016 [RefID 3447]). Similarly, hepatic lipid accumulation was associated with hypomethylation of
2582 lipogenic genes and increased transcription factor Nrf2 signalling (binding to Srebp-1c promoter) in
2583 mouse offspring (at week 39) following BPA exposure via dams (25 µg/kg bw per day, i.p. from GD8
2584 to PND16) and drinking water (until PND35) (Shimpi et al., 2017 [RefID 6685]). Sex-specific modulation
2585 of hepatic DNA methylation and gene expression were studied in offspring of Sprague Dawley (SD) rats
2586 exposed to BPA via dams (100 µg/kg bw per day from GD6 to PND21) (Strakovsky et al., 2015 [RefID
2587 6914]). At PND1, BPA altered methylation and transcription factor binding within the Cpt 1a gene was
2588 observed in males but not in females suggesting epigenetic changes in the male hepatic β-oxidation
2589 capacity. Males at PND110 had microvesicular steatosis particularly when fed with a high-fat diet after
2590 weaning (Strakovsky et al., 2015 [RefID 6914]). In contrast, no changes in DNA methylation were
2591 found in livers of female mouse offspring (at 23 weeks) exposed to BPA via dams (3, 10, 30, 100, 300,
2592 1000, and 3000 µg/kg bw per day; from 2 weeks before mating through gestation and lactation) and

2593 fed a high-fat diet starting from week 17 to the end of the experiment (van Esterik et al., 2014 [RefID
2594 7494]).

2595 Diabetes was studied in rat offspring exposed via dams (50 µg/kg bw per day; throughout gestation
2596 and lactation) (Ma et al., 2013 [RefID 4748]). BPA-induced insulin resistance observed at week 21 was
2597 preceded by hypermethylation and a reduction in the expression of hepatic glucokinase at week 3,
2598 possibly indicating an impact on glucose metabolism and an increased risk of developing insulin
2599 resistance and type 2 diabetes mellitus. Perreault et al. (2013) [RefID 5797] reported that male mice
2600 treated with an acute oral dose of 50 µg/kg bw or the same dose daily over 2 weeks had a reduced
2601 glucokinase activity.

2602 Metabolomics based approaches were used in numerous studies addressing BPA-induced alterations of
2603 hepatic metabolite profiles, changes in carbohydrate and lipid metabolism and related gene expressions
2604 in the liver. Metabolomic profiling of rat urine was performed by Chen MJ et al. (2014) [RefID 1054]
2605 following gavage treatment with BPA at 50 or 50000 µg/kg bw per day for 8 weeks. Increases in biotin
2606 and riboflavin excretion and in the formation of the hepatic methyl donor S-adenosylmethionine along
2607 with enhanced expression of hepatic methionine adenosyltransferases (*Mat1a*, *Mat2a*) were observed
2608 dose dependently while increased levels of methylated products (e.g. 3-methylhistidine) in rat urine
2609 were only reported for the high dose, indicating protein catabolism. Another metabolome study was
2610 performed in male mouse offspring exposed to low BPA doses (0.025, 0.25, or 25 µg BPA/kg bw per
2611 day, from GD8 to PND16) (Cabaton et al., 2013 [RefID 776]). Metabolite profiles in livers at PND21
2612 revealed increased levels of taurine (TAU), glutamate (GLU) and glutathione along with decreased levels
2613 of lactate, glucose, glycogen, phosphatidylcholine and glycerophosphocholine at the highest dose
2614 tested. Dose-dependent and time-dependent metabolic changes in rat livers from offspring exposed
2615 perinatally to BPA (subcutaneous doses: 0.25, 2.5, 25, or 250 µg/kg bw per day) were studied by
2616 Tremblay-Franco et al. (2015) [RefID 7285] at PND21, 50, 90, 140 and 200). Changes often showed
2617 no clear dose-response and were for some metabolites also observed at the low BPA doses, e.g. for
2618 increases in phosphorylcholine and choline.

2619 Metabolic changes related to carbohydrate and lipid metabolism were addressed in several *in vivo*
2620 studies. Plasma lipid profiles, liver transcriptomic analysis and gene expression were performed in adult
2621 male mice exposed to BPA (5, 50, 500 and 5000 µg/kg bw per day) via drinking water for 8 months
2622 (Marmugi et al., 2014 [RefID 4884]). The increased plasma glucose (but not insulin) levels at the two
2623 highest doses and enhanced cholesterol levels (not dose dependent) along with a hepatic
2624 overexpression of genes related to cholesterol biosynthesis indicate BPA-induced hyperglycaemia,
2625 glucose intolerance, hypercholesterolemia and increased cholesterol biosynthesis. In a study on male
2626 rat offspring perinatally exposed to BPA at 40 µg/kg bw per day (Jiang et al., 2014 [RefID 3190])
2627 changes were observed in mitochondrial fatty acid metabolism at 3 weeks. Reduced ATP production at
2628 weeks 15 and 26 was reported along with increased ROS generation, upregulation of genes related to
2629 lipogenesis and fatty accumulation/steatosis in liver. In contrast to these findings, long-term exposure
2630 of male rats (fed a standard or a high-fat diet) to BPA (50 µg/kg bw per day) had no effect on total
2631 cholesterol or total triglycerides and the known mechanisms (Peroxisome proliferator-activated
2632 receptors (PPARs) and Sterol regulatory element-binding proteins (SREBPs)) involved in the regulation
2633 of hepatic lipid metabolism (Ding et al., 2013 [RefID 1621]). Differential effects of BPA and other
2634 bisphenols (100 µg/kg bw per day) on lipid accumulation and glucose homeostasis in adult female
2635 mouse offspring (exposed from GD7 to PND21) at PND35 were reported by Meng Z et al. (2018) [RefID
2636 12708]. BPA treatment resulted in an increased relative content of two out of 13 studied fatty acids
2637 (i.e. C20:0 and C20:1n9) and affected genes involved in the fatty acid transport. García-Arévalo et al.
2638 (2014) [RefID 2193] studied liver triglyceride accumulation and glucose homeostasis in adult male
2639 mouse offspring exposed to BPA via dams (subcutaneously injected with 10 µg/kg bw per day from
2640 GD9 to GD16). BPA increased the hepatic triglyceride content and *Pparγ* mRNA expression in livers of
2641 mice fed a regular diet but not in those on a high-fat diet, while mRNA expression of *Cd36* involved in
2642 fatty acid uptake was decreased by BPA with both diets. Liver histology of Watanabe Heritable Hyper-
2643 Lipidemic (WHHL) rabbits treated with 400 µg BPA/kg bw per day for 12 weeks revealed mild steatosis
2644 and inflammatory cell infiltration in the liver (Fang et al. 2014, RefID 1914). The authors also report on
2645 BPA-induced increases of mRNA expression of genes related to the ER pathway, to lipid metabolism
2646 and to liver inflammation. Reddivari et al. (2017) [RefID 6131] observed liver inflammation in rabbit

2647 offspring exposed to BPA (200 µg/kg bw per day) from GD15 to PND7 and discussed an association
2648 with changes in the gut microbiota and gut metabolites, e.g. reduced short chain fatty acid levels.

2649 Analysing male fetal mouse livers following BPA exposure (50 µg or 50000 µg/kg bw per day until
2650 GD18.5) Susiarjo et al. (2017) [RefID 7023] identified increased bile acid and tryptophan levels at the
2651 higher dose. Elevated tryptophan levels were also observed in the maternal livers and the authors
2652 discussed a potential link to a disturbed glucose metabolism and gestational diabetes. Galyon et al.
2653 (2017) [RefID 2131] studied the hepatic insulin signalling in rat offspring exposed to BPA during
2654 gestation and lactation via drinking water consumption by dams (at 239 ± 8 µg/kg bw per day and at
2655 466±33 µg/kg bw per day, respectively). This BPA treatment resulted in impaired glucose tolerance (at
2656 6 weeks and 6 months) and altered protein levels of components of insulin signalling (at 3 weeks) in
2657 male, but not in female offspring.

2658 Mechanisms leading to dysregulated hepatic lipid metabolism were further investigated *in vitro*. Lin Y
2659 et al. (2017) [RefID 4338] reported a BPA-induced increase in serum and hepatic triglyceride via an
2660 enhanced expression of Srebf1 and lipogenesis-related genes in male post-weaning mice treated with
2661 50 µg BPA/kg bw per day for 90 days. These effects were also observed in BPA (>100 nM, 48 h)-
2662 treated human HepG2 cells and were mediated by a downregulation of microRNA, miR-192. Cabaton
2663 et al. (2018) [RefID 11650] reported on metabolomic changes in HepG2 induced by BPA (1 pM, 1nM,
2664 1µM). The endogenous metabolite variations were concentration-specific, indicating significant effects
2665 on several amino acids (at all BPA concentrations) and on reduced glutathione (GSH) at the lowest BPA
2666 concentration. The authors concluded that BPA modulated major metabolic routes involved in cellular
2667 functions and detoxification processes. In another study (Hélie-Toussaint et al., 2014 [RefID 2676]),
2668 HepG2 cells were treated with BPA concentrations between 1 fM and 1 µM for 4 days but only 1pM BPA
2669 increased the accumulation of triglyceride while glucose uptake was not affected. In contrast to these
2670 data, Peyre et al. (2014) [RefID 5812] reported an increase in lipid accumulation in HepG2 cells only
2671 at high BPA concentrations (>10 µM; 72 h). Lv Q et al. (2017) [RefID 4697] showed that in livers of
2672 male rats fed BPA containing diets (corresponding to 0.5 or 5 µg/kg bw per day), lipid accumulation
2673 increased together with the number of Kupffer cells (KC) transformed toward a pro-inflammatory M1
2674 phenotype which may also participate in hepatic lipid deposition *in vivo*. A high concentration of BPA
2675 (10 µM) in primary KC cultures also induced this change of KC phenotype and enhanced the levels and
2676 the secretion of inflammatory factors. In addition, conditional medium from BPA-treated KC was shown
2677 to increase lipid accumulation in HepG2 cells. Using a long-term cell culture model with human
2678 hepatoma HepaRG cells (Bucher et al., 2017 [RefID 743]), BPA treatment (0.2–2000 nM) for 3 weeks
2679 induced an increase in neutral lipids and triglycerides accumulation at 2 nM; however, the expression
2680 of enzymes involved in lipid and carbohydrate homeostasis remained unchanged. Similarly, lipid
2681 accumulation in rat hepatoma FaO cells (Grasselli et al., 2013 [RefID 2386]) were observed at high BPA
2682 concentrations (≥100 nM; 24 h), interfering with the pathways involved in lipid oxidation and secretion
2683 but without an increase in the expression of lipogenic genes. Yang SM et al. (2017) [RefID 8398]
2684 reported on the association of lipid accumulation and dysregulated autophagy in mice treated with 5–
2685 500 µg BPA/kg bw per day for 8 weeks and further investigated lipid accumulation and the mechanisms
2686 involved in the reduced autophagy induction and autophagosome accumulation (via the mammalian
2687 target of rapamycin (mTOR) pathway) in HepG2 cells, however *in vitro* only a high BPA concentration
2688 (10 µM) was effective.

2689 Several rat studies addressed BPA-induced oxidative stress associated with liver toxicity. Adult male
2690 rats treated with a high dose of BPA (10000 µg/kg bw per day) via drinking water over 60 days
2691 (Mahmoudi et al., 2018 [RefID 12656]) showed increases in thiobarbituric acid reactive substances
2692 (TBARS, a marker of lipid peroxidation), total antioxidant capacity and lipid peroxidation along with
2693 decreases in catalase (CAT) and superoxide dismutase activities in the liver. Concomitantly, the hepatic
2694 levels of total cholesterol and triglyceride as well as parameters indicating liver damage (ALT, AST, ALP,
2695 LDH and TNF-α) were clearly increased. In another study (Vahdati Hassani et al., 2017 [RefID 2614])
2696 male rats treated with 500 µg/kg bw per day for 30 days had elevated serum activities of AST and LDH,
2697 increased serum levels of triglyceride, high density lipoprotein (HDL) cholesterol and glucose as well as
2698 increased hepatic level of 8-isoprostane and a decreased level of reduced glutathione and also showed
2699 periportal inflammation in the liver. At the same dose level, the authors reported increases in elements
2700 of mitogen-activated protein kinase (MAPK) pathways, MAPK-activated protein kinase (MAPKAPK) and
2701 AKT activation and upregulated microRNA (miR-122) transcript levels in liver. In a follow-up study

2702 Vahdati Hassani et al. (2018) [RefID 11240] observed in livers of male rats treated with the same BPA
2703 dose an indication of oxidative stress (increased malondialdehyde (MDA), decreased glutathione levels).
2704 In addition, BPA-induced alterations in the expression of (phospho)proteins involved in homocysteine,
2705 fatty acid and carbohydrate metabolism and antioxidant defence in liver were reported. In a study by
2706 Wei et al. (2014) [RefID 7890] hepatic gene expression of genes and/or proteins were investigated in
2707 male rats perinatally exposed to BPA (50 µg/kg bw per day; GD0 to PND21). While BPA induced mild
2708 steatosis and altered insulin signalling in livers on a standard diet, BPA worsened the hepatic damage
2709 of a high-fat diet (lipid accumulation, inflammation, mild fibrosis; increased activities of AST, ALT, ALP
2710 and gamma-glutamyltransferase (GGT)). These findings were associated with indications of an
2711 enhanced oxidative stress in liver (mainly in the high-fat diet BPA group) as well as increased mRNA
2712 expression of hepatic genes associated with lipogenesis, fatty acid oxidation, and gluconeogenesis. An
2713 increased expression of two genes linked to oxidative stress was reported by Kazemi et al. (2016b)
2714 [RefID 3442] in adult male rats treated with BPA at 5, 25 or 125 µg/kg bw per day for 35 days: HO-1
2715 (heme catabolism) was induced dose dependently as a monotonic dose response (MDR) while
2716 GADD45B (related to cell cycle, apoptosis and DNA repair) showed a NMDR. The same group (Kazemi
2717 et al., 2017 [RefID 3441]) reported with the same experimental design a BPA-induced increase of
2718 malonaldehyde as an indicator for oxidative stress along with histopathological findings in the liver but
2719 surprisingly with a marked decrease in the serum levels of ALP and AST in BPA-treated rats. Thilagavathi
2720 et al. (2017) [RefID 9247] investigated the antioxidant status of the liver of adult female rats treated
2721 with 10, 50 or 100 µg BPA/kg bw per day for 12 weeks. They observed a dose-dependent decrease of
2722 the activities of superoxide dismutase, CAT and glutathione peroxidase (GPx) along with a decreased
2723 glutathione content in the liver in all treatment groups. In addition, they reported on a decrease in
2724 hepatic cytochrome P450 and cytochrome b5 (phase I detoxification) with a NMDR. Esplugas et al.
2725 (2018) [RefID 11900] studied oxidative DNA damage (8-OHdG) in livers of male mice exposed to a
2726 single subcutaneous dose of BPA (25 µg/kg bw per day) at PND10. The authors hypothesised that CYP-
2727 mediated modifications of BPA may give rise to oxidative damage. They observed a reduced hepatic
2728 expression of CYP1A2 but 8-OHdG was not significantly altered in treated mice at 2 months of age.

2729 Using HepG2 cells treated with 100 nM BPA for 72 hours Vidyashankar et al. (2014) [RefID 7469]
2730 reported a marked cytotoxicity (56%) along with indications of oxidative stress, i.e. GSH content,
2731 reduced activities of antioxidant enzymes (CAT, GPx, superoxide dismutase (SOD)), lipid peroxidation
2732 and impaired mitochondrial function, i.e. reduced ATP production and mitochondrial membrane
2733 potential. An upregulation of CYP2C9 expression in HepG2 cells was reported with treatment of BPA
2734 (10, 100 and 1000 nM) through an ER α -mediated transcriptional activation (Xu JY et al., 2017 [RefID
2735 8203]). This upregulation may alter metabolism in human liver.

2736 Involvement of mitochondria in the induction of BPA-induced apoptosis of liver cells has been studied
2737 by Xia et al. (2014) [RefID 8103] in male rat offspring exposed to BPA from G0 to PND21 (50 µg/kg
2738 bw per day). The authors observed liver toxicity (increase in serum ALT at week 21) and hepatic
2739 apoptosis at week 15 and 21 associated with increased activities of caspase 3 and caspase 9 and an
2740 elevated release of cytochrome *c* from mitochondria in hepatocytes suggesting a mitochondrial
2741 apoptosis pathway. In addition, *in vitro* experiments with mitochondria isolated from untreated neonatal
2742 rat liver and then exposed to BPA further supported the hypothesis that apoptosis is induced by
2743 alteration of the mitochondrial ultrastructure and the release of proteins.

2744 In conclusion, many MoA studies addressed several potential mechanisms of BPA in liver and liver cells.
2745 It was proposed that epigenetic changes via DNA methylation may have an impact on different
2746 signalling pathways related to lipid and carbohydrate metabolism. In addition, oxidative stress may be
2747 related to impaired mitochondrial function and liver toxicity.

2748 Lung effects

2749 Among the HOC General toxicity, the cluster Lung effects was considered. Based on animal studies, the
2750 CEP Panel assigned a likelihood level of ALAN of BPA effects (increase in lung weight) at high doses in
2751 male rats in the exposure periods developmental, developmental and adult, and adult.

2752 No evidence was available regarding the MoA of BPA for this effect in the human studies.

2753 Nine MoA studies were considered, five within the mammalian stream and four in the *in vitro* stream.
2754 Most of these studies addressed changes related to allergic/inflammatory responses. An *in vivo* MoA

2755 study in mice (Hijazi et al., 2015 [RefID 2707]) demonstrated that BPA retards fetal lung maturation,
2756 as evidenced by diminished alveolar airspace and thickened septa which are both hallmarks of lung
2757 immaturity. This immaturity was characterised by aberrant alveolar epithelial type I cell differentiation.
2758 The authors concluded that the effects of BPA are likely to be mediated through the glucocorticoid
2759 signalling pathway: this point was further investigated in another *in vitro* MoA study carried out by the
2760 same authors (Hijazi et al., 2017 [RefID 2708]).

2761 In conclusion, data obtained from MoA studies suggest that BPA can delay fetal lung maturation
2762 evaluated through reduced alveolar airspace and thickened septa. Both these findings are related to an
2763 increase in the lung weight and can explain the results of the animal studies.

2764 **Thyroid gland effects**

2765 Among the HOC General toxicity, the cluster Thyroid gland effects was considered.

2766 Based on animal studies, the CEP Panel assigned an overall likelihood level of As Likely As Not (ALAN)
2767 for BPA effects following developmental (C-cell hyperplasia, ultimobranchial cysts) and developmental
2768 and adult exposure (C-cell hyperplasia, follicular-cell hyperplasia).

2769 No evidence was available regarding the MoA of BPA for this effect in the human studies.

2770 Four MoA studies were considered, two in rodents and two in *in vitro* stream. These studies addressed
2771 mainly carcinogenic effects of BPA on the thyroid gland. An animal (rat) study (Zhang J et al., 2017
2772 [RefID 8770]) reported that chronic administration of BPA alone did not increase thyroid carcinoma
2773 incidence. However, the study indicated that BPA could enhance the susceptibility to thyroid carcinoma
2774 stimulated by N-bis(2-hydroxypropyl)nitrosamine (DHPN) and iodine excess. ER α is probably involved
2775 in the proliferation effect of BPA. An *in vitro* study (Zhang YH et al., 2017 [RefID 8883]) reported that
2776 BPA mediates oestradiol-like effects by binding to nuclear oestrogen receptors (ER α and ER β), and also
2777 promotes growth by binding to oestrogen membrane receptor (mER α and GPR30) and activating the
2778 AKT/mTOR pathway, ultimately altering gene expression to stimulate proliferation of thyroid cancer
2779 cells. Porreca et al. (2016) [RefID 5892] investigated the effects of BPA on rat follicular thyroid cell line
2780 (FRTL-5). The authors concluded that while BPA does not directly cause genetic damage, it may
2781 contribute to thyroid cell damage by impairing DNA repair and efficiency, and, likely, to thyroid
2782 carcinogenesis in cooperation with other endogenous or external factors.

2783 In conclusion, data obtained from the few MoA studies available, indicated potential mechanisms that
2784 are responsible for an increase in the proliferation of thyroid cells, supporting the limited evidence of
2785 hyperplastic changes observed in the *in vivo* animal study. Moreover, it is suggested that BPA could
2786 enhance the susceptibility to thyroid carcinoma in combination with other factors.

2787 **Parathyroid gland effects**

2788 Among the HOC General toxicity, the cluster parathyroid gland effects was considered.

2789 Based on animal studies, the CEP Panel assigned a likelihood level of ALAN of BPA effects in the
2790 developmental and developmental and adult exposure periods.

2791 No evidence was available regarding the MoA of BPA for this effect in the human, *in vitro* or animal
2792 studies.

2793 **Pituitary gland effect**

2794 Among the HOC General toxicity, the cluster 'pituitary gland effects' was considered.

2795 Based on animal studies, the CEP Panel assigned a likelihood level of ALAN of BPA effects in the
2796 exposure periods developmental and developmental and in adulthood.

2797 No evidence was available regarding the MoA of BPA for this effect in the human, *in vitro* or animal
2798 studies.

2799 **Adrenal gland effects**

2800 Among the HOC General toxicity, the cluster adrenal gland effects was considered.

2801 Based on animal studies, the CEP Panel assigned a likelihood level of ALAN of BPA effects in the
2802 developmental exposure period, and Not Likely in the developmental and adult exposure period.

2803 No evidence was available regarding the MoA of BPA for this effect in the human, *in vitro* or animal
2804 studies.

2805 **Bone marrow effects**

2806 Among the HOC General toxicity, the cluster 'bone marrow effects' was considered.

2807 Based on animal studies, the CEP Panel assigned a likelihood level of Not Likely of BPA effects in the
2808 developmental exposure period, and of ALAN in the developmental and adult exposure period.

2809 No evidence was available regarding the MoA of BPA for this effect in the human, *in vitro* or animal
2810 studies.

2811 **Haematological effects**

2812 Among the HOC General toxicity, the cluster 'haematological effects' was considered.

2813 Based on animal studies, the CEP Panel assigned a likelihood level of Not Likely of BPA effects in the
2814 developmental exposure period, of ALAN in the developmental and adult exposure period, and of
2815 Inadequate evidence in adulthood.

2816 No evidence was available regarding the MoA of BPA for this effect in the human, *in vitro* or animal
2817 studies.

2818 **3.1.2.5. Conclusion on hazard identification for General toxicity of BPA**

2819 In the 2015 EFSA opinion on BPA (EFSA CEF Panel, 2015) BPA effects in liver and kidney were judged
2820 as Likely based on results from multigeneration studies in mice and rats. Increases in liver and kidney
2821 weight were considered relevant systemic effects of BPA (EFSA 2007; EFSA CEF Panel 2010 and 2015)
2822 as they were associated in rodents with hepatocellular hypertrophy and nephropathy, respectively.
2823 Based on a WoE approach changes in the mean relative kidney weight in a two-generation toxicity
2824 study in mice were considered as the most critical effect at low doses. The kidney data were taken
2825 forward for a BMD–response modelling resulting in a BMDL₁₀ of 8,960 µg/kg bw per day. Using
2826 toxicokinetic data the BMDL₁₀ value was converted into a HED of 609 µg/kg bw per day which was
2827 considered the lowest value to be used for the derivation of a TDI (EFSA CEF Panel, 2015).

2828 The available new information that has been evaluated in the current evaluation is in line with these
2829 earlier observations on kidney and liver toxicity. An increase of absolute kidney weight along with
2830 changes in clinical biochemistry was reported in male rats (females not tested) treated with a dose of
2831 10000 µg/kg bw per day for 8 weeks during adulthood while no changes in kidney weight were observed
2832 from 2.5 to 25000 µg/kg bw per day during the other exposure periods, except for an increase in
2833 relative weight in males (but not in females) at 50000 and 250000 µg/kg bw per day treated from PND6
2834 for 90 days. Histological findings in kidney (renal tubule cysts, nephropathy and hyperplasia of the
2835 transitional epithelium) at lower doses (2.5 to 2500 µg/kg bw per day) following BPA exposure during
2836 developmental life stages until weaning or adulthood were not consistently observed in the evaluated
2837 studies and therefore judged as ALAN. Regarding liver weight changes, inconsistent outcomes were
2838 reported and therefore this effect was considered as Not Likely (developmental exposure until weaning)
2839 or ALAN (developmental and adult and adult exposure). Histological changes in liver (congestion,
2840 degeneration, inflammation, necrosis and steatosis) were observed in two rat studies during adult
2841 exposure to BPA at 10000 µg/kg bw per day while exposure during the developmental until weaning or
2842 adulthood periods resulted in histological findings (angiectasis, cystic degeneration,
2843 hepatodiaphragmatic nodules, mononuclear cell infiltration, fatty change) with unclear dose–responses
2844 between 2.5 to 2500 µg/kg bw per day. Therefore, the CEP Panel considered the histological effects in
2845 female or male rat liver which were not consistently reported in the studies ALAN. Several changes in
2846 clinical chemistry parameters potentially associated with liver toxicity (liver enzyme activities, bilirubin
2847 levels) following adult exposure to BPA at 10000 mg/kg bw per day indicated likely effects. However,
2848 the results from studies with developmental until weaning or adulthood exposure are not consistent
2849 regarding these findings and were judged as ALAN.

2850 In three MoA studies on kidney effects, indications for oxidative stress as a potential pathogenetic
2851 mechanism for the weak evidence of kidney damage were observed. Similarly, BPA-induced oxidative

2852 stress was suggested to be associated with liver toxicity. Other studies reported evidence of epigenetic
2853 effects via hepatic DNA methylation for changes in lipid and carbohydrate metabolism in liver.

2854 Considering the other clusters in this HOC there were only few effects reported in the WoE assessment
2855 (effects at very high doses of BPA, i.e. increase of relative lung weight and hypercellularity in bone
2856 marrow, inconsistent effects, i.e. hyperplastic changes in thyroid, parathyroid and pituitary gland and
2857 hypertrophic changes in adrenal gland, and effects without a clear dose-response (in haematology)
2858 that were all judged as ALAN or Not Likely.

2859 Overall, none of the endpoints in the HOC General toxicity were considered Likely or Very Likely and
2860 therefore, none of them was taken forward for BMD analysis.

2861 **3.1.3. Immunotoxicity**

2862 **3.1.3.1. Epidemiological studies**

2863 For the HOC Immunotoxicity, a total of nine studies was appraised by the CEP Panel. The details of the
2864 appraisals (internal validity) are reported in Annex B.

2865 *Identification of the clusters to be considered for WoE*

2866 On the basis on the approach described in Chapter 2.3.2 'Definition of Health Outcome Categories and
2867 Clusters', the following Clusters (C) and Exposure periods (Exp) was brought forward to WoE analysis:

- 2868 • C: Asthma/allergy
- 2869 – Exp: Childhood
- 2870 – Exp: Pregnancy

2871

2872 *WoE of the relevant clusters*

2873 The main information extracted from the studies included in relevant clusters in the HOC
2874 Immunotoxicity are summarised in Annex C. The outcome of the weight of the evidence is described
2875 in the text below and presented in a tabulated format in Annex D.

2876 **Cluster Asthma/allergy**

2877 A large number of allergy-related endpoints was studied in the eight longitudinal studies assessing
2878 different exposure windows and all using spot urine samples (Donohue et al., 2013 [RefID 10918]; Kim
2879 KN et al., 2014 [RefID 3531]; Spanier et al., 2014b [RefID 6862]; Gascon et al., 2015 [RefID 2206];
2880 Wang JI et al., 2016 [RefID 7635]; Vernet et al., 2017 [RefID 7452]; Zhou AF et al., 2017 [RefID 9013];
2881 Buckley et al., 2018 [RefID 11645]).

2882 The observed heterogeneity for endpoint definitions was considerable, including asthma (n = 3),
2883 wheezing (n = 3), chest infections (n = 1), allergic diseases (n = 1), atopy (n = 1), rhinitis (n = 1),
2884 atopic dermatitis (n = 1), FEV₁ (n = 1), PC20 (n = 1), IgE (n = 1), and FENO (n = 1). Statistically
2885 significant associations in more than one study were observed for asthma and wheezing. In the text
2886 below, the assessed longitudinal studies are described in brief. Their detailed description and risk of
2887 bias assessment are provided in Annexes C and D.

2888 Exposure during pregnancy

2889 Seven cohort studies (Donohue et al., 2013 [RefID 10918]; Spanier et al., 2014b [RefID 6862]; Gascon
2890 et al., 2015 [RefID 2206]; Vernet et al., 2017 [RefID 7452]; Zhou AF et al., 2017 [RefID 9013]; Buckley
2891 et al., 2018 [RefID 11645]; Liao et al. (2016) [RefID 4266]) assessed the association between BPA
2892 exposure measured in pregnancy and allergy-related endpoints with a cumulative sample size of 2836
2893 participants. The populations under study were of comparable sample size but varied in their
2894 characteristics; there were four studies including a European-descent population. BPA exposure was
2895 measured via a single spot urine sample in all seven studies and varied between studies. Thus, the
2896 currently available longitudinal epidemiological evidence is characterised by a small number of studies,

2897 suboptimal exposure assessment and considerable heterogeneity in the assessed populations, exposure
2898 levels and endpoints.

2899 Donohue et al. (2013) [RefID 10918] used a birth cohort to investigate the association between pre-
2900 natal BPA exposure and wheeze, asthma and increased FENO in African-American and Dominican
2901 children in the USA followed up until the age of 11 years old (n = 568). No statistically significant
2902 associations were observed.

2903 Likewise, Spanier et al. (2014b) [RefID 6862] assessed pre-natal BPA exposure in a population of
2904 European ancestry (HOME study, n = 398) and parent-reported wheeze (5-year follow-up) and forced
2905 expiratory volume in the first second of expiration (FEV₁) at age 4 and 5 years. Statistically significant
2906 associations were observed for BPA exposure at 16 weeks gestation and for FEV₁ at 4 years and wheeze
2907 at 5 years [Odds Ratio (OR), 95% CI; 1.79, 1.16–2.78], but not for BPA exposure at 26 weeks gestation
2908 or mean gestational BPA exposure and wheeze at 5 years or FEV₁ at 5 years.

2909 Gascon et al. (2015) [RefID 2206] in the INMA (Infancia y Medio Ambiente (Environment and
2910 Childhood) Project)) birth cohort (n = 657) evaluated whether pre-natal exposure to BPA and
2911 phthalates increases the risk of respiratory and allergic outcomes (chest infections, bronchitis, wheeze,
2912 eczema, asthma, IgE) in children (7-year follow-up). Pre-natal BPA levels were statistically significantly
2913 associated with wheeze [Relative Risk (RR), 1.20; 95% CI, 1.03–1.40], chest infections (RR, 1.15; 95%
2914 CI, 1.00–1.32) and bronchitis (RR, 1.18; 95% CI, 1.01–1.37).

2915 Vernet et al. (2017) [RefID 7452] reporting on the EDEN birth cohort (n = 587) assessed the association
2916 between pre-natal BPA exposure (among 9 other phenols and 11 other phthalates) and respiratory
2917 outcomes related to allergy (FEV₁, asthma, bronchitis, wheezing). No statistically significant associations
2918 were observed.

2919 Zhou AF et al. (2017) [RefID 9013] evaluated the association between BPA concentrations collected at
2920 delivery and eczema and wheeze in infants at age 6 months (n = 412). BPA was associated with an
2921 increased risk of allergic diseases (OR, 1.21; 95% CI, 1.02–1.47).

2922 Buckley et al. (2018) [RefID 11645] in the Mount Sinai Children's Environmental Health Study evaluated
2923 the pre-natal exposure to BPA (among other phenol and phthalate biomarkers) and its association to
2924 asthma, wheeze and skin atopy at ages 6 and 7 years (n = 164). Overall, no statistically significant
2925 association was observed. For asthma, a statistically significant association for boys was observed
2926 (diagnosis, OR 3.04, 95% CI, 1.38–6.68; emergency room visits, OR 3.28, 95% CI 1.15–9.34).

2927 Liao et al. (2016) [RefID 4266] in a birth cohort conducted in Taiwan (n = 250) addressed the
2928 association between pre-natal BPA exposure (cord blood) and production of TNF- α , IL-6 and IL-10 after
2929 stimulating mononuclear cells with Toll-like receptor ligands (TLR1–4 and 7/8), bacteria from
2930 nasopharyngeal specimens and the incidence of infections. Significant associations were identified for
2931 TLR3-stimulated and TLR4-stimulated TNF- α response as well as for TLR7/8-stimulated IL-6 response,
2932 but not for infection or bacterial colonisation during the first year of life.

2933 On the basis of the above, the CEP Panel concluded that the evidence for a positive association between
2934 BPA exposure during pregnancy and allergy is ALAN.

2935 Exposure during childhood

2936 Four cohort studies (Donohue et al., 2013 [RefID 10918]; Kim KN et al., 2014 [RefID 3531]; Spanier
2937 et al., 2014b [RefID 6862]; Wang IJ et al., 2016 [RefID 7635]) assessed the association between BPA
2938 exposure measured in childhood and allergy-related endpoints with a cumulative sample size of 1,546
2939 participants. The populations under study were of comparable sample size but varied in their
2940 characteristics; the one study including a European-descent population (Spanier et al., 2014b [RefID
2941 6862]) included 398 children in USA. BPA exposure was measured via a single spot urine sample in all
2942 four studies and varied between studies; in the study including individuals of European descent the
2943 total BPA geometric mean ($\mu\text{g/g}$ of creatinine) was 2.4 (95% CI, 2.1–2.7; range, 0.5–293.6). Thus, the
2944 currently available longitudinal epidemiological evidence is characterised by a small number of studies,
2945 suboptimal exposure assessment and considerable heterogeneity in the assessed populations, exposure
2946 levels and endpoints.

2947 Donohue et al. (2013) [RefID 10918] implemented a birth cohort to investigate the association between
 2948 BPA exposure (3, 5 and 7 years) and wheeze, asthma and increased FENO in African-American and
 2949 Dominican children in the USA followed up until the age of 11 years old (n = 568). No statistically
 2950 significant associations were observed.

2951 Spanier et al. (2014b) [RefID 6862] is the only study in this group including a population of European
 2952 ancestry (HOME study, n = 398). Urine samples were collected annually, and parent-reported wheeze
 2953 was assessed every 6 months for 5 years along with FEV₁ at age 4 and 5 years. No statistically significant
 2954 associations were observed.

2955 Kim KN et al. (2014) [RefID 3531] included older children (n = 127, age range 7–8 years) in Korea that
 2956 were assessed three times every 2 years. The association between BPA concentration at 7–8 years of
 2957 age and wheezing, asthma and PC20 at ages up to 11–12 years were examined. A statistically significant
 2958 association was observed for wheezing (OR 2.48; 95% CI 1.15–5.31) and asthma (HR 2.13; 95% CI
 2959 1.51–3.00) and PC20 (beta 22.33; P = 0.02).

2960 Wang IJ et al. (2016) [RefID 7635] evaluated 453 children in Taiwan (Childhood Environment and
 2961 Allergic Diseases Study). Urinary BPAG levels at age 3 were statistically significantly associated with
 2962 asthma at age 6 (OR, 95% CI; 1.27, 1.04–1.55) and IgE.

2963 On the basis of the above, the CEP Panel concluded the evidence for a positive association between
 2964 BPA exposure during childhood and allergy is ALAN.

2965 Overall conclusions

2966 On the basis of the above, the CEP Panel concluded that the evidence for a positive association between
 2967 BPA exposure and asthma/allergy is ALAN.

2968 *Cross-sectional studies*

2969 Six cross-sectional studies investigated the relationship between BPA exposure (maternal and fetal
 2970 serum, placenta) and immunity-related endpoints (Spanier et al., 2014a [RefID 6861]; Ashley-Martin
 2971 et al., 2015 [RefID 281]; Savastano et al., 2015 [RefID 6495]; Ferguson et al., 2016a [RefID 1988];
 2972 Lin et al., 2018 [RefID 12522]; Youssef et al., 2018 [RefID 13583]). Three of them assessed immunity
 2973 biomarkers (Ashley-Martin et al., 2015 [RefID 281]; Savastano et al., 2015 [RefID 6495]; Ferguson et
 2974 al., 2016a [RefID 1988]) and another three studies examined asthma-related endpoints, including the
 2975 assessment of lung function (Spanier et al., 2014a [RefID 6861]; Lin et al., 2018 [RefID 12522]; Youssef
 2976 et al., 2018 [RefID 13583]). The immunity biomarkers under study included IgE, thymic stromal
 2977 lymphopoietin (TSLP), IL-33, IL-1 β , IL-6, IL-10, TNF- α , plasma monocyte chemoattractant protein 1
 2978 and C-reactive protein (CRP). Across the multiple analyses performed in these studies, statistically
 2979 significant results were observed for IL-6 in two studies of populations of European ancestry (n = 76,
 2980 n = 482) (Savastano et al., 2015 [RefID 6495]; Ferguson et al., 2016a [RefID 1988]) and TNF α in a
 2981 small study (n = 76) (Savastano et al., 2015 [RefID 6495]). All three studies that assessed allergic
 2982 disorders of the lung included children and yielded statistically significant results. The results from these
 2983 cross-sectional studies on asthma supports the findings from the longitudinal studies reviewed above.

2984 **3.1.3.2. Animal studies**

2985 For the HoC Immunotoxicity a total of 42 studies was appraised by the CEP Panel. The details of the
 2986 appraisals (internal and external validity) are reported in Annex E.

2987 The endpoints for each study identified as relevant are reported in Annex F. These include also the
 2988 endpoints identified as key in the uncertainty analysis in the 2015 opinion (EFSA CEF Panel, 2015,
 2989 Section 4.3.2), except for 'inflammation of the uterus' (pyometra and macrophage infiltration) and
 2990 serum parameters (histamin and β -hexosamidase) for which no new data were available in the current
 2991 assessment. For more details see Annex A, Section 2.5.

2992 The key endpoints in the uncertainty analysis in the 2015 EFSA opinion were the following:

- 2993 • Effects on serum parameters (IgE).
- 2994 • Lung effects following intraperitoneal sensitisation and inhalatory challenge to ovalbumin (OVA)
- 2995 (airway hyperreactivity, eosinophils in lavage).

- 2996 • Lung effects following mucosal sensitisation and inhalatory challenge to OVA (inflammation
2997 severity, eosinophils in lavage, neutrophils, lymphocytes and T lymphocytes in lavage).
- 2998 • Lung effects following mucosal sensitisation and inhalatory challenge to OVA and
2999 Lipopolysaccharides (LPS) (inflammation severity, eosinophils in lavage, neutrophils,
3000 lymphocytes and T lymphocytes in lavage).
- 3001 • Lung effects following intraperitoneal sensitisation and inhalatory challenge to OVA and alum
3002 (eosinophils in lavage, neutrophils and lymphocytes in lavage, airway resistance, airway
3003 elastance).

3004 *Identification of clusters of relevant endpoints*

3005 The immune system is oriented at offering defence to exogenous agents, notably microorganisms, to
3006 the host, and preventing deleterious consequences of such pathogens. It operates by a first line of
3007 defence, also called innate immune system, which comprises mainly phagocytic and non-specific
3008 cytotoxic cells. While killing and degrading the pathogens, antigens are presented by antigen-presenting
3009 (dendritic) cells to the antigen-specific arm of the immune system, i.e. lymphocytes that have specific
3010 receptors on them that recognise the antigens, and while doing so, expand by proliferation, mature
3011 and exert functions such as specific antibody production or specific cytotoxic activity, which terminates
3012 the infection. The adaptive immune response includes actions by T cells (i.e. cell-mediated immunity)
3013 and B cells (i.e. humoral immunity). Pathogen-specific T cells and B cells, after activation, undergo
3014 intensive cell proliferation (i.e. clonal expansion) so there exists a large number of cells that can react
3015 to the current threat. Thus, the immune system has the necessary and powerful mechanisms to
3016 eradicate threats but must also be tightly regulated to avoid inappropriate reactions.

3017 While defence depends on damage inflicted on the invading pathogens, collateral damage to the tissues
3018 of the host may also be inflicted. Whereas inflammation is in principle oriented at destroying the
3019 invading pathogen, inflammation may also be adverse to the host as damage to the inflamed tissues
3020 may be a consequence. Also, an overreaction of the specific arm of the immune system may occur, i.e.
3021 immune responses to agents that are not adverse by themselves but are only adverse because of the
3022 consequence of the immune reaction. Such responses are called allergic responses.

3023 Based on the studies available and the nature of the immune system, the relevant immune outcomes
3024 identified in the appraised studies were grouped into five clusters:

- 3025 • Innate immunity
- 3026 • Cellular immunity
- 3027 • Humoral immunity
- 3028 • Inflammation
- 3029 • Allergic lung inflammation.

3030 **Innate immunity**

3031 The endpoints considered relevant for the cluster Innate immunity are the cells of the innate immune
3032 system, i.e. monocytes/macrophages, natural killer cells, antigen-presenting dendritic cells and their
3033 products, such as the antimicrobial molecule lysozyme produced by macrophages. Reduced cell
3034 numbers or activity of the cells may result in reduced resistance to pathogens or reduced induction of
3035 acquired immunity and should be regarded as adverse. The specific endpoints that were included for
3036 the effects on BPA on innate immunity were lysozyme activity, mast cells CysLT, mast cells PGD₂, mast
3037 cell TNF- α , mast cells IL-13, monocytes/macrophages, MHC class II⁺ cells, NK cells, NKT cells and
3038 dendritic cells, G-CSF.

3039

3040 **Cellular immunity**

3041 T cells have a central role in both cell-mediated and humoral immune responses of the adaptive immune
3042 system. T cells, so-called because they are primarily produced in the thymus, aid in the immune
3043 response by recruiting or activating other immune cells (i.e. T helper cells, CD4⁺) or by directly killing

3044 infected cells (i.e. cytotoxic T cells, CD8⁺). They recognise antigens presented by antigen-presenting
3045 cells (APCs) via the surface-expressed T-cell receptor (TCR). In the case of antigens processed by APCs
3046 by the exogenous pathway, CD4⁺ T cells bear antigen that has been processed and expressed in the
3047 groove of the MHC-II molecules, whereas other antigens found within cells, e.g. target cells, are
3048 processed through an endogenous pathway and are delivered by MHC-I to TCR of CD8⁺ cells. Multiple
3049 phenotypes of both CD4⁺ and CD8⁺ T cells have been identified with different functions. CD4⁺ cells
3050 are divided into Th1, Th2, Th9, Th17 and T regulatory (Treg) groups, each with a specific profile of
3051 cytokine production. CD8⁺ T cells are comprised of Tc1 and Tc2 subpopulations with cytokine profiles
3052 similar to Th1 and Th2 cells. The functions of T cells include promotion of inflammation by cytokine
3053 production (Th1 and Th17 cells); helping B cells (Th2 cells); regulation of immunosuppressive responses
3054 (Treg) and killing of unwanted target cells (cytotoxic T lymphocytes). The specific endpoints that were
3055 included for assessing the effects on BPA on cellular immunity were spleen weight, spleen histology,
3056 spleen proliferation, spleen total cell number, T-cell proliferation, Th17 cells, Treg cells, Th1 cells, Th
3057 cells (CD4⁺ cells), Tc cells (CD8⁺ cells), CD25⁺ T cells, IFN- γ (not in lung), IL-4 (not in lung), IL-5 (not
3058 in lung), IL-13 (not in lung), IL-17 (not in lung), IL-21 (not in lung), IL-23 (not in lung). It should be
3059 noted that the spleen contains basically all immune cells, including B cells, T cells, NK cells,
3060 macrophages, etc. Therefore, in reality, effects on the spleen could indicate effects on the cellular
3061 immunity (T cells), humoral immunity (B cells and antibody production) and innate immunity (NK cells,
3062 macrophages).

3063

3064 **Humoral immunity**

3065 Humoral immunity is provided by antigen-specific antibodies. These are produced by plasma cells,
3066 which are differentiated antigen-specific B cells. B cells may recognise antigens, upon which they
3067 proliferate, mature and produce antibodies, initially IgM antibodies. Under regulation by helper T
3068 lymphocytes isotype switching takes place, and instead of IgM, IgG, IgA and IgE may be produced.
3069 IgM, after recognising the antigens on the pathogen to which it is specific, may bind complement and
3070 destroy the pathogen. IgA in mucosa may bind the pathogens they recognise, hence bind the pathogens
3071 to the mucus and subsequently use the mucus as a vehicle to dispose of the microbes. IgE is one of
3072 the isotypes produced and potentially affected by BPA exposure, but typically this antibody is associated
3073 with allergic reactions and is therefore included in the cluster allergic lung inflammation. The specific
3074 endpoints that were included for the effects on BPA on humoral immunity were IgA⁺ cells, IgA, IgM
3075 production and B-cell proliferation of spleen cells (e.g. using LPS or Pokeweed mitogen as mitogen).

3076

3077 **Inflammation**

3078 Inflammation is a broad term referring to the presence of active immune cells at a site of infection,
3079 which is part of the normal process of pathogen destruction. In addition, tissue damage, by the release
3080 of DAMPs (damage-associated molecular patterns), can also trigger inflammation (i.e. sterile
3081 inflammation). Inflammation is a common response to a variety of stressors, including xenobiotics.
3082 Common features of the inflammatory process include tissue resident cell activation, release of pro-
3083 inflammatory mediators and leukocyte recruitment/activation. Hence, xenobiotics can elicit prolonged,
3084 severe and/or inappropriate inflammatory responses that play a causal role in the progression of
3085 biological events resulting in an adverse effect. The specific endpoints that were included for the effects
3086 on BPA on inflammation were IL-1, IL-6, IL-12p70, IL-22, IL-31, TNF- α , VEGF, lung stromal cell-derived
3087 factor 1 (SDF1), neutrophils, eosinophils and colonic inflammation score.

3088

3089 **Allergic lung inflammation**

3090 Allergic lung inflammation may be brought about by serum IgE levels, specific for certain respiratory
3091 allergens. When IgE, bound on the membrane of mast cells through specific IgE receptors, is cross-
3092 linked by antigens on the allergen, the mast cells release various products such as histamine and
3093 serotonin as well as inflammatory mediators. This in turn leads to attraction of inflammatory cells, such
3094 as eosinophils, causing damage in the respiratory tract. Inflammation may ultimately lead to reduced
3095 capacity of the respiratory function of the lungs. Production of IgE is regulated by an array of cytokines,

3096 released from T lymphocytes as well as from other cell types, such as the mast cells or the inflammatory
3097 cells themselves. In addition, the recruitment of inflammatory cells is regulated by pro-inflammatory
3098 mediators, produced by different cell types. The specific endpoints that were included for the effects
3099 on BPA on allergic lung inflammation were lung IL-4, lung IL-13, lung IL-17, lung IL-33, lung TNF- α ,
3100 lung (serum anti-OVA) IgE, lung inflammatory score, lung CysLT, lung KC, lung RANTES, lung cellularity
3101 in bronchoalveolar lavage (BAL) (total cells, macrophages, neutrophils, eosinophils, lymphocytes).

3102 *WoE of the clusters of relevant endpoints*

3103 The main information extracted from the studies addressing relevant endpoints in the HOC
3104 Immunotoxicity are summarised in Annex G. The outcome of the WoE is described in the text below
3105 and presented in a tabulated format in Annex H.

3106 The clusters of the effects of BPA on Immunity considered for the WoE assessment were the following:

- 3107 • Innate immunity
- 3108 • Cellular immunity
- 3109 • Humoral immunity
- 3110 • Inflammation
- 3111 • Allergic lung inflammation.

3112 As a general approach for the WoE and subsequent selection of endpoints to be taken forward for risk
3113 characterisation, it should be noted that not all parameters in a cluster or subcluster need necessarily
3114 to show effects in the same direction, to still prove an effect. This is inherent to the nature of the
3115 immune system which is a regulatory network with checks and balances. Whereas all intermediate
3116 endpoints may shed light on the likelihood of the cluster to be affected by BPA, many of these are not
3117 specific for the eventual adverse outcome and sometimes not even predictive. For this reason, in case
3118 a (sub)cluster identifies a likely effect, apical endpoint parameters within this cluster were taken forward
3119 for BMD analysis rather than these intermediate ones. Intermediate endpoints comprise predominantly
3120 cytokines or other factors mediating communication between different components of the immune
3121 system.

3122

3123 **Innate immunity**

3124 Within this cluster, six studies in mice were identified, of which five studies dealt with exposure during
3125 development and one with exposure during adulthood. Furthermore, four studies in rats were identified,
3126 two of which dealt with exposure during development, three with exposure during development and
3127 adulthood, and one during growth phase/young age. Note that some studies cover different stages of
3128 life (i.e. both exposure and endpoint measurement timing).

3129 Developmental exposure (pre-natal and/or post-natal until weaning)

3130 The evidence that investigated the effect of exposure during development on innate immunity included
3131 seven studies: five in mice, two in rats. Of the mouse studies there were two allocated to Tier 1: Malaisé
3132 et al. (2018) [RefID 11172] and Rogers et al. (2017) [RefID 6257], two to Tier 2: Malaisé et al. (2017)
3133 [RefID 4815] and Patel et al. (2015a) [RefID 5696] and one to Tier 3: Bodin et al. (2014) [RefID 623].
3134 The rat studies were both allocated to Tier 1: NTP Clarity Report (2018)/Camacho et al. (2019) [RefID
3135 11370] and Li J et al. (2018a) [RefID 12460], part of the NTP Clarity study. There are no endpoints in
3136 this cluster showing a consistent effect following BPA exposure during developmental phases of life.
3137 The only parameter that showed a consistent effect was the decrease in the production of lysozyme.
3138 This effect was shown in two papers: Malaisé et al. (2017) [RefID 4815] and Malaisé et al. (2018)
3139 [RefID 11172] in which C3H/HeN male mice in Malaisé et al. (2017) [RefID 4815] and female mice in
3140 Malaisé et al. (2018) [RefID 11172] were exposed to 50 $\mu\text{g}/\text{kg}$ bw per day from gestational day 15 until
3141 weaning. Measurements were done at PND45 and 170 in Malaisé et al. (2017) [RefID 4815] and PND50
3142 in Malaisé et al. (2018) [RefID 11172]. Both papers were from the same scientific group and may in
3143 fact have been provided by one study. In addition, there was only one dose of BPA used.

3144 Therefore, the CEP Panel assigned a likelihood level of ALAN to the innate immunity effects of BPA
3145 during the developmental exposure period, hence none of the endpoints was taken forward for BMD
3146 analysis. However, they were considered in the uncertainty analysis (see Appendix D).

3147 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

3148 Three studies in rats investigated the effect of exposure during development and adulthood on innate
3149 immunity. All were allocated in Tier 1: NTP Clarity Report (2018)/Camacho et al. (2019) [RefID 11370],
3150 Li J et al. (2018a) [RefID 12460] and Li J et al. (2018b) [RefID 12461]. Both Li papers belong to the
3151 NTP Clarity Grantee Studies. No consistent effects were shown. In detail, BPA (0, 2.5, 25, 250, 2500 or
3152 25000 µg/kg bw per day) was administered daily by gavage in 0.3% carboxymethylcellulose vehicle to
3153 NCTR (National Centre for Toxicological Research) Sprague–Dawley rats from GD6 through the start of
3154 parturition and then directly to pups from the day after birth until termination at 1 or 2 years
3155 (continuous-dose group). Overall, no effects were identified. For this reason, the CEP Panel decided
3156 that the effect of BPA on innate immunity during developmental and adult stages is Not Likely, so, none
3157 of the endpoints was taken forward for BMD analysis.

3158 Growth phase/young age exposure

3159 The studies that investigated the effect of exposure during the growth phase on innate immunity
3160 included one study in rats: Ogo et al. (2018) [RefID 11201], which was allocated to Tier 2. This study
3161 evaluated the effect of BPA on macrophages following exposure at young age. In this study, rats were
3162 exposed to 20 µg/kg bw or 200 µg/kg bw per day during PND36–66. No effects of BPA on macrophages
3163 were observed.

3164 As no effects were observed, the CEP Panel assigned a likelihood level of Not Likely to the innate
3165 immunity effects of BPA during the growth phase/young age exposure period, so none of the endpoints
3166 was taken forward for BMD analysis.

3167 Adult exposure (after puberty)

3168 A single Tier 2 study investigated the effect of exposure during adulthood on innate immunity: Cetkovic-
3169 Cyslje et al. (2017) [RefID 916]. In this study, male C57BL/6 mice were orally exposed to 1 or 10 mg
3170 BPA/L (corresponding to 160 and 1600 µg/kg bw per day) starting at 4 weeks of age; diabetes was
3171 induced at 9 weeks of age with streptozotocin (STZ). The innate parameters investigated (macrophages
3172 and natural killer cells) either showed no effect, or some statistically significant differences but without
3173 a clear dose–response relationship. As the study measured different endpoints, the CEP Panel judged
3174 this effect as Not Likely.

3175 The CEP Panel assigned a likelihood level of Not Likely to the innate immunity effects of BPA during the
3176 adult exposure period, so, none of the endpoints was taken forward for BMD analysis.

3177 Indirect (germline) exposure

3178 No studies were available for this exposure period.

3179 Overall cluster selection of the endpoints/studies for BMD analysis for innate immunity

3180 Overall, the CEP Panel assigned a likelihood level of ALAN, Not Likely, Not Likely and Not Likely to innate
3181 immunity effects of BPA in the exposure periods developmental exposure, developmental and adult
3182 exposure, growth phase/young age and adult exposure, respectively. The overall likelihood across all
3183 exposure periods, i.e. the highest likelihood given in the cluster innate immunity, was ALAN.

3184 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
3185 Likely effect of BPA in any exposure period, therefore, none of the endpoints was taken forward for
3186 BMD analysis.

3187

3188 **Cellular immunity**

3189 Within this cluster, 10 studies in mice were identified, of which six studies dealt with exposure during
3190 development, one with exposure during development and adulthood and three during adulthood.
3191 Furthermore, eight studies in rats were identified, five of which dealt with exposure during

3192 development, three with exposure during development and adulthood, and one with exposure during
3193 the adult phase. Note that some studies cover different stages of life.

3194 Developmental exposure (pre-natal and/or post-natal until weaning)

3195 The studies that investigated the effect of exposure during development on cellular immunity included
3196 six studies in mice and five in rats. Of the six studies in mice, three were allocated to Tier 1: Luo et al.
3197 (2016) [RefID 4679], Malaisé et al. (2018) [RefID 11172] and O'Brien et al. (2014a) [RefID 5462] ;
3198 two were allocated in Tier 2: Malaisé et al. (2017) [RefID 4815] and Patel et al. (2015a) [RefID 5696];
3199 and one was allocated in Tier 3: Bodin et al. (2014) [RefID 623]. Of the five studies in rats, four were
3200 allocated in Tier 1: Lejonklou et al. (2017) [RefID 3975], NTP Clarity Report (2018)/Camacho et al.
3201 (2019) [RefID 11370], Dunder et al. (2018) [RefID 11866] and Li AJ et al. (2018) [RefID 12460]; and
3202 one study was allocated to Tier 3: Tarapore et al. (2017) [RefID 7128].

3203 Following developmental exposure to BPA, a consistent dose-related increase in Th17 cells and
3204 associated cytokines (i.e. IL-17, IL-21 and IL-23) was observed at doses as low as 100 nM in drinking
3205 water (equivalent to 4.75 µg/kg bw per day) by Luo et al. (2016) [RefID 4679]. In this study, pregnant
3206 dams were exposed to BPA (10, 100 or 1000 nM) in drinking water from GD0 to PND21 (equivalent to
3207 0.475, 4.75 and 47.5 µg/kg bw per day). Offspring were analysed at PND21 and PND42.

3208 Three other studies support these findings, showing an effect in the same direction: Malaisé et al.
3209 (2017) [RefID 4815] (50 µg/kg bw per day, from GD15 until weaning, males measured at PND45 and
3210 170), Bodin et al. (2014) [RefID 623] (0.1, 1 and 10 mg/L drinking water, equivalent to 30, 300 and
3211 3000 µg/kg bw per day during pregnancy, and 45, 450 and 4500 µg/kg bw per day during lactation,
3212 from mating until weaning, females were investigated at 7 and 11 weeks) and Malaisé et al. (2018)
3213 [RefID 11172] (pregnant mice were orally exposed to BPA 50 µg/kg bw per day from day 15 of
3214 pregnancy until weaning, measures were taken at PND50 only in females). Whereas other parameters,
3215 including IFN-γ, IL-13 and other T-cell subpopulations were not consistently affected (Bodin et al., 2014
3216 [RefID 623]; Malaisé et al., 2017 [RefID 4815]; O'Brien et al., 2014a [RefID 5462]). This should not be
3217 considered as inconsistency, as under cellular immunity many cells with different functions are included.
3218 Th17 cells play a critical role in the induction of the tissue inflammation and tissue destruction, common
3219 to many immune-mediated diseases, like psoriasis, rheumatoid arthritis, multiple sclerosis,
3220 inflammatory bowel disease and asthma.

3221 The CEP Panel assigned a likelihood level of Likely to the cellular immunity effect of BPA during the
3222 developmental exposure period. Since the likelihood level for this cluster is Likely for Th17 cells (Luo et
3223 al., 2016 [RefID 4679]), this endpoint was taken forward for BMD analysis (see Chapter 3.2.1) and
3224 uncertainty analysis (see Appendix D). The endpoints IL-17, IL-21 and IL-23, also Likely, were not
3225 taken forward for BMD analysis because these parameters can be triggered by different stimuli,
3226 including physiological stimuli, and are not considered very close to the apical endpoint (allergic lung
3227 inflammation).

3228 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

3229 The studies that investigated the effect of exposure during development and adulthood on cellular
3230 immunity included three studies in rats and one in mice. The studies in rats, Li J et al. (2018b) [RefID
3231 12461], Li AJ et al. (2018) [RefID 12460] and NTP Clarity Report (2018)/Camacho et al. (2019) [RefID
3232 11370], were allocated to Tier 1. The mouse study, Gear and Belcher (2017) [RefID 2230], was
3233 allocated to Tier 3.

3234 Following developmental and adult exposure, no consistent effects were observed. Some effects were
3235 identified, while most of these alterations were found to be transient and not dose dependent (note:
3236 Th17 cells were not evaluated in this exposure period).

3237 As some effects were identified in one study only, while most of these alterations were found to be
3238 transient and not dose dependent, the CEP Panel assigned a likelihood level of Not Likely to the cellular
3239 immunity effects of BPA during the developmental and adult exposure period, so, none of the endpoints
3240 was taken forward for BMD analysis.

3241 Growth phase/young age exposure

3242 No studies were available for this exposure period.

3243 Adult exposure (after puberty)

3244 The studies that investigated the effect of exposure during adulthood on cellular immunity included
3245 three studies in mice and one in rats. Of the studies in mice, two were allocated to Tier 1: Dong et al.
3246 (2013) [RefID 1676] and DeLuca et al. (2018) [RefID 11805], and one in Tier 2: Cetkovic-Cyrlje et al.
3247 (2017) [RefID 916]. The study in rats is allocated to Tier 3: Özaydin et al. (2018a) [RefID 12853].

3248 Overall, no consistent effects were observed following adult exposure, and the CEP Panel assigned a
3249 likelihood level of Not Likely to the cellular immunity effects of BPA during adult exposure period, so,
3250 none of the endpoints was taken forward for BMD analysis.

3251 Indirect (germline) exposure

3252 No studies were available for this exposure period.

3253 Overall cluster selection of the endpoints/studies for BMD analysis for cellular immunity

3254 Overall, the CEP Panel assigned a likelihood level of Likely to cellular immunity effects of BPA in the
3255 exposure period developmental exposure, and Not Likely to developmental and adult and to adult only
3256 exposure. The overall likelihood across all exposure periods, i.e. the highest likelihood given in the
3257 cluster cellular immunity, was Likely.

3258 The CEP Panel considered that the evidence from the studies available showed a Likely effect of BPA
3259 during the developmental exposure period for the endpoint Th17 cells (Luo et al., 2016 [RefID 4679]),
3260 therefore, this endpoint was taken forward for BMD analysis (see Chapter 3.2.1).

3261

3262 Humoral Immunity

3263 In the cluster Humoral Immunity only two studies were included. One study, part of the NTP CLARITY
3264 study, Li J et al. (2018b) [RefID 12461], investigated the effects of BPA exposure during development
3265 in rats. A second study, Malaisé et al. (2018) [RefID 11172], investigated the effects of BPA during
3266 development and adulthood in mice.

3267 Developmental exposure (pre-natal and/or post-natal until weaning)

3268 The effect of BPA exposure on humoral immunity following developmental exposure was investigated
3269 in one study in mice (Malaisé et al. (2018) [RefID 11172]). For local IgA parameters the study was
3270 allocated to Tier 3; for systemic IgA parameters, the study was allocated to Tier 1. Pregnant mice were
3271 exposed from GD15 until weaning to BPA 50 µg/kg bw per day. The IgA endpoints were investigated
3272 in female mice at PND50 on which a consistent decrease was noted. However, the endpoints were all
3273 in one study only and are not independent from each other. In addition, only one dose was applied in
3274 this study.

3275 The CEP Panel assigned a likelihood level of ALAN to the humoral immunity effects of BPA during the
3276 developmental exposure period, so, none of the endpoints was taken forward for BMD analysis.
3277 However, they were considered in the uncertainty analysis (see Appendix D).

3278 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

3279 The effect of BPA exposure on humoral immunity following development and adulthood exposure was
3280 investigated in one study in rats, Li J et al. (2018b) [RefID 12461], allocated to Tier 1. Following
3281 developmental and adult exposure animals were dosed by oral gavage with BPA (2.5, 25, 250, 2500 or
3282 25000 µg/kg bw per day) and were euthanised on PND21, 90, 6 months and 1 year. No consistent
3283 effects on LPS-induced proliferation were observed: an increase was observed in females at 2500 µg/kg
3284 bw per day at PND90, while a decrease was observed in males at 2.5 µg/kg bw per day, and no effects
3285 at 6 months and 1 year. The effects on LPS-induced proliferation were transient with no dose–response.
3286 Hence, this effect was judged as Not Likely.

3287 In addition, statistically significantly different values of IgM production were found, but inconsistent
3288 between the sexes, and without a clear dose–response. The CEP Panel considered these as chance
3289 findings and decided the study did not show a likely effect neither for IgM production nor cell
3290 proliferation.

3291 The CEP Panel assigned a likelihood level of Not Likely to the humoral immunity effects of BPA in the
3292 developmental and adult exposure period, so, none of the endpoints was taken forward for BMD
3293 analysis.

3294 Growth phase/young age

3295 No studies were available for this exposure period.

3296 Adult exposure (after puberty)

3297 No studies were available for this exposure period.

3298 Indirect (germline) exposure

3299 No studies were available for this exposure period.

3300 Overall cluster selection of the endpoints/studies for BMD analysis for humoral immunity

3301 Overall, the CEP Panel assigned a likelihood level of ALAN and Not Likely to humoral immunity effects
3302 of BPA in the exposure periods developmental exposure and developmental and adult exposure,
3303 respectively. The overall likelihood across all exposure periods, i.e. the highest likelihood given in the
3304 cluster humoral immunity was ALAN.

3305 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
3306 Likely effect of BPA in any exposure period, therefore, none of the endpoints was taken forward for
3307 BMD analysis.

3308

3309 **Inflammation**

3310 In this cluster, following developmental exposure to BPA a total of five studies (four in mice and one in
3311 rats) were allocated; two studies in rats following developmental and adult exposure, one study
3312 following exposure during the growth phase and four studies (two in mice, one in rabbits and one in
3313 rats) following adult exposure.

3314 Developmental exposure (pre-natal and/or post-natal until weaning)

3315 The studies that investigated the effect of exposure during development on inflammation included five
3316 studies, four in mice and one in rats. Of the four studies in mice, two were allocated to Tier 1: Luo et
3317 al. (2014) [RefID 4660] and Luo et al. (2016) [RefID 4679], one paper was allocated to Tier 2: Malaisé
3318 et al. (2017) [RefID 4815] and one study was allocated to Tier 3: Bodin et al. (2014) [RefID 623]. The
3319 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) was allocated to Tier 1. These
3320 five studies covered the parameters 'numbers of neutrophils', 'numbers of eosinophils' as well as
3321 'expression of several interleukins' (IL-1, IL-6, IL-22, TNF- α). Effects were reported at doses as low as
3322 0.34 $\mu\text{g}/\text{kg}$ bw per day in Luo et al. (2016) [RefID 4679] and 50 $\mu\text{g}/\text{kg}$ bw per day in Malaisé et al.
3323 (2017) [RefID 4815], but the effects were not seen in all the studies, were not dose related, and some
3324 studies only included one dose (Malaisé et al., 2017 [RefID 4815]; Luo et al., 2014 [RefID 4660]).

3325 As there is insufficient evidence to support an effect, having only a trend in a decrease in neutrophils,
3326 the CEP Panel assigned a likelihood level of Not Likely to the inflammation adverse effects of BPA during
3327 the developmental exposure period, so, none of the endpoints was taken forward for BMD analysis.

3328 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

3329 The studies that investigated the effect of exposure during development and adulthood on inflammation
3330 were two studies in rats: NTP Clarity Report (2018)/Camacho et al. (2019) [RefID 11370] and Ben-
3331 Jonathan et al. (2018) (NTP Grantee study) [RefID 13786], both allocated to Tier 1. In both studies,
3332 BPA (0, 2.5, 25, 250, 2500, and 25000 $\mu\text{g}/\text{kg}$ bw per day) was administered daily by gavage in 0.3%
3333 carboxymethylcellulose vehicle to NCTR Sprague–Dawley rats from GD6 through the start of parturition
3334 and then directly to pups from the day after birth until termination at one or 2 years (continuous-dose
3335 group).

3336 No effects on neutrophils were found in the CLARITY Core Study NTP Clarity Report (2018)/Camacho
3337 et al. (2019) [RefID 11370]. In the same study, a decrease in blood eosinophils at 250 $\mu\text{g}/\text{kg}$ bw per
3338 day was found, both in males and females, in the continuous-dose group at interim sacrifice, without a

3339 dose–response; no effects were observed at other time points. In the other study, Ben-Jonathan et al.
3340 (2018) (NTP Grantee study) [RefID 13786], no change in IL-6 was observed.

3341 The CEP Panel assigned a likelihood level of Not Likely to the inflammation effects of BPA during the
3342 developmental and adult exposure period, so, none of the endpoints was taken forward for BMD
3343 analysis.

3344 Growth phase/young age exposure

3345 One study in rats, Ogo et al. (2018) [RefID 11201], that was allocated to Tier 1 investigated the effect
3346 of exposure during young age on inflammation. An effect was noted on inflammation at the tissue level
3347 in the epididymis in a single rat study, characterised by an increase in IL-6 and neutrophils, following
3348 exposure during the growth phase to 20 µg/kg bw or 200 µg/kg bw per day, at PND36–66.

3349 The CEP Panel assigned a likelihood level of Likely to the inflammation effects of BPA in the growth
3350 phase/young age exposure period. Since the likelihood level for this cluster is Likely for the endpoint
3351 neutrophils in epididymis (Ogo et al., 2018 [RefID 11201]), this was taken forward for BMD analysis
3352 (see Chapter 3.2.1). As the effect on endpoint IL-6 in epididymis, also likely, can be triggered by
3353 different stimuli including physiological stimuli and it is not considered very close to the apical endpoint
3354 (inflammation), it was decided not to use this endpoint for BMD analysis.

3355 Adult exposure (after puberty)

3356 The studies that investigated the effect of exposure during adulthood on inflammation included two
3357 studies in mice, one study in rabbits and one in rats. Both studies in mice were allocated to Tier 2:
3358 Cetkovic-Cyrlje et al. (2017) [RefID 916], DeLuca et al. (2018) [RefID 11805]. The study in rabbits,
3359 Fang et al. (2014) [RefID 1914], was allocated to Tier 1. The study in rats, Özyaydin et al. (2018a)
3360 [RefID 12853], was allocated to Tier 2. The four studies after adult exposure covered the parameters
3361 IL-1 α , IL-6, IL-12p70, IL-31, SDF-1 α , TNF- α , VEGF, as well as the colon inflammation score. The only
3362 dose–response reported is in Özyaydin et al. (2018a) [RefID 12853], in which a dose-related increase in
3363 IL-6 was observed in male rats exposed to 50 and 500 µg/kg bw per day. In addition, in the adult
3364 exposure period, there is inconsistency between different species, models or strains used.

3365 Due to insufficient evidence, studies performed only at one dose and Tier 2 studies showing no effects,
3366 the CEP Panel assigned a likelihood level of Not Likely to the inflammation effects of BPA during the
3367 adult exposure period, so, none of the endpoints was taken forward for BMD analysis.

3368 Indirect (germline) exposure

3369 No studies were available in this exposure period.

3370 Overall cluster selection of endpoints/studies for BMD analysis for Inflammation

3371 Overall, the CEP Panel assigned a likelihood level of Not Likely, Not Likely, Likely and Not Likely to
3372 inflammation effects of BPA in the exposure periods developmental exposure, developmental and adult
3373 exposure, growth phase/young age exposure and adult exposure, respectively.

3374 The CEP Panel considered that the evidence from the studies available showed a Likely effect of BPA
3375 in the exposure period growth phase/young age for the endpoint neutrophils in epididymis (Ogo et al.,
3376 2018 [RefID 11201]), therefore, this endpoint was taken forward for BMD analysis (see Chapter 3.2.1).

3377 The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster
3378 inflammation, was Likely.

3379

3380 **Allergic lung inflammation**

3381 The cluster allergic lung inflammation included four studies in mice, of which two dealt with exposure
3382 during development, and one with exposure during adulthood.

3383 Developmental exposure (pre-natal and/or post-natal until weaning)

3384 The studies that investigated the effect of exposure during development on allergic lung inflammation
3385 included two studies in mice allocated to Tier 2: O'Brien et al. (2014a) [RefID 5462] and O'Brien et al.
3386 (2014b) [RefID 5463].

3387 In the study of O'Brien et al. (2014b) [RefID 5463], mice were dosed at 0.05 µg, 50 µg, 50000 µg/kg
3388 diet (equivalent to 0.0075 µg, 7.5 µg, 7500 µg/kg bw per day), from 2 weeks before mating until
3389 PND21. Animals were tested at 6 months. The study investigated effects on mast cell mediators (CysLT,
3390 IL-13, PGD2 and TNF-α). The CEP Panel judged effects on CysLT, IL-13 and TNF-α as ALAN as all values
3391 were significantly increased, even if there was not a clear dose–response. For PGD2, there was instead
3392 a clear dose-related increase, hence this effect was judged as Likely. As this effect can be triggered by
3393 different stimuli including physiological stimuli, and it is not considered very close to the apical endpoint
3394 (allergic lung inflammation), it was decided not to use this endpoint for BMD analysis.

3395 In the study O'Brien et al. (2014a) [RefID 5462], mice were dosed at 0.05 µg, 50 µg, 50000 µg/kg diet
3396 (equivalent to 0.0075 µg, 7.5 µg, 7500 µg/kg bw per day) 1–2 weeks before mating until PND21.
3397 Animals were tested at 3 months (12 weeks). Ovalbumin-specific IgE was dose-relatedly increased in
3398 both sexes. This effect was therefore judged as Very Likely and the CEP Panel decided to take this
3399 effect forward for BMD analysis (see Chapter 3.2.1).

3400 In the same study, lung inflammatory parameters were investigated, i.e. lung cellularity, IL-13, CysLT,
3401 IL-17, IL-4, lung inflammatory score, RANTES and TNF-α. Decreased CysLT and IL-17 were considered
3402 by the CEP Panel as Likely effects, observed in both sexes and with the same dose–responses. Since
3403 this parameter can be triggered by different stimuli including physiological stimuli and it is not
3404 considered very close to the apical endpoint of allergic lung inflammation, it was decided not to use
3405 this endpoint for BMD analysis.

3406 Lung cellularity, IL-13, IL-4, TNF-α and lung inflammation score were judged as ALAN, as these
3407 parameters were either affected in one sex only or there was no clear dose–response.

3408 The CEP Panel considered the effects on RANTES as Not Likely, as the only changes were observed at
3409 the lowest dose, in females only.

3410 The CEP Panel assigned a likelihood level of Likely to the allergic lung inflammation effects of BPA
3411 during the developmental exposure period. Since the likelihood level for this allergic lung inflammation
3412 is Very Likely for the endpoint ovalbumin-specific IgE (O'Brien et al., 2014a [RefID 5462]) this was
3413 taken forward for BMD analysis (see Chapter 3.2.1). The endpoints mast cell PGD2, lung CysLT and
3414 lung IL-17, also judged as likely, were not taken forward for BMD analysis because these endpoints can
3415 be triggered by different stimuli, including physiological stimuli. In addition, these endpoints are not
3416 considered very close to the apical endpoint (allergic lung inflammation). However, they will be
3417 considered in the uncertainty analysis (see Appendix D).

3418 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

3419 No studies reporting exposure pre-natally and post-natally in pups until adulthood were available.

3420 Growth phase/young age exposure

3421 No studies were available in this exposure period.

3422 Adult exposure (after puberty)

3423 There was one study that investigated the effect of exposure during adulthood on allergic lung
3424 inflammation, which was allocated to Tier 1, Tajiki-Nishino et al. (2018) [RefID 13221]. In this study,
3425 IL-4, IL-33 and eosinophils in the BAL fluid were measured. A dose-dependent increment in IL-4 and
3426 IL-33 was observed in the lung of mice sensitised and challenged to toluene diisocyanate and exposed
3427 to BPA at 60 and 200 µg/kg bw per day, which were judged as Likely effects.

3428 The CEP Panel assigned a likelihood level of Likely to the allergic lung inflammation effects of BPA
3429 during the adult exposure period. Since the likelihood level for this allergic lung inflammation is Likely
3430 for eosinophils in the bronchioalveolar lavage and that this endpoint represents a very close to an
3431 adverse apical endpoint, this effect was taken forward for BMD analysis (see Chapter 3.2.1). The
3432 cytokine endpoints were also assigned Very Likely or Likely but were not taken forward for BMD analysis
3433 because these endpoints can be triggered by different stimuli, including physiological stimuli. In
3434 addition, these endpoints are not considered very close to the apical endpoint (allergic lung
3435 inflammation). However, they will be considered in the uncertainty analysis (see Appendix D).

3436 Indirect germline exposure

3437 No studies were available in this exposure period.

3438 Overall cluster selection of the endpoints/studies for BMD analysis for allergic lung inflammation

3439 Overall, the CEP Panel assigned a likelihood level of Likely to allergic lung inflammation effects of BPA
 3440 in the exposure periods developmental exposure and adult exposure. The overall likelihood across all
 3441 exposure periods, i.e. the highest likelihood given in the cluster allergic lung inflammation, was Likely.

3442 The CEP Panel considered that the evidence from the studies available showed a Very Likely effect of
 3443 BPA in the developmental exposure period for the endpoint serum OVA-specific IgE (O'Brien et al.,
 3444 2014a [RefID 5462]) and a Likely effect of BPA in the adult exposure period for the endpoint eosinophils
 3445 in the BAL (Tajiki-Nishino et al., 2018 [RefID 13221]), therefore, these endpoints were taken forward
 3446 for BMD analysis (see Chapter 3.2.1).

3447 3.1.3.3. Integration of likelihoods from human and animal studies

3448 Table 9 presents the overall likelihood per cluster for the human and animal stream separately, as well
 3449 as the integration of the likelihoods from the human and animal studies for Immunotoxicity.

3450 **Table 9:** Integration of the human and animal studies 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	
<i>Overall likelihood: ALAN</i>	<i>Overall likelihood: Likely</i>	<i>Likely</i>
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	
	<i>Overall likelihood: Likely</i>	<i>Likely</i>
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	
	<i>Overall likelihood: ALAN</i>	<i>ALAN</i>
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	
	<i>Overall likelihood</i>	<i>ALAN</i>	<i>ALAN</i>
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	
	<i>Overall likelihood</i>	<i>Likely</i>	<i>Likely</i>

3451

3452 **3.1.3.4. *In vitro* and mechanistic studies**3453 *Cellular immunity*

3454 The mechanisms along which BPA influences the immune system are largely unknown, and this applies
3455 to all components of the immune system, including acquired cellular immunity.

3456 Since there was no direct human evidence available for the cluster cellular immunity, the overall
3457 likelihood of effects of BPA for this cluster was scored based on the animal evidence. As indicated in
3458 the WoE section, the CEP Panel considered that the evidence from the studies available indicated a
3459 Likely effect of BPA on cellular immunity during the developmental exposure period (see Chapter
3460 3.1.3.2).

3461 Exposure to low-dose BPA (4.75 µg/kg bw per day) during gestation and lactation led to a sustained,
3462 sex-specific and dose-dependent increase in Th17 cells in the offspring mice mediated by specific
3463 alteration of their transcription factor and regulatory cytokines IL-17, IL-21 and IL-23 (Luo et al., 2016
3464 [RefID 4679]). The study by Malaisé et al. (2018) [RefID 11172] showed deficient dendritic cell
3465 maturation in the lamina propria in the intestine and spleen after exposure to 50 µg/kg bw per day
3466 during gestational and lactating phases, which may be the basis for the effects on regulatory T cells in
3467 the lamina propria and subsequent effects on the Th17 cells systemically. Also, direct effects of BPA on
3468 lymphocytes may play a role, as Cipelli et al. (2014) [RefID 1252] reported effects of *in vitro* exposure
3469 at 100 nM BPA on proliferation and the ATP content of human leukaemia T-cell lymphoblasts (Jurkat
3470 cells), in which oestrogen receptor 2 (ER2) and oestrogen-related receptor-α (ERRA) appeared to be
3471 involved.

3472 Whereas *in vitro* studies included BPA concentrations of 100 nM, it should be mentioned that these
3473 concentrations may still be quite high compared with the actual internal exposure in humans and
3474 mechanisms identified by *in vitro* studies may therefore not necessarily all be operational.

3475 *Humoral Immunity*

3476 There was no direct human evidence available for the cluster humoral immunity. Thus, the overall
3477 likelihood of effects of BPA for this cluster was scored based on the animal evidence. The CEP Panel
3478 considered that the evidence from the animal studies available did not show a Likely effect of BPA on
3479 humoral immunity in any exposure period. Only during development, an effect of BPA exposure on IgA
3480 production was judged as Likely, but this was found in only one study and not supported by other
3481 parameters, hence the CEP Panel considered this effect as ALAN. It should be noted that reagents (i.e.
3482 antibodies involved in allergic reactions) were not included in this cluster but are dealt with in the cluster
3483 allergic lung inflammation, as IgE is a crucial component of allergic lung inflammation. Obviously, IgE
3484 is a component of humoral immunity, under strict regulation of T cells, hence under regulation of the
3485 cellular immune system. In the current section, the humoral immune aspects other than IgE and IgG1
3486 are being dealt with.

3487 It should be noted that like IgE, IgA is also regulated by cellular immune components and even if the
3488 effect on IgA was not sufficiently convincing to justify a judgement Likely for the cluster, effects on
3489 both IgE (dealt with under allergic lung inflammation) and IgA do indicate dysregulation of humoral
3490 immunity. An effect of 50 µg/kg bw per day of BPA on IgA may be associated with influences of BPA
3491 on the intestinal barrier functions, as suggested by Malaisé et al. (2018) [RefID 11172].

3492 Mechanisms underlying humoral immunity were studied in humans by Chailurkit et al. (2016) [RefID
3493 924], who investigated associations of BPA exposure with autoantibodies (antithyroglobulin,
3494 antithyropoxidase and antithyrotrophin receptor) and found a significant trend for an association of
3495 antithyroglobulin and antithyropoxidase with BPA exposure. These autoantibodies again are under
3496 regulation of cellular immunity. In addition, their findings may lend further support to an effect on
3497 humoral immunity, brought about by suppression of T-cell regulation and subsequent enhancement of
3498 Th-17 mechanisms, leading to enhanced responses as indicated in the section on cellular immunity.

3499 *Allergic lung inflammation*

3500 After the integration of the human and animal evidence, the overall likelihood of effects of BPA for the
3501 cluster allergic lung inflammation was scored Likely (see Chapter 3.1.3.2).

3502 The CEP Panel considered that the evidence from the animal studies available showed a Very Likely
3503 effect of BPA in the developmental exposure period for the endpoint serum OVA-specific IgE and a
3504 Likely effect of BPA in the adult exposure period for the endpoint eosinophils in the BAL. Even if effects
3505 of exposure in the cluster humoral immunity were judged as ALAN, especially after exposure during
3506 development, these effects are in line with the judgement on effects on allergic lung inflammation, that
3507 are especially evident after exposure during development. This supports the susceptibility of the
3508 developing immune system, even if in this current cluster of allergic lung inflammation, also effects
3509 were noted after exposure to BPA during adulthood. A study performed after intratracheal instillation
3510 of BPA, Koike et al. (2018) [RefID 12355], found an exacerbation of ovalbumin-induced lung
3511 inflammation and specific IgE responses by of BPA exposure, by enhancing Th2 responses via disruption
3512 of the immune system, further supporting this finding. A possible hypothesis for allergic lung
3513 inflammation was put forward by Tajiki-Nishino et al. (2018) [RefID 13221], who suggested that
3514 bronchial epithelial cells and TSLP may activate APCs resulting in stimulation of Th cells and subsequent
3515 exacerbation of local cytokine level, found after exposure to 0.06 mg/kg bw per day at adulthood and
3516 IgE production. Cross-linking of IgE on mast cells triggers these mast cells to release their mediators.
3517 O'Brien et al. (2014b) [RefID 5463] showed that perinatal BPA exposure to doses from 7.5 ng/kg bw
3518 per day displayed a long-term influence on mast cell-mediated production of pro-inflammatory
3519 mediators associated with asthma and global DNA methylation levels, supporting the role for mast cells
3520 in pulmonary inflammation associated with allergic airway disease into adulthood.

3521 In addition to regulatory effects on IgE production by mediators from bronchial epithelium, also direct
3522 inflammatory effects of mediators from bronchial fibroblasts may play a role in an eventual lung
3523 inflammation. Mahemuti et al. (2016) [RefID 4784] exposed human fetal lung fibroblasts (HFLF) to 100
3524 µM BPA *in vitro* and observed that BPA affects the release of growth differentiation factor-15 (GDF15),
3525 ET-1, interleukin-6 (IL-6) and interferon γ-induced protein 10 (IP-10), as well as phosphorylation of
3526 nuclear factor kappa B (NF-κB) p65. The effects were reported at concentrations higher than the cut-
3527 off for inclusion of these studies and may not be relevant for the human situation.

3528 Throughout the studies of effects of BPA on the immune system, there seems to be an age dependency,
3529 i.e. a more pronounced effect after exposure during development compared with effects of exposure
3530 during later stages in life. This is in accordance with findings by Petzold et al. (2014) [RefID 5811].
3531 These authors observed an asthma-promoting effect after life-long exposure, including during
3532 pregnancy and breast feeding, to 5 µg/mL in drinking water during pregnancy (equivalent to 0.9 µg/kg
3533 bw per day). After BPA exposure of adult mice during sensitisation to ovalbumin, reduced allergic
3534 responses were noted. This reduction could be reverted using the glucocorticoid receptor (GR)
3535 antagonist RU486.

3536 *Innate immunity*

3537 There was no direct human evidence available for the cluster innate immunity. Thus, the overall
3538 likelihood of effects of BPA for this cluster was scored based on the animal evidence. The CEP Panel
3539 considered that the evidence from the animal studies available showed overall ALAN effects.

3540 A reduction in the functional parameter lysozyme was shown by Malaisé et al. (2017) [RefID 4815] and
3541 Malaisé et al. (2018) [RefID 11172]. An increase in plasma G-CSF was reported by Rogers et al. (2017)
3542 [RefID 6257] in an experimental model of multiple sclerosis. In that study, the increase in C-CSF was
3543 associated with increased number of circulating neutrophils compared with vehicle treatment. Blocking
3544 C-CSF by a monoclonal antibody resulted in a decreased incidence and severity of Experimental Allergic
3545 Encephalomyelitis. This suggests that the mechanism by which gestational BPA exposure increases risk
3546 for autoimmunity could be through priming macrophages that produce G-CSF after activation and
3547 subsequent mobilisation of neutrophils by G-CSF. Of note, following adult exposure, was the increased
3548 number of antigen-presenting (dendritic) cells observed after intratracheal exposure to BPA by Koike
3549 et al. (2018) [RefID 12355], which may be in line with the effects on allergic lung inflammation.

3550 With respect to *in vitro* studies and the mechanistic understanding that go far beyond the *in vivo* studies
3551 included in this cluster, the effect of BPA on innate immunity was investigated in the most relevant cell
3552 types: macrophages, dendritic cells, neutrophils and mast cells. *In vitro* studies were conducted using
3553 both primary cells from humans and rodents or cell lines (e.g. THP-1, U936). Studies indicate that BPA
3554 can directly act on innate immune cells modulating cytokine production, with results showing increased
3555 pro-inflammatory cytokines and decreased anti-inflammatory cytokines at a concentration of 100 nM
3556 (Couleau et al., 2015 [RefID 1316]), at 10 nM (Liu YZ et al., 2014 [RefID 4533]) and at 100 nM (Chen
3557 Y et al., 2018 [RefID 11728]); decreased phagocytosis at a concentration of 1 nM (Couleau et al., 2015
3558 [RefID 1316]) and of 100 µM (Berntsen et al., 2018 [RefID 11063]). Increased ROS were observed by
3559 Michalowicz et al. (2015) [RefID 5083] but only at concentrations higher than 0.3 µM.

3560 As indicated above, it should be mentioned that the concentrations used for *in vitro* studies may be
3561 quite high compared with the actual internal exposure in humans and mechanisms identified by these
3562 *in vitro* studies may therefore not necessarily all be operational.

3563 Overall, the mechanistic elucidation of the BPA effect was minimal, however, several studies provide
3564 evidence for these effects to be induced by modulation of the extracellular signal-related kinase
3565 (ERK)/NF-κB signalling pathway observed by Herz et al. (2017) [RefID 2698] at a concentration of 1
3566 nM, Couleau et al. (2015) [RefID 1316] and Liu YZ et al. (2014) [RefID 4533] at a concentration of 100
3567 nM and O'Brien et al. (2014c) [RefID 5464] at concentrations from 1 nM, potentially mediated via ERs
3568 (ERα/β and the membrane receptor GPER or GPR30). Furthermore, Michalowicz et al. (2015) [RefID
3569 5083] and Neri et al. (2015) [RefID 5363] suggest that BPA is capable of damaging innate immune
3570 cells through oxidative stress and DNA damage leading to apoptosis and necrosis at concentrations
3571 ranging from 6.57 nM to 0.3 µM. Finally, O'Brien et al. (2014c) [RefID 5464] studied the effects of BPA
3572 *in vitro*, demonstrating that BPA could enhance mast cell release at concentrations of 100 nM and that
3573 this could be mediated partly through the ERK pathway and extracellular Ca²⁺ concentrations, but not
3574 dependent on an ER-mediated mechanism. This latest study supports the role for mast cells in
3575 pulmonary inflammation associated with allergic airway diseases (O'Brien et al., 2014b [RefID 5463];
3576 Petzold et al., 2014 [RefID 5811]; Koike et al., 2018 [RefID 12355]; Tajiki-Nishino et al., 2018 [RefID
3577 13221]).

3578 Liao et al. (2016) [RefID 4266], using a human birth cohort, investigated the effect of pre-natal
3579 exposure to BPA on TLR-induced cytokine responses in neonates. Production of TNF-α, IL-6 and IL-10
3580 was evaluated after stimulating mononuclear cells with TLR ligands (TLR1–4 and TLR7/8). Although
3581 the study did not yield a statistically significant result for the epidemiological risk of infection during
3582 early infancy, they report an association between cord blood BPA concentration and suppressed TLR3-
3583 stimulated and TLR4-stimulated TNF-α response and TLR7/8-stimulated IL-6 response. Overall, the
3584 studies on innate immunity cells support *in vivo* evidence indicating an immune de-regulation and
3585 possibly increased susceptibility to develop inflammatory reactions.

3586 *Inflammation*

3587 There was no human evidence available for the cluster inflammation. Thus, the overall likelihood of
3588 effects of BPA for this cluster was scored based on the animal evidence. The CEP Panel considered that
3589 the evidence from the animal studies available showed overall Likely effects.

3590 Some endpoints included in this cluster, e.g. pro-inflammatory cytokines, have been discussed in the
3591 previous Chapter, as produced by innate immune cells.

3592 Effects following growth phase/young age were shown in the epididymis by Ogo et al. (2018) [RefID
3593 11201] after exposure from 20 µg/kg bw per day, as a clear indication of increased IL-6 and neutrophils.

3594 Regarding cytokine production, overall the findings support an increase in the production of pro-
3595 inflammatory cytokines, but with inconsistent results or results obtained in a single-dose study. At
3596 adulthood, an increase in IL-6 was observed in two studies from 50 µg/kg bw per day (Özaydin et al.,
3597 2018a [RefID 12853]; Fang et al., 2014 [RefID 1914]), with no effect in another study (Cetkovic-Cvrlje
3598 et al., 2017 [RefID 916]). Increased TNF-α was found in two studies (Özaydin et al., 2018a [RefID
3599 12853]; Fang et al., 2014 [RefID 1914]), while a decrease was observed in Cetkovic-Cvrlje et al. (2017)
3600 [RefID 916] after exposure to 1.6 mg/kg bw per day. Increases in IL-1a, IL-12p70, IL-31, VEGF were
3601 reported in a single-dose study of 50 µg/kg bw per day (DeLuca et al., 2018 [RefID 11805]) and in
3602 SDF1a at 0.084 ng/kg bw per day in Koike et al. (2018) [RefID 12355]. The increase in pro-inflammatory
3603 cytokines is consistent with *in vitro* results, as also described in the previous section.

3604 Two mechanistic studies in humans reported inflammation markers in Asian populations. Song et al.
3605 (2017) [RefID 6815] supplemented an *in vitro* study with evidence from epidemiological studies and
3606 investigated the association between urinary BPA levels and well known inflammation-related markers
3607 including WBC, CRP, IL-10, ALT, AST and γ-GTP. Significant positive associations between BPA level
3608 and WBC, ALT and γ-GTP levels were found. Yang et al. (2016) [RefID 8375] also supplemented their
3609 *in vivo* and *in vitro* investigations with evidence from epidemiological studies on ALT, γ-GT and high-
3610 sensitivity C-reactive protein (hs-CRP), leptin and TNF-α. BPA was associated with inflammation markers
3611 (leptin, TNF-α) in lean subjects but not in overweight/obese subjects and stratified analyses suggested
3612 a possible attenuation by sex and BMI.

3613 In addition, there are studies indicating modulation of signalling pathways at concentrations equal to
3614 or below 100 nM (e.g. ERK, JNK, NF-κB) by Couleau et al. (2015) [RefID 1316] at 100 nM, Liu YZ et al.
3615 (2014) [RefID 4533] at 10 nM, Song et al. (2017) [RefID 6815] at 100 nM and Zhu et al. (2015) [RefID
3616 9100] at 10 nM; modulation of cytokine gene expression and secretion by Camarca et al. (2016) [RefID
3617 805] at 0.1 nM, Couleau et al. (2015) [RefID 1316] at 100 nM, Liu YZ et al. (2014) [RefID 4533] at 10
3618 nM, Zhu et al. (2015) [RefID 9100], Chen Y et al. (2018) [RefID 11728] at 100 nM, Li Q et al. (2018)
3619 [RefID 12475] at 8.76 nM, Tajiki-Nishino et al. (2018) [RefID 13221] at 1 nM; modulation of histone
3620 methylation by Li Q et al. (2018) [RefID 12475] at 8.76 nM and modulation of ER signalling pathways,
3621 where effects were reversed by ERa/b antagonists by Couleau et al. (2015) [RefID 1316] at 100 nM,
3622 Liu YZ et al. (2014) [RefID 4533] at 10 nM, Chen Y et al. (2018) [RefID 11728] at 100 nM. In contrast
3623 to the latter findings, Chakhtoura et al. (2017) [RefID 927] found no effects on cytokine expression
3624 and no influence on dendritic cell maturation at concentrations as low as 0.05 nM, while the positive
3625 control treatment at the same concentration with β-E2 did.

3626 Again, whereas *in vitro* studies included BPA concentrations of 100 nM, it should be mentioned that
3627 these concentrations may still be quite high compared with the actual internal exposure in humans and
3628 mechanisms identified may therefore not necessarily all be operational. Nevertheless, taking all results
3629 together, many of the studies reviewed highlighted a possible role of BPA in inflammatory processes.
3630 Modulation of ERK1/2 phosphorylation, NF-κB activation, modulation of the ERs, GR and androgen
3631 (AR), as well as cytokine/chemokine secretion, are relatively common hypothesised mechanisms for
3632 the effects observed.

3633 *Concluding remarks*

3634 Different components of the immune system seem to be affected. Effects may be on non-specific cells
3635 belonging to the immune system or influencing the immune system, such as APCs and epithelial cells,
3636 that through presentation of antigens to T lymphocytes or release of mediators, influence the regulatory
3637 homeostasis of the immune system, suppressing T regulatory cells and stimulating Th17 cells, leading
3638 to enhanced production of IgE. IgE, after being cross-linked at the surface of mast cells, may lead to
3639 the release of inflammatory mediators, that in concert with inflammatory mediators from other cell
3640 types, may lead to inflammatory reactions, including those in the respiratory tract. Such increases in
3641 inflammation have also been observed in the epididymis after exposure to BPA, which may in part
3642 follow similar mechanisms. It is currently not clear how BPA interacts with the various cells comprising

3643 the immune system or cells such as epithelial cells or fibroblasts, of which the mediators influence the
3644 immune system, but a role for GR, ER2 and the ERRA and subsequent activation of transcription factors
3645 may be plausible. The response to BPA may differ according the experimental condition. This refers to
3646 the exposure regimen, i.e. the exposure at different life stages as well as the exposure in relation to
3647 the allergenic challenge. It is likely that such conditions will also have an impact on effects in humans.

3.1.3.5. Conclusion on hazard identification for Immunotoxicity of BPA

3649 In the 2015 EFSA opinion on BPA (EFSA CEF Panel, 2015) it was stated that based on human studies,
3650 there were indications that BPA may be linked to immunological outcomes, although a causal link could
3651 not be established. In addition, studies in animals lent support to the possibility of immune effects.
3652 Effects of concern were especially increase in IgE and allergic lung inflammation, but like the human
3653 studies, also animal studies suffered from shortcomings, for which reason the CEF Panel did not take
3654 these effects forward for the risk characterisation carried out in 2015. In a later statement in 2016,
3655 after evaluating two additional studies of effects of exposure to BPA on the immune system, in which
3656 potential allergic conditions were investigated, the CEF Panel confirmed its position that the studies
3657 available at that time suggested effects on the immune system, but that the studies were not sufficiently
3658 robust to take them forward for risk characterisation (EFSA CEF Panel, 2016).

3659 The available information that has been evaluated in the current evaluation is in line with these earlier
3660 observations and indicates now more firmly that there is a hazard with respect to adverse outcomes of
3661 exposure on the immune system, notably an effect on cellular immunity and parameters indicating
3662 allergic lung inflammation. Even if mechanisms along which the immune system is affected by BPA are
3663 not clear, it is clear from the studies shedding some light on these mechanisms, that effects may be on
3664 non-specific cells, such as APCs and epithelial cells, that through presentation of antigens to T
3665 lymphocytes or release of mediators influence the regulatory homeostasis of the immune system. This
3666 may lead to suppression of T regulatory cells and subsequent stimulation of Th17 cells, leading to
3667 enhanced production of IgE. IgE, after being cross-linked at the surface of mast cells, may lead to the
3668 release of inflammatory mediators, that in concert with inflammatory mediators from other cell types
3669 may lead to inflammatory reactions (see Chapter 3.1.3.4 on mechanisms of immunotoxicity of BPA).
3670 Whereas the parameters investigated mainly comprised intermediate endpoints, such as interleukins,
3671 mast cell mediators and specific antibodies related to allergy, and while indications for inflammation (as
3672 visualised by histology and cellular infiltrations and shown by broncho-alveolar lavage) were noted, the
3673 eventual disease endpoint of altered lung functionality has been investigated in only a few studies.
3674 These seemed to indicate an effect, although were not judged to be of high quality.

3675 In addition to effects in the respiratory tract, an inflammatory effect was also seen in the epididymis
3676 after exposure to BPA, which may be brought about by partly similar mechanisms. Based on the
3677 available information, the effects in the cluster innate immunity were judged as ALAN, but an increased
3678 number of antigen-presenting (dendritic) cells was observed after intratracheal exposure to BPA, which
3679 underscores the effect that BPA can have on the homeostasis of the immune system. It is currently not
3680 clear how BPA interacts with the various cells comprising the immune system or cells such as epithelial
3681 cells or fibroblasts, of which the mediators influence the immune system, but a role for GR, ER2 and
3682 ERRA and subsequent activation of transcription factors may be plausible.

3683 Overall, the CEP Panel considers that a hazard exists for adverse effects of BPA on the immune system,
3684 that may result, depending on the dose, most likely in inflammatory reactions such as in the respiratory
3685 tract. Whereas the developing immune system is generally considered more vulnerable to toxic damage
3686 than the fully mature immune system, effects were noted both after exposure during developmental
3687 stages as well as at adulthood, hence the hazard exists throughout the different life stages.

3688 Using a WoE approach, the CEP Panel assigned a likelihood level of Likely to BPA-induced effects on
3689 the Th17 cells, on the neutrophils in epididymis, on eosinophils in the BAL, and of Very Likely to BPA-
3690 induced effects on serum OVA-specific IgE. Therefore, these endpoints were brought forward for BMD
3691 analysis (see Chapter 3.2.1).

3.1.4. Metabolic effects

3693 3.1.4.1. Epidemiological studies

3694 For the HOC Metabolic, a total of 27 studies was appraised by the CEP Panel. The details of the
3695 appraisals (internal validity) are reported in Annex B.

3696 *Identification of the clusters to be considered for WoE*

3697 On the basis on the approach described in Chapter 2.3.2 'Definition of Health Outcome Categories and
3698 Clusters', the following Clusters (C) and Exposure periods (Exp) were brought forward to WoE analysis:

- 3699 • C: Obesity
 - 3700 – Exp: Adulthood
 - 3701 – Exp: Pregnancy
 - 3702 – Exp: Childhood
- 3703 • C: Cardiometabolic effects
 - 3704 – Exp: Pregnancy
- 3705 • C: Thyroid effects
 - 3706 – Exp: Pregnancy
- 3707 • C: Type 2 diabetes mellitus
 - 3708 – Exp: Adulthood
- 3709 • C: Gestational Diabetes Mellitus
 - 3710 – Exp: Adulthood.

3711 *WoE of the relevant clusters*

3712 The main information extracted from the studies included in relevant clusters in the HOC Metabolic are
3713 summarised in Annex C. The outcome of the WoE is described in the text below and presented in a
3714 tabulated format in Annex D.

3715 **Cluster Obesity**

3716 A large number of obesity-related endpoints was studied in the 13 epidemiological longitudinal studies
3717 assessing different exposure periods and all using spot urine samples. The observed heterogeneity for
3718 population characteristics and endpoint definitions was considerable. The associations under study
3719 reached statistical significance for waist circumference (n = 3) (Valvi et al., 2013 [RefID 7384]; Hoepner
3720 et al., 2016 [RefID 2732]; Hao et al., 2017 [RefID 9400]), central obesity (n = 1) (Hao et al., 2017
3721 [RefID 9400]) and annual weight gain (n = 1) (Song Y et al., 2014 [RefID 6839]). In the text below,
3722 the assessed studies are described in brief. Their detailed description and risk of bias assessment are
3723 provided in Annexes C and D.

3724 Exposure during pregnancy

3725 Eleven publications corresponding to nine cohort studies assessed the association between BPA
3726 exposure measured during pregnancy and obesity-related endpoints with a cumulative sample size of
3727 3,536 participants (Valvi et al., 2013 [RefID 7384]; Ashley-Martin et al., 2014 [RefID 280]; Braun et
3728 al., 2014b [RefID 709]; Buckley et al., 2016 [RefID 748]; Hoepner et al., 2016 [RefID 2732]; Vafeiadi
3729 et al., 2016 [RefID 7368]; Bae et al., 2017 [RefID 350]¹⁵; Perng et al., 2017 [RefID 5792]; Watkins et
3730 al., 2017a [RefID 7875]; Yang TC et al., 2017 [RefID 8408]; Junge et al., 2018 [RefID 12262]). The
3731 populations under study were of small (<500) sample size except for one study that included 1,237
3732 participants (Ashley-Martin et al., 2014 [RefID 280]); three studies studied European populations (Valvi

¹⁵ This study considered as a cross-sectional study in the EFSA supporting publication 'Implementation of the evidence-based risk assessment for the re-evaluation of Bisphenol A: preparatory work on cross-sectional studies' (AERU, University of Hertfordshire, 2020) was recategorized by the EFSA WG on BPA re-evaluation as a cohort study and therefore considered in the WoE assessment.

3733 et al., 2013 [RefID 7384]; Vafeiadi et al., 2016 [RefID 7368]; Junge et al., 2018 [RefID 12262]), while
3734 four studies studied populations in North America (Ashley-Martin et al., 2014 [RefID 280]; Braun et al.,
3735 2014b [RefID 709]; Buckley et al., 2016 [RefID 748]; Hoepner et al., 2016 [RefID 2732]) and one study
3736 was conducted in Mexico (Watkins et al., 2017a [RefID 7875]; Yang TC et al., 2017 [RefID 8408]; Yang
3737 et al., 2018 [RefID 13545]). BPA exposure was measured via spot urine sampling in all eight studies
3738 and was comparable across studies. The assessed endpoints that were related to obesity were BW,
3739 obesity, BMI (n = 6), rapid growth, weight change, waist circumference (n = 2), skinfold thickness, fat
3740 mass (n = 2), leptin and adiponectin. The follow-up of the included studies ranged from 1 to 14 years.
3741 For FMI, % body fat and waist circumference, statistically significant associations were observed in one,
3742 one and two studies, respectively. None of the six studies (8 publications) that assessed BMI reported
3743 statistically significant findings; moreover, all six studies used different follow-up points rendering a
3744 formal synthesis uninformative. Of note, results on BMI and overweight risk coming from the INMA
3745 study that were presented by Valvi et al. (2013) [RefID 7384] were corroborated by Valvi et al. (2013)
3746 [RefID 7384] using a 7-year follow-up. Thus, the currently available longitudinal epidemiological
3747 evidence is characterised by a small number of small studies, suboptimal exposure assessment and
3748 considerable heterogeneity in the assessed endpoints. Moreover, there are no studies to replicate the
3749 observed 'positive' associations; for waist circumference, an endpoint that emerged from the studies
3750 assessing exposure during adulthood, the two available studies that reported statistically significant
3751 results used fundamentally different follow-up points (14 months and 7 years) and their respective
3752 results cannot be consolidated.

3753 On the basis of the above, the CEP Panel concluded that a positive association between exposure to
3754 BPA during pregnancy and obesity is Not Likely.

3755 Exposure during childhood

3756 Three small studies (Lee et al., 2013 [RefID 3911]; Braun et al., 2014b [RefID 709]; Vafeiadi et al.,
3757 2016 [RefID 7368]) in Greece, USA and South Korea investigated the association between BPA exposure
3758 in pregnancy and childhood and obesity-related endpoints. No statistically significant associations were
3759 observed.

3760 On the basis of the above, it is concluded that the evidence for a positive association between BPA
3761 exposure during childhood and obesity is Not Likely.

3762 Exposure during adulthood

3763 Three cohort studies (Song Y et al., 2014 [RefID 6839]; Rönn et al., 2015 [RefID 6277]; Hao et al.,
3764 2017 [RefID 9400]) assessed the association between BPA exposure measured in adulthood and
3765 obesity-related endpoints with a cumulative sample size of 2,759 participants. The populations under
3766 study were of comparable sample size but varied in their characteristics; the one study of a European
3767 population (Rönn et al., 2015 [RefID 6277]) included 70-year-old community dwellers in Sweden. BPA
3768 exposure was measured via a single spot urine sample in two studies, via serum sampling in one study
3769 and exposure levels varied between studies; in the study including individuals from Europe the total
3770 BPA median interquartile range was 3.9 (2.08–6.57) ng/mL for men and 2.1 (1.97–6.51) ng/mL for
3771 women. The assessed endpoints were central obesity, annual weight gain, waist circumference, fat
3772 trunk/leg ratio, subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT),
3773 visceral/subcutaneous adipose tissue ratio (VAT/SAT), fat (leg), fat (trunk) and body fat. The follow-up
3774 of the included studies ranged from 2 to 10 years and no single endpoint was assessed in more than
3775 one study. For the incidence of central obesity, annual weight gain and waist circumference, statistically
3776 significant associations were observed. For the body fat and fat distribution indices, no statistically
3777 significant effects were observed. Thus, the currently available longitudinal epidemiological evidence is
3778 characterised by a small number of studies, suboptimal exposure assessment and considerable
3779 heterogeneity in the assessed populations, exposure levels and endpoints. Moreover, there are no
3780 studies to replicate the observed positive associations between exposure to BPA and obesity.

3781 On the basis of the above, the CEP Panel concluded that a positive association between exposure to
3782 BPA during adulthood and obesity is ALAN.

3783 Overall conclusions

3784 On the basis of the above, the CEP Panel concluded that the evidence for an association between BPA
3785 exposure and obesity is ALAN.

3786

3787 **Cluster Cardiometabolic effects**

3788 Exposure during pregnancy

3789 Three cohort studies (Vafeiadi et al., 2016 [RefID 7368]; Bae et al., 2017 [RefID 350]; Perng et al.,
3790 2017 [RefID 5792]) assessed cardiovascular risk factors related to the metabolic syndrome (total
3791 cholesterol, LDL, HDL, triglycerides, diastolic blood pressure, systolic blood pressure and pulse
3792 pressure). The follow-up of these studies ranged from 4 to 14 years.

3793 One study (Bae et al., 2017 [RefID 350]) reported respectively a statistically significant increase of
3794 diastolic blood pressure and a statistically significant decrease of systolic blood pressure, while no
3795 statistically significant results were found as regards to pulse pressure. The other two studies (Vafeiadi
3796 et al., 2016 [RefID 7368]; Perng et al., 2017 [RefID 5792]) did not find any statistically significant
3797 results related to the measured endpoints (total cholesterol, LDL, HDL, triglycerides, systolic blood
3798 pressure and diastolic blood pressure).

3799 The currently available longitudinal epidemiological evidence is characterised by a small number of
3800 small studies and suboptimal exposure assessment. One study reports two conflicting statistically
3801 significant results on blood pressure, while the other studies do not report any statistically significant
3802 results.

3803 Overall conclusions

3804 On the basis of the above, the CEP Panel concluded that the evidence for a positive association between
3805 BPA exposure and cardiometabolic effects is Not Likely.

3806

3807 **Cluster Thyroid effects**

3808 Three cohort studies assessed the association between BPA exposure measured in spot urine samples
3809 and thyroid hormones' profile using different exposure periods. In the text below, the assessed studies
3810 are described in brief. Their detailed description and risk of bias assessment related to these studies
3811 are provided in Annexes C and D. Of these, one study evaluated BPA exposure during adulthood (Aung
3812 et al., 2017 [RefID 303]) and it was not considered further in the WoE as it represents a single entry
3813 for this cluster. In summary, the limited currently available epidemiological evidence does not put
3814 forward a thyroid-related endpoint as a critical one for risk assessment.

3815 Exposure during pregnancy

3816 Two birth cohort studies in USA (Chevrier et al., 2013 [RefID 1154]; Romano et al., 2015 [RefID 6270])
3817 assessed whether maternal BPA concentrations in spot urine samples during pregnancy were associated
3818 with neonatal (cord blood) TSH (n = 364) or neonatal thyroid hormones (n = 249). No statistically
3819 significant associations were observed.

3820 On the basis of the above, it is concluded that the evidence for a positive association between BPA
3821 exposure during pregnancy and thyroid effects is Not Likely.

3822 Overall conclusions

3823 On the basis of the above, the CEP Panel concluded that the evidence for an association between BPA
3824 exposure and abnormal thyroid function is Not Likely.

3825

3826 **Cluster Type 2 diabetes mellitus (T2DM)**

3827 Six studies (four cohort, two case-control) (Lee et al., 2013 [RefID 3911]; Sun et al., 2014 [RefID
3828 6986]; Hu et al., 2015 [RefID 2818]; Bi et al., 2016 [RefID 571]; Watkins et al., 2016 [RefID 7874];
3829 Shu et al., 2018 [RefID 13119]) assessed the association between BPA exposure measured in spot
3830 urine samples and endpoints related to T2DM using different exposure periods. Their detailed

3831 description and risk of bias assessment related to these studies are provided in Annexes C and D. Of
3832 these, two studies evaluated BPA exposure during pregnancy (Watkins et al., 2016 [RefID 7874]) and
3833 during childhood (Lee et al., 2013 [RefID 3911]), respectively, and they were not considered further in
3834 the WoE as they represent single entries for this cluster.

3835 Exposure during adulthood

3836 Four studies evaluated BPA exposure during adulthood and were considered in the WoE (Sun et al.,
3837 2014 [RefID 6986]; Hu et al., 2015 [RefID 2818]; Bi et al., 2016 [RefID 571]; Shu et al., 2018 [RefID
3838 13119]). Their detailed description and risk of bias assessment related to these studies are provided in
3839 Annexes C and D. In summary, the limited currently available epidemiological evidence does not put
3840 forward a T2DM-related endpoint as a critical one for risk assessment.

3841 Two cohorts (Hu et al., 2015 [RefID 2818]) and two case-control studies (Sun et al., 2014 [RefID
3842 6986]; Shu et al., 2018 [RefID 13119]) assessed the association between BPA exposure measured in
3843 adulthood and T2DM-related endpoints with a cumulative sample size of 4504 participants. The
3844 populations under study were of various sample sizes and varied in their characteristics. The assessed
3845 endpoints were T2DM and eGFR in T2DM patients. The largest studies were a nested case-control
3846 study within the Nurses' Health Study and the Nurses' Health Study II (NHSII) (n = 1942) in a
3847 population of predominantly European ancestry (Sun et al., 2014 [RefID 6986]) and a cohort study in
3848 a China (n = 2209, n cases = 242) (Bi et al., 2016 [RefID 571]). BPA levels were statistically
3849 significantly associated with incident-T2DM only in NHSII and after adjustment for BMI [per quartile
3850 OR (95%CI), 1.34 (0.70, 2.27), 1.91 (1.11, 3.29), 2.08 (1.17, 3.69)]. Conversely, Bi et al. (2016) [RefID
3851 571] reported no significant association of risk of incident T2D with BPA. Based on the above, the
3852 currently available longitudinal epidemiological evidence is characterised by a small number of studies,
3853 suboptimal exposure assessment and considerable heterogeneity in the assessed populations, exposure
3854 levels and endpoints. Moreover, there are no studies to replicate the observed partially 'positive'
3855 associations.

3856 On the basis of the above, the CEP Panel concluded that a positive association between BPA exposure
3857 during adulthood and T2D is ALAN.

3858 Overall conclusions

3859 On the basis of the above, the CEP Panel concluded that a positive association between BPA exposure
3860 and type 2 diabetes is ALAN.

3861

3862 **Cluster Gestational diabetes mellitus**

3863 Exposure during adulthood

3864 Three cohort studies (Bellavia et al., 2018 [RefID 11590] n = 350; Fisher et al., 2018 [RefID 11946]
3865 n = 232 and Shapiro et al., 2015 [RefID 6605] n = 1885) with their follow-up restricted within
3866 pregnancy assessed the association between BPA exposure measured in spot urine samples (n = 2) or
3867 serum (n = 1) and endpoints related to GDM. Their detailed description and risk of bias assessment
3868 related to these studies are provided in Annexes C and D. All three studies include participants from
3869 Europe or North America and they shared similarities for their population characteristics and endpoint
3870 definitions. No association under study reached statistical significance.

3871 On the basis of the above, the CEP Panel concluded that a positive association between BPA exposure
3872 during adulthood and GDM is Not Likely.

3873 Overall conclusions

3874 On the basis of the above, the CEP Panel concluded that a positive association between BPA exposure
3875 and gestational diabetes mellitus is Not Likely.

3876 *Cross-sectional studies*

3877 **Obesity**

3878 A total of 20 studies (Nicolucci et al., 2013 [RefID 5391]; Choi et al., 2014 [RefID 1185]; Eng et al.,
3879 2014 [RefID 1817]; Ko et al., 2014 [RefID 10443]; Wells et al., 2014 [RefID 7921]; Agay-Shay et al.,
3880 2015 [RefID 54]; Akin et al., 2015 [RefID 93]; Andra and Makris, 2015 [RefID 197]; Geens et al., 2015
3881 [RefID 2238]; Milic et al., 2015 [RefID 5098]; Pornkunwilai et al., 2015 [RefID 5889]; Savastano et al.,
3882 2015 [RefID 6495]; Xue et al., 2015 [RefID 8263]; Corbasson et al., 2016 [RefID 1292]; Metwally et
3883 al., 2016 [RefID 9673]; Do et al., 2017 [RefID 1640]; Hong et al., 2017 [RefID 2762]; Li J et al., 2017
3884 [RefID 4058]; Liu et al., 2017 [RefID 11165]; Charisiadis et al., 2018 [RefID 11691]) examined cross-
3885 sectionally the relationship between BPA exposure and obesity, BMI and body composition
3886 measurements. Of those, nine studies were conducted in children or adolescents (Nicolucci et al., 2013
3887 [RefID 5391]; Choi et al., 2014 [RefID 1185]; Eng et al., 2014 [RefID 1817]; Wells et al., 2014 [RefID
3888 7921]; Agay-Shay et al., 2015 [RefID 54]; Akin et al., 2015 [RefID 93]; Pornkunwilai et al., 2015 [RefID
3889 5889]; Xue et al., 2015 [RefID 8263]; Li J et al., 2017 [RefID 4058]). The populations under study were
3890 diverse in terms of sample size, age, sex, comorbidities, exposure levels and geographical origin. The
3891 endpoints assessed also varied considerably and included obesity, overweight, weight, BMI, insulin,
3892 insulin resistance, body fat, fat mass, lean mass, hip and waist circumference, waist to height ratio,
3893 adiponectin, adipokine hormones, leptin. Multiple analyses were performed per study. The cross-
3894 sectional studies in adults were generally small and some yielded statistically significant results.
3895 Although, the associations were not entirely consistent in terms of directionality, findings from some
3896 individual studies could be interpreted as being indicative of an association with obesity. The results
3897 from these cross-sectional analyses are in accordance with the results from the few prospective cohorts
3898 on obesity. Of course, with only a single measure of BPA cross-sectionally it seems biologically
3899 implausible that short time exposure quantified at that point in time could affect obesity risk. An
3900 alternative explanation for these cross-sectional findings is that those with obesity may be differently
3901 exposed to BPA through difference in lifestyle or behaviour. Alternatively, these associations may also
3902 reflect biological differences in rate of excretion of BPA. Studies relying on repeated measures of BPA
3903 would to some extent be able to address these limitations.

3904 **Cardiometabolic effects**

3905 Thirteen cross-sectional studies assessed the association between BPA exposure and blood pressure
3906 and vascular health (Sabanayagam et al., 2013 [RefID 6353]; Khalil et al., 2014 [RefID 3467]; Ko et
3907 al., 2014 [RefID 10443]; Shiue, 2014 [RefID 6705]; Aekplakorn et al., 2015b [RefID 47]; Lin et al.,
3908 2015 [RefID 4307]; Wang TG et al., 2015 [RefID 7755]; Metwally et al., 2016 [RefID 9673]; Turgut et
3909 al., 2016 [RefID 7330]; Kataria et al., 2017 [RefID 3407]; Menale et al., 2017 [RefID 5026]; Mouneimne
3910 et al., 2017 [RefID 5237]; Amin et al., 2018 [RefID 11498]). The available cross-sectional evidence on
3911 blood pressure is not characterised by consistency, while the small studies related to biomarkers of
3912 cardiovascular health used heterogeneous endpoints. Eleven cross-sectional studies (Eng et al., 2014
3913 [RefID 1817]; Ko et al., 2014 [RefID 10443]; Lin et al., 2015 [RefID 4307]; Savastano et al., 2015
3914 [RefID 6495]; Turgut et al., 2016 [RefID 7330]; Milosevic et al., 2017 [RefID 5101]; Mouneimne et al.,
3915 2017 [RefID 5237]; Amin et al., 2018 [RefID 11498]; Carlsson et al., 2018 [RefID 11070]; Lee I et al.,
3916 2018 [RefID 12428]; Mohsen et al., 2018 [RefID 12746]) investigated the relationship between BPA
3917 exposure and lipid profile characterised by various population characteristics and sample sizes. Of these,
3918 only a few studies showed statistically significant associations between BPA exposure and total
3919 cholesterol, LDL, triglycerides and dyslipidemia.

3920 **Thyroid effects**

3921 A total of 10 studies (Sriphrapradang et al., 2013 [RefID 6870]; Wang TG et al., 2013 [RefID 7753];
3922 Wang N et al., 2015 [RefID 7702]; Aker et al., 2016 [RefID 92]; Chailurkit et al., 2016 [RefID 924];
3923 Minatoya et al., 2017b [RefID 5106]; Park et al., 2017 [RefID 5656]; Zhou ZZ et al., 2017 [RefID 9089];
3924 Przybyla et al., 2018 [RefID 12920]; Sanlidag et al., 2018 [RefID 13042]) examined cross-sectionally
3925 the relationship between BPA exposure and endpoints related to thyroid function. Of those, three
3926 studies were conducted in children or adolescents (Wang N et al., 2015 [RefID 7702]; Minatoya et al.,
3927 2017b [RefID 5106]; Sanlidag et al., 2018 [RefID 13042]) and one study was conducted in pregnant
3928 women (Aker et al., 2016 [RefID 92]). The populations under study were diverse in terms of sample
3929 size, age, sex, comorbidities, exposure levels and geographical origin. The endpoints assessed also
3930 varied considerably and included TSH, Triiodothyronine (T₃), Thyroxine (T₄), thyroid autoimmunity,
3931 antithyroglobulin, antithyropoxidase, antithyrotrophin receptor, thyroid volume, thyroid nodules,
3932 hyperthyroidism, euthyroidism, nodular goitre, papillary thyroid carcinoma and thyroid secretory

3933 capacity. Multiple analyses were performed per study. Overall, the associations reported in these studies
3934 are Not Likely to be causal.

3935 **Type 2 diabetes mellitus (T2DM)**

3936 A total of 18 studies (Sabanayagam et al., 2013 [RefID 6353]; Ahmadkhaniha et al., 2014 [RefID 67];
3937 Beydoun et al., 2014 [RefID 551]; Aekplakorn et al., 2015a [RefID 46]; Savastano et al., 2015 [RefID
3938 6495]; Bi et al., 2016 [RefID 571]; Piecha et al., 2016 [RefID 5831]; Tai and Chen, 2016 [RefID 7070];
3939 Turgut et al., 2016 [RefID 7330]; Chailurkit et al., 2017 [RefID 11073]; Hong et al., 2017 [RefID 2762];
3940 Kataria et al., 2017 [RefID 3407]; Menale et al., 2017 [RefID 5026]; Mouneimne et al., 2017 [RefID
3941 5237]; Carlsson et al., 2018 [RefID 11070]; Dallio et al., 2018 [RefID 11782]; Li AJ et al., 2018 [RefID
3942 12448]; Verstraete et al., 2018 [RefID 13308]) examined cross-sectionally the relationship between
3943 BPA exposure and endpoints related to type 2 diabetes mellitus (T2DM). Of those, three studies were
3944 conducted in children or adolescents (Kataria et al., 2017 [RefID 3407]; Menale et al., 2017 [RefID
3945 5026]; Carlsson et al., 2018 [RefID 11070]). The populations under study were diverse in terms of
3946 sample size, age, sex, comorbidities, exposure levels and geographical origin. The endpoints assessed
3947 also varied considerably and included diabetes, impaired fasting glucose, haemoglobin A1c,
3948 hyperinsulinemia, insulin levels, glucose homeostasis, 2-hour post-loading plasma glucose, insulin
3949 resistance, Homeostatic Model Assessment (HOMA), and resistin. Multiple analyses were performed per
3950 study. Overall, the associations reported in these studies are Not Likely to be causal.

3951 **Gestational diabetes mellitus (GDM)**

3952 Two studies (Robledo et al., 2013 [RefID 6218]; Wang X et al., 2017 [RefID 7773]) examined cross-
3953 sectionally the relationship between BPA exposure and endpoints related to gestational diabetes. One
3954 of the studies showed a statistically significant association between higher urinary BPA concentrations
3955 and reduced risk of GDM (Wang X et al., 2017 [RefID 7773]), while the other one yielded statistically
3956 non-significant findings (Robledo et al., 2013 [RefID 6218]). Overall, the associations reported in these
3957 studies are Not Likely to be causal.

3958 **3.1.4.2. Animal studies**

3959 For the HoC Metabolic effects a total of 82 studies were appraised by the CEP Panel. The details of the
3960 appraisals (internal and external validity) are reported in Annex E.

3961 The endpoints for each study identified as relevant in this opinion are reported in Annex F. The data of
3962 these studies used for the WoE are shown in Annex G.

3963 Glucose regulation was already a key endpoint in the uncertainty analysis in the 2015 EFSA opinion
3964 (EFSA CEF Panel, 2015, Section 4.3.2). For more details see Annex A, Chapter 2.5.

3965 *Identification of clusters of relevant endpoints*

3966 The clusters of relevant endpoints assessed in the studies on effects of BPA on metabolism were the
3967 following:

- 3968 • Obesity
- 3969 • Fat deposition in the liver
- 3970 • Glucose regulation
- 3971 • Blood lipids
- 3972 • Uric acid
- 3973 • Type 1 diabetes mellitus (T1DM)
- 3974 • Other metabolic hormones
- 3975 • Thyroid hormones.

3976 **Obesity**

3977 Environmental chemicals may both increase obesity (have obesogenic effect) and decrease obesity
3978 (have a toxic effect), which both are considered adverse. Obesity is associated with multiple metabolic

3979 alterations that are risk factors for diabetes mellitus with its consequences due to macrovascular and
3980 microvascular changes, such as cardiovascular diseases, and is related to non-alcoholic fatty liver
3981 disease (NAFLD) with the risk to develop non-alcoholic steatohepatitis (NASH) (Napoli and Pozzilli,
3982 2014). Whereas non-alcoholic liver disease is reversible, NASH may progress to cirrhosis and liver
3983 cancer (Willebrords et al., 2015). It is believed that insulin resistance in the adipose tissue, liver and
3984 skeletal muscle is crucial in the pathogenesis of these metabolic abnormalities. Adipocytes are key
3985 regulators of whole-body energy homeostasis and altered adipose tissue glucose metabolism is also an
3986 important cause of insulin resistance and metabolic dysfunction. Adipose tissue contributes to the
3987 development of obesity-related glucose abnormalities through excessive release of free fatty acids
3988 (FFA), adipokines, cytokines and macrophage infiltration, causing inflammation. Increased body weight
3989 and body fat mass are also linked to several types of cancer, i.e. colorectal, liver and postmenopausal
3990 breast cancer (WCRF/AICR, 2018).

3991 All the endpoints in the clusters are interrelated and may potentially affect humans adversely. Although
3992 there are several slightly different definitions on the metabolic syndrome, obesity, insulin resistance,
3993 atherogenic dyslipidemia and hypertension comprise the metabolic syndrome, and are risk factors for
3994 cardiovascular disease and T2DM (Huang, 2009). Several of these endpoints are included as metabolic
3995 endpoints in this WoE.

3996 The specific endpoints that were included for the effects of BPA in the cluster obesity from the metabolic
3997 studies were body weight, BMI, body fat mass, visceral adipocyte size and non-visceral adipocyte size.

3998 **Body weight** was regarded as a transversal endpoint¹⁶. Data on body weight were therefore collected
3999 from the HOC Metabolic effects, as well as from General toxicity and Reproductive toxicity. Body weight
4000 was recorded at one or several time points and as body weight gain over time.

4001 **Body mass index (BMI)** is defined in humans as the body mass in kg divided by the square of the
4002 body height, expressed as kg/m² (Batsis et al., 2016). Persons with BMI > 25 are categorised as
4003 overweight, whereas persons with BMI > 30 are categorised as obese. In the studies on mice, BMI was
4004 defined as $[BW/(BL)^2] \times 100$, where BW is body weight (in g) and BL is body length (in cm). The BL
4005 was measured from nose to anus.

4006 **Body fat mass** was used as a term to describe fat masses measured in the whole or large parts of the
4007 body by equipment such as Echo Magnetic Resonance Imaging System (ECoMRI), dual energy X-ray
4008 absorptiometry (DEXA) or computed tomography scans (CT) in the studies assessed in this opinion, as
4009 opposed to measurements of individual adipose tissues (fat pads).

4010 Adipose tissue is a central metabolic organ, however, unlike other organs it is compartmentalised into
4011 individual depots distributed throughout the body. In humans, intra-abdominal fat, often referred to as
4012 visceral adipose tissue, which surrounds the inner organs, is associated with increased risk of insulin
4013 resistance and dyslipidemia and is regarded as an independent risk factor for type 2 diabetes mellitus,
4014 hypertension and all-cause mortality (Chusyd et al., 2016). In contrast, the upper-body subcutaneous
4015 adipose tissue may be beneficial to health due to its ability to act as long-term fat (energy) storage
4016 site, protecting against ectopic fat depositions in organs such as liver and pancreas and the associated
4017 lipotoxicity. Regarding individual fat deposits in the body, there is some controversy regarding the
4018 terminology used for the various fat pads, and the correlation between the various fat pads, in both
4019 their composition and function, in humans versus those in rodents (Chusyd et al., 2016). In the papers
4020 assessed in this opinion, the expression **visceral white adipose tissue (WAT) weight** was used for
4021 weight of various adipose tissues in the abdominal area of rodents, specified in the papers as
4022 retroperitoneal, gonadal/perigonadal/perigonadal WAT (pWAT), epididymal/epididymal WAT (eWAT),
4023 ovarian/periovarian, parametrial, renal/perirenal and mesenterial adipose tissues or fat pads. Weight of
4024 WAT outside of the abdominal area, called **non-visceral WAT weight**, was specified in the papers as
4025 inguinal, subcutaneous and subcutaneous mammary – caudal. However, complicating matters even
4026 more, there are also data indicating that individual adipose tissue depots are independent organs,
4027 developing from different precursor populations and serving different metabolic functions. For instance,

¹⁶ Transversal endpoints refer to endpoints relevant in more than one HOC and evaluated collectively.

4028 there is growing support for the idea that even within visceral fat deposits subpopulations of adipocytes
4029 could have beneficial metabolic effects (Schoettl et al., 2018).

4030 Brown adipose tissue (BAT) protects against hypothermia in small mammals and newborn human
4031 infants through thermogenesis but was thought to be absent in adult humans (Wang GX et al., 2015).
4032 However, recent studies using positron emission tomography (PET) demonstrated that metabolically
4033 active BAT is present in some adults. Activation of BAT leads to increased energy expenditure, reduced
4034 adiposity and lower plasma glucose and lipid levels, thus, contributing to better homeostasis. Brown fat
4035 is emerging as a promising target for therapeutic intervention in obesity and metabolic disease (Betz
4036 and Enerbäck, 2018). Activation of BAT in humans is associated with marked improvement in metabolic
4037 parameters such as levels of FFA and insulin sensitivity. In older persons, the amount of BAT was
4038 inversely correlated with BMI (Cypess et al., 2009). Because changes of the weight of brown fat tissue
4039 are of unclear relevance, data on BAT were not included in the assessment.

4040 In the studies assessed in this opinion, data on non-visceral BAT weight, comprised of interscapular or
4041 unspecified BAT weight, were available.

4042 It was decided that in this assessment, only endpoints considered biologically relevant and statistically
4043 significant or showed a clear trend in the data in at least one Tier 1 or Tier 2 study were included in
4044 the WoE (see Annex A). Although statistically significant in some studies, the data on visceral or non-
4045 visceral WAT weight or non-visceral BAT weight were considered as Tier 3 because blinding was not
4046 mentioned in any such papers, which is important to avoid bias for the manual excised and weighed
4047 adipose tissues or fat pads. Hence, because there were no Tier 1 or Tier 2 studies showing significant
4048 effects on these endpoints, the available data on these endpoints were not included in the WoE.

4049 There are principally two ways of fat accumulation in the adipocytes in the body, either that the
4050 adipocytes increase in number, without increasing in size (hyperplastic morphology) or they increase in
4051 size, because of a larger fat content per cell (hypertrophic morphology) (Tandon et al., 2018). Increased
4052 adipocyte size is associated with adverse health effects, such as insulin resistance, diabetes and
4053 cardiovascular disease, both in visceral and subcutaneous, i.e. non-visceral, fat (Tandon et al., 2018).
4054 The data on adipocyte size were also divided into two endpoints. Visceral adipocyte size was specified
4055 in the papers as size of visceral, retroperitoneal, perigonadal/gonadal WAT, epididymal, periovarian,
4056 perirenal/perirenal WAT and mesenteric adipocytes. Non-visceral adipocyte size was specified as size
4057 of adipocytes obtained from subcutaneous, inguinal and interscapular WAT. Results were also included
4058 in the endpoint non-visceral adipocyte size if it was not stated in the publication from which fat pad the
4059 adipocytes were obtained.

4060 **Fat deposition in the liver**

4061 The endpoints considered for fat deposition in the liver were liver cholesterol and liver triglycerides.
4062 One role of the liver in fat metabolism includes oxidising triglycerides to produce energy (Trefts et al.,
4063 2017). The liver breaks down fatty acids and uses the break down product acetoacetate and β -
4064 hydroxybutyrate as energy supply. The liver also exports acetoacetate and β -hydroxybutyrate into the
4065 blood which transports them into tissues outside the liver where it is converted into acetyl-CoA, which
4066 then enters the citric acid cycle and is oxidised in the mitochondria for energy. The liver also synthesises
4067 lipoprotein, and it is the major site for converting excess carbohydrates and proteins into fatty acids
4068 and triglyceride, which are exported and stored in adipose tissue. The liver also synthesises large
4069 quantities of cholesterol and phospholipids. Some of this is packaged with lipoproteins and made
4070 available to the rest of the body. The remainder is excreted in bile as cholesterol or after conversion to
4071 bile acids.

4072 The endpoint liver fat % is relevant as fat accumulation in the liver may have adverse health effects. It
4073 is normal for the liver to contain some fat. However, if >5% of the liver's weight is fat, then it is called
4074 a fatty liver (steatosis). The condition is often called NAFLD, which is an umbrella term for a range of
4075 liver conditions affecting people who drink little to no alcohol, but have too much fat stored in their
4076 liver cells (Kneeman et al., 2012). It is often associated with obesity, type 2 diabetes mellitus,
4077 dyslipidaemia and insulin resistance. The more severe form of NAFLD is called NASH, characterised by
4078 inflammation and enlargement of the liver. This may progress to advanced scarring (cirrhosis) and liver
4079 failure. Because there were no Tier 1 or Tier 2 studies showing significant effects or a trend for liver
4080 fat %, the available data on this endpoint were not included in the WoE.

4081 **Glucose regulation**

4082 Insulin is a hormone that regulates lipid and carbohydrate metabolism and energy homeostasis (Wilcox,
4083 2005). After food intake, insulin is secreted by the β -cells of the pancreas, and it regulates the blood
4084 glucose to stay in the normal range by facilitating the uptake of glucose by glucose transporters in the
4085 liver and in other cells in the body. Insulin resistance is a condition where the cells, in particular in muscles,
4086 fat and liver have a reduced response to insulin, which leads to a reduced uptake of glucose from the
4087 blood into the cells and hence, hyperglycaemia. To compensate, the β -cells in pancreas produce more
4088 insulin, causing high blood levels of insulin (hyperinsulinemia). Since insulin is important to regulate the
4089 blood glucose levels, the relationship between glucose and insulin is tight and the effects on both are
4090 evaluated together. Impaired glucose regulation is a risk factor linked to adverse health effects, and
4091 insulin resistance is one of the components in metabolic syndrome (Huang, 2009). Obesity is a risk
4092 factor for developing insulin resistance.

4093 Glucose regulation was assessed by several endpoints in the metabolic papers. **Glucose levels** were
4094 measured in full blood, serum or plasma in animals that were fasting, fed or this information was not
4095 given in the papers. **Insulin levels** were measured in serum or plasma in fasting or fed animals or this
4096 information was not given. In addition to measurements of existing levels of glucose and insulin,
4097 functional tests were also performed. In the **glucose tolerance test (GTT)**, glucose was administered
4098 via oral, intraperitoneal (i.p.) or intravenous (i.v.) route or the route was not stated in the papers, and
4099 changes in glucose levels over time were measured. In **insulin tolerance test (ITT)**, insulin was
4100 injected via the i.p. route and changes in glucose levels over time were measured. The data in GTT and
4101 ITT are expressed as AUC of the glucose levels measured over time. In GTT, impaired glucose regulation
4102 is seen as higher and prolonged levels of glucose in the blood after a glucose challenge, whereas in
4103 ITT, it is seen as higher levels of glucose decreasing slower over time after the insulin challenge.

4104 **Pancreas weight** is also considered a metabolic endpoint, since insulin and glucagon are produced in
4105 the β -cells and α -cells of the pancreas, respectively (Wilcox, 2005). The endpoint **β -cell morphometry**
4106 was given in the papers as 'insulin-stained β -cells as ratio of β -cells/total pancreas', β -cell mass, β -cell
4107 fraction (%), insulin-reactive cells in pancreas (%) or H-score (taking degree of staining into account).
4108 The endpoint **α -cell morphometry** was given as 'glucagon-stained α -cells as ratio of α -cells/total
4109 pancreas' or α -cell mass.

4110 **Blood lipids**

4111 The endpoints indicating a possible effect on metabolism of lipids were cholesterol (in some studies
4112 also reported as 'total cholesterol'), HDL cholesterol, LDL cholesterol, triglycerides and FFA.

4113 Impaired lipid metabolism (dyslipidemia) is one component of metabolic syndrome (Huang, 2009).
4114 Depending on the various definitions of metabolic syndrome, dyslipidemia is defined with increased
4115 values of triglycerides and decreased HDL cholesterol, outside of certain ranges. The adversity of
4116 elevated cholesterol is due to the fact that it is a risk factor for cardiovascular disease in humans,
4117 involved in the development of atherosclerosis (Avci et al., 2018). The adversity of elevated cholesterol
4118 has to be judged on the relationship between its subfraction, HDL cholesterol, which has a protective
4119 function on cardiovascular disease in humans, and LDL cholesterol, which is related to an increased risk
4120 for cardiovascular events (Avci et al., 2018). Thus, HDL cholesterol and LDL cholesterol should be
4121 assessed together. In the animal studies, mainly rodents have been investigated, in which HDL levels
4122 are higher than LDL levels, which is opposite to the situation in humans (Kaabia et al., 2018). Also,
4123 non-human primates had higher HDL than LDL levels. Hence, the predictive value of results in these
4124 species might be in question. FFA cause both insulin resistance and inflammation in the major insulin
4125 target tissues, skeletal muscle, liver and endothelial cells, and thus, are an important link between
4126 obesity, insulin resistance, inflammation and the development of T2DM, hypertension, dyslipidaemia,
4127 disorders of coagulation and atherosclerotic vascular disease (Boden, 2008).

4128 **Uric acid**

4129 The effects of BPA on **uric acid in serum, urine or liver** were assessed as elevated uric acid is
4130 associated with obesity and data in experimental animals indicate that purine catabolism in adipose
4131 tissue could be enhanced in obesity (Tsushima et al., 2013). Uric acid is the degradation product of the

4132 metabolism of purines. Purines are constituents of the diet which contains nucleic acids. They are also
4133 endogenously formed, partly by *de novo* purine biosynthesis.

4134 Increased level of **uric acid in blood** (hyperuricaemia) can result from increase of the rate of purine
4135 biosynthesis *de novo* in the liver, by increased dietary uptake and by a decrease in renal clearance of
4136 uric acid or a combination of the processes. Another cause for increased levels of uric acid may be
4137 increase in glucagon levels due to glucagon-induced catabolism of proteins.

4138 In humans, uric acid is the final end product of purine metabolism and is excreted by the kidneys as
4139 **uric acid in the urine** or via intestine. In some species, including rats and mice, most of the uric acid
4140 is further metabolised by the enzyme uricase to allantoin. Because in humans uricase is mutated and
4141 non-functional, and the ionic form urate is extensively reabsorbed in the renal tubuli, the urate acid
4142 levels in plasma are ten-fold higher than those of other mammals (Mandal and Mount, 2015).

4143 Hyperuricaemia is a frequent finding in patients with hypertension, partly explained by the effect of
4144 drugs (i.e. diuretics) given against hypertension. A positive association exists between serum urate and
4145 body weight. In an obese mouse model (*ob/ob*), adipose tissue could produce and secrete uric acid
4146 through the activity of xanthine oxidoreductase (XOR), catalysing purines to uric acid, and the
4147 production was enhanced in obesity (Tsushima et al., 2013). There are also some findings indicating
4148 an association between hyperlipidaemia and hyperuricaemia without a biological explanation.
4149 Hyperuricaemia is noted in patients with degenerative vascular disease and after acute myocardial
4150 infarction, however, the interrelationship is caused probably both by effects of the drugs given in those
4151 conditions and by effects of the underlying disease on urate excretion (Emmerson, 1978; Fang and
4152 Alderman, 2000; Strasak et al., 2008). Hyperuricemia is also strongly associated with insulin resistance
4153 syndrome, an established risk factor for T2DM, and individuals with higher serum uric acid, including
4154 younger adults, had a higher future risk of T2DM (Bhole et al., 2010). Uric acid is mainly produced in
4155 the liver, and both higher baseline and increased serum uric acid have been identified as risk factors
4156 for developing fatty liver (Jensen et al., 2018).

4157 **Type 1 Diabetes Mellitus (T1DM)**

4158 In T1DM, an autoimmune response gradually destroys the β -cells in the pancreas which leads to a
4159 severely reduced insulin production with the consequence of continuously elevated blood glucose
4160 (Katsarou et al., 2017). T1DM develops most often in young people but can also appear in adults, and
4161 if not well medically treated may lead to microvascular and macrovascular complications. Among the
4162 papers on metabolic effects, effects of BPA were only studied as the incidence of T1DM in specific mice
4163 models, such as diabetes-prone non-obese diabetic (NOD) mice or mice with streptozotocin-induced
4164 T1DM.

4165 **Other metabolic hormones**

4166 Hormones related to obesity, glucose regulation and lipid metabolism were also among the metabolic
4167 endpoints. Adiponectin and leptin levels were measured in serum or plasma, glucagon was measured
4168 in serum and resistin in plasma.

4169 **Leptin** and **adiponectin** are cytokines produced by adipocytes (Ghantous et al., 2015). Generally,
4170 obesity is associated with high levels of the circulating leptin and low levels of adiponectin. Leptin's
4171 physiological role is to regulate hunger by inducing a feeling of satiety. Studies show that lack of leptin
4172 causes severe obesity because of the resulting lack of appetite control and is strongly linked with insulin
4173 resistance and with T2DM (Facey et al., 2017).

4174 Leptin is produced also by other cells such as cardiomyocytes and vascular smooth muscle cells. It may
4175 mechanistically be involved in causing the cardiovascular risks linked to obesity (Ghantous et al., 2015).
4176 However, as it has also been shown that it may have beneficial effects on reperfusion of ischaemic
4177 damage. (Zhang WF et al., 2019) Thus, it mediates adverse and beneficial effects in humans. In the
4178 context of this assessment, leptin may also be seen as an indicator of the number of adipocytes.

4179 Adiponectin is produced by brown and white adipose tissue and is inversely related to metabolic and
4180 cardiovascular diseases (Ghantous et al., 2015). Adiponectin levels in plasma are inversely correlated
4181 with adiposity and directly correlated with insulin sensitivity. It contributes to the normal functioning of
4182 the cardiovascular system and can be regarded as a cardioprotective hormone (Ghantous et al., 2015).

4183 In the context of this assessment, adiponectin may also be seen as an indicator of the number of
4184 adipocytes.

4185 **Glucagon** is produced by the α -cells in the pancreas and has opposite effects of insulin. It activates
4186 adenylate cyclase in the liver, which is the first step in transforming glycogen to glucose in the liver
4187 cells (Finan et al., 2020). Glucose is released into the blood and increases the blood glucose level.
4188 However, glucagon is also involved in the gluconeogenesis from proteins. It was hypothesised that
4189 dysregulated α -cell function and increased glucagon in the blood were essential contributors to
4190 hyperglycaemia. However, newer studies indicated that glucagon has a more complex biology than
4191 previously thought (Finan et al., 2020).

4192 **Resistin** is mainly secreted by adipocytes in rodents and in peripheral blood mononuclear cells (PBMC)
4193 and macrophages in humans (Parreno et al., 2018). It plays an important role in many mechanisms in
4194 rodent studies, including insulin resistance, lipid metabolism and inflammation. In a systematic review
4195 and meta-analysis, it was shown that in persons with T2DM and obesity, the resistin levels were
4196 positively associated with insulin resistance in persons with increased levels of resistin in their blood,
4197 but not in those with normal blood resistin levels (Su et al., 2019). Serum resistin was involved in the
4198 pathogenesis of arteriosclerosis (Parreno et al., 2018). In addition, serum resistin levels were increased
4199 in patients with hypertension, coronary heart disease and cerebrovascular disease and are related to
4200 the development and worsening of heart failure.

4201 **Thyroid hormones**

4202 T_3 and T_4 , as well as TT_3 , TT_4 , FT_3 , FT_4 and reverse T_3 /total T_4 (rT_3/TT_4) ratio, were measured in
4203 serum. TSH was also measured in the publications but was not included among the relevant endpoints
4204 in this assessment, because no Tier 1 or 2 studies showed statistically significant effects or a clear
4205 trend.

4206 A reciprocal interaction between the hypothalamus–pituitary–thyroid axis and the adipose tissue is
4207 required for proper homeostasis of energy balance (Ceccarini et al., 2015). In both lean and obese
4208 subjects, thyroid hormones T_3 and T_4 (and TSH) levels are strongly influenced by the individual
4209 nutritional status. Furthermore, obesity can be associated with variations of circulating thyroid
4210 hormones T_3 and T_4 (and TSH). Whether obesity is a risk factor for thyroid diseases (e.g. autoimmunity
4211 or cancer) is still under debate. It is well established that hypothyroidism may induce obesity, but the
4212 relationship may be bidirectional. Obesity was significantly associated with an increased risk of
4213 hypothyroidism in a recent systematic review and meta-analysis (Song et al., 2019).

4214 The association between thyroid hormones and cardiovascular conditions has been well studied,
4215 specifically, the effects of hypothyroidism on cardiomyopathy and hyperthyroidism with arrhythmias,
4216 and the literature demonstrated a clear correlation between hypothyroidism, even subclinical, and
4217 cardiac dysfunction (Khan et al., 2020). However, there were mixed findings when studying patients
4218 with hyperthyroidism and heart failure.

4219 Thyroid hormones were regarded as transversal endpoints¹⁷.

4220 *WoE of the clusters of relevant endpoints*

4221 The main information extracted from the studies addressing relevant endpoints in the HOC Metabolic
4222 effects are summarised in Annex G. The outcome of the weight of the evidence is described in the text
4223 below and presented in a tabulated format in Annex H.

4224 In the WoE, the metabolic endpoints were divided into several exposure categories: developmental
4225 (pre-natal and/or post-natal until weaning), developmental and adult (pre-natal and/or post-natal in
4226 pups until adulthood), growth phase/young age and adult exposure (after puberty), indirect (germline)
4227 exposure.

4228 A majority of the metabolic studies had an experimental design with maternal exposure, i.e. the
4229 offspring that were studied for the metabolic endpoints were exposed to BPA during their developmental

¹⁷ Transversal endpoints refer to endpoints relevant in more than one HOC and evaluated collectively.

4230 phase, as embryos when the dams were exposed during pregnancy and/or through mother's milk, when
4231 the dams were exposed during the lactation period. In some studies, the BPA exposure of the offspring
4232 also continued after the weaning, or they were not treated, but observed for a period after weaning
4233 before termination. In some studies, the metabolic effects were compared between animals on a
4234 standard chow diet and a high-fat diet (HFD) or high-fat/high cholesterol diet.

4235 The **clusters** of metabolic endpoints assessed in the studies on effects of BPA on metabolism were the
4236 following:

- 4237 • Obesity
- 4238 • Fat deposition in the liver
- 4239 • Glucose regulation
- 4240 • Blood lipids
- 4241 • Uric acid
- 4242 • Type 1 diabetes mellitus (T1DM)
- 4243 • Metabolic hormones.

4244 Body weight and thyroid hormones were considered as transversal endpoints, and the data on them
4245 were collected from the HOCs Metabolic effects, General toxicity and Reproductive and developmental
4246 Toxicity, and from Metabolic effects and Reproductive and developmental Toxicity, respectively.

4247 **Obesity**

4248 The specific endpoints that were assessed for the effects of BPA in the cluster obesity were body weight,
4249 BMI, body fat mass, visceral adipocyte size and non-visceral adipocyte size.

4250 Body weight was treated as a transversal endpoint in this opinion. Data on this transversal endpoint
4251 were collected from the HOCs Metabolic effects, General toxicity and Reproductive toxicity. This resulted
4252 in very high numbers of studies on body weight for most of the exposure periods. Therefore, the WoE
4253 of this endpoint was done by comparing the number of studies demonstrating no effects, increasing
4254 effects or decreasing effects on body weight, because it was not possible to compare all the various
4255 study parameters and outcomes in detail simultaneously for such high numbers of studies. In addition,
4256 the tier allocation and the range of doses administered in each category of effects were taken into
4257 consideration.

4258 In this assessment, a total of 114 studies (i.e. unique RefIDs) reported data included in the cluster
4259 obesity. Many of the studies reported more than one result, i.e. effects on different endpoints, doses,
4260 time points, sex and sometimes also on different diets or on two strains, and in some studies also data
4261 from more than one exposure period. Thus, the number of individual results reported is much higher
4262 than the number of studies.

4263 Within the cluster obesity, 85 studies were on mice, of which 32 studies had exposure in the
4264 developmental until weaning period, 13 had exposure during the developmental until adulthood period,
4265 13 had exposure during the growth phase, 24 were exposed as adults and three had germline exposure.
4266 Of the 96 studies on rats, 36 studies had exposure during the developmental until weaning period, 13
4267 had exposure during the developmental until adulthood period, eight had exposure during the growth
4268 phase, 35 were exposed as adults and four had germline exposure. In addition, nine studies were on
4269 other animal species. One study was on Mongolian gerbils, with exposure during the developmental
4270 until weaning period, three studies were on rabbits exposed as adults, two studies were on sheep, both
4271 with exposure during the developmental until weaning period, and three studies were on monkeys; one
4272 study on common marmosets, exposed as adults, and two studies on rhesus macaques, one had
4273 exposure during the developmental until weaning period and one as adults.

4274 Developmental exposure (pre-natal and/or post-natal until weaning)

4275 For the endpoint body weight, there were 59 studies in which the exposure of the animals was under
4276 the developmental until weaning period; 27 studies on mice, 30 studies on rats and one study each on
4277 Mongolian gerbils and monkeys (rhesus macaques). In Park et al. (2018) [RefID 12869], it was not
4278 clear if the mice were exposed to 10000 µg/kg bw per day by i.p. injection or by gavage for the age

4279 nine to 21 weeks. Thus, the dose and/or administration route of these two studies could not be taken
4280 into consideration in the WoE. In Rubin et al. (2017) [RefID 6319] and Kass et al. (2015) [RefID 3402],
4281 BPA was given via subcutaneous osmotic minipumps, and in Ke et al. (2016) [RefID 3447], BPA was
4282 given by subcutaneous injections, thus, the doses were converted to oral doses in these studies. In
4283 Junge et al. (2018) [RefID 12262], Meng Y et al. (2018) [RefID 12707] and Desai et al. (2018) [RefID
4284 11817], BPA doses in µg/kg bw per day were calculated from intake of drinking water.

4285 There were 23 studies that did not show any effects on body weight at all. Of these, there were 10
4286 studies on mice, of which there were four, three and three studies, respectively, in Tier 1 (Taylor et al.,
4287 2018 [RefID 13239]; MacKay et al., 2017 [RefID 4767]; Tucker et al., 2018 [RefID 13275]; Meng Y et
4288 al., 2018 [RefID 12707]), Tier 2 (Ke et al., 2016 [RefID 3447]; Meng Z et al., 2018 [RefID 12708]; Shi
4289 et al., 2018 [RefID 13099]) and Tier 3 (Bodin et al., 2014 [RefID 623]; Eckstrum et al., 2018 [RefID
4290 11874]; Hijazi et al., 2018 [RefID 2707]). There were 11 studies on rats (of which there were six, four
4291 and one studies, respectively, in Tier 1 (Cao et al., 2015 [RefID 831]; Ding et al., 2014 [RefID 1620];
4292 Lejonklou et al., 2017 [RefID 3975]; Lejonklou et al., 2016 [RefID 3974]; Ferguson SA et al., 2014
4293 [RefID 1998]; Lind et al., 2017 [RefID 4350]), Tier 2 (Leung et al., 2017 [RefID 3990]; Spörrndly-Nees
4294 et al., 2018 [RefID 13164]; Kass et al., 2015 [RefID 3402] and Santamaria et al., 2016 [RefID 6448])
4295 and Tier 3 (Tarapore et al., 2017 [RefID 7128]). In addition, there was one study on Mongolian gerbils
4296 (de Lima et al., 2015 [RefID 1470]), which was Tier 2, and one study on monkeys (rhesus macaques)
4297 (Calhoun et al., 2014 [RefID 798]), which was Tier 2. Kass et al. (2015) [RefID 3402] contained two
4298 separate experiments, with pre-natal or perinatal exposure. The doses that were found not to have an
4299 effect on body weight were in the range 0.05–50000 µg/kg bw per day.

4300 There were 21 studies that showed increased body weight after BPA exposure. Of these, there were
4301 nine studies on mice, of which there were one, four and four studies respectively, in Tier 1 (van Esterik
4302 et al., 2014 [RefID 7393]), Tier 2 (Malaisé et al., 2017 [RefID 4815]; Junge et al., 2018 [RefID 12262];
4303 Susiarjo et al., 2015 [RefID 7022] and Wang W et al., 2014 [RefID 7759]) and Tier 3 (Rubin et al.,
4304 2017 [RefID 6319]; Ziv-Gal et al., 2015 [RefID 9143]; Dobrzynska et al., 2015 [RefID 1644] and Patel
4305 et al., 2013 [RefID 5697]). There were 12 studies on rats, of which eight, four and zero studies in Tier
4306 1 (Hass et al., 2016 [RefID 2610]; Jiang et al., 2014 [RefID 3190]; Song SZ et al., 2014 [RefID 6829];
4307 Desai et al., 2018 [RefID 11817]; Dunder et al., 2018 [RefID 11866]; Xia et al., 2014 [RefID 8103];
4308 Quan et al., 2017 [RefID 6025] and Uchtmann et al., 2019 [RefID 13784]), Tier 2 (Ma et al., 2013
4309 [RefID 4748]; Mao et al., 2017 [RefID 4865]; Zhang et al., 2013 [RefID 8798] and Anteur et al., 2016
4310 [RefID 13773]) and Tier 3, respectively. The doses that were found to increase the body weight were
4311 in the range 0.45–10000 µg/kg bw per day.

4312 There were 15 studies that showed decreased body weight after BPA exposure. Of these, there were
4313 eight studies on mice, of which there were one, four and three studies in Tier 1 (van Esterik et al., 2014
4314 [RefID 7393]), Tier 2 (Malaisé et al., 2017 [RefID 4815]; Suglia et al., 2016 [RefID 6943]; Susiarjo et
4315 al., 2015 [RefID 7022] and Kremmentsov et al., 2013 [RefID 3692]) and Tier 3 (Kalb et al., 2016 [RefID
4316 3312]; Dobrzynska et al., 2015 [RefID 1644] and Patel et al., 2013 [RefID 5697]), respectively. There
4317 were seven studies on rats, of which three, four and zero studies were in Tier 1 (NTP Clarity Report,
4318 2018/Camacho et al., 2019 [RefID 11370]; Bernardo et al., 2015 [RefID 533] and Brandt et al., 2014
4319 [RefID 700]), Tier 2 (Greenberg, 2018 (NTP Grantee study) [RefID 13785]; Chang et al., 2016 [RefID
4320 965]; Santos-Silva et al., 2018 [RefID 13047] and Anteur et al., 2016 [RefID 13773]) and Tier 3,
4321 respectively. The doses that were found to decrease the body weight were in the range 4–25000 µg/kg
4322 bw per day.

4323 The decreasing effect observed in Dobrzynska et al. (2015) [RefID 1644] was with 20000 µg/kg bw per
4324 day, and in Greenberg (2018) (NTP Grantee study) [RefID 13785] with 25000 µg/kg bw per day, i.e.
4325 above the cut-off value of 10000 µg/kg bw per day.

4326 There was approximately the same number of studies that did not find any effects of BPA on body
4327 weight (23), (10 Tier 1, nine Tier 2, four Tier 3), as found an increased effect (i.e. an obesogenic effect)
4328 (21), (nine Tier 1, eight Tier 2, four Tier 3) and also quite a high number of studies that observed
4329 decreasing body weight (i.e. potentially a toxic effect) (15) (four Tier 1, eight Tier 2, three Tier 3). The
4330 dose ranges were also more or less in the same orders between these three categories of effects. Thus,
4331 the CEP Panel judged this endpoint as ALAN.

4332 For the endpoint BMI, there were one study in mice in which the exposure of the animals was under
4333 the developmental until weaning period. In the Tier 3 study (Patel et al., 2013 [RefID 5697]), F0 dams
4334 were exposed from GD11.5 to pups' weaning (PND21) with an assumed dose of 0.5, 5.0 or 200 µg/kg
4335 bw per day. At weaning, there was no effects of any doses on BMI for F1 female offspring, whereas
4336 BMI was decreased for F1 males with 200 µg/kg bw per day only. At months 1–4, F1 females had
4337 decreased BMI with 200 µg/kg bw per day at 2 months of age only, and increased BMI with 5.0 µg at
4338 4 months only, whereas F1 males had decreased BMI 200 µg/kg bw at 1 month only and increased
4339 BMI with 5.0 µg/kg bw at 4 months only. At termination, there were no effects on BMI with any doses
4340 either for females or males. The CEP Panel judged this evidence as Inadequate.

4341 For the endpoint body fat mass, there were six studies in which the exposure of the animals was during
4342 the developmental until weaning period. Of these, three studies were on mice, two studies were on
4343 rats and one study on sheep.

4344 Two mouse studies were Tier 2 (Susiarjo et al., 2015 [RefID 7022] and Junge et al., 2018 [RefID
4345 12262]) and one study was Tier 3 (Rubin et al., 2017 [RefID 6319]). In Susiarjo et al. (2015) [RefID
4346 7022], F0 dams were exposed to 10 or 10000 µg/kg bw per day from 2 weeks before mating and
4347 through pregnancy and lactation. At PND98–PND117, whole body fat % was increased in male offspring
4348 with both doses, whereas in female offspring, there were no effects of either dose. In Junge et al.
4349 (2018) [RefID 12262], BPA doses in µg/kg bw per day were calculated from intake of drinking water.
4350 F0 dams were exposed to 0.45 µg/kg bw per day from 1 week before mating to giving birth. In F1 male
4351 and female offspring combined, whole body fat mass as % of bw was increased (+53%). In the mouse
4352 study Rubin et al. (2017) [RefID 6319], BPA was given via subcutaneous osmotic minipumps, thus, the
4353 doses were converted to oral doses, and the mice were exposed to BPA in two different periods. First,
4354 F0 dams were exposed to 60, 600, 5600 or 55600 µg/kg bw per day (converted oral doses) from GD8
4355 to LD16. The overall effect of treatment (all doses) on whole body fat (g) was increased in F1 males
4356 and females, but not statistically significantly different from controls for any individual doses in either
4357 sex. In males, the strongest effect was with 5,600 µg/kg bw per day (increased 19%, 20% and 17.7%
4358 at PND50, 90 and 130, respectively). The overall effect of treatment (all doses) on whole body fat (%)
4359 was increased in F1 males and females, but not significantly different from controls for any individual
4360 doses in either sex. In males, the strongest effect was with 5600 µg/kg bw per day (increased 15%,
4361 17% and 13% vs. controls at PND50, 90 and 130, respectively). When the mice were exposed to the
4362 same doses from GD8 to LD16 and PND21–PND35 (Rubin et al., 2017 [RefID 6319]), the overall
4363 treatment effect (all doses) on whole body fat mass (g) or with any individual doses were not significant
4364 in F1 males. In F1 females, the overall treatment effect on body fat mass (g) was increased, and the
4365 dose 600 µg/kg bw was increased vs. controls and increased vs. 60 and 56000 µg/kg bw at PND141.
4366 The overall treatment effect on whole body fat (%) was increased in F1 males and females, but there
4367 were no significant differences between the doses in F1 males. For whole body fat (%) in F1 females,
4368 600 µg/kg bw was significantly increased vs. 60 µg/kg bw, and nearly significantly increased vs. control
4369 and 56000 µg/kg bw with P adjusted for hyperactive females ($p = 0.007$).

4370 In the Tier 1 rat study Desai et al. (2018) [RefID 11817], BPA doses in µg/kg bw per day were calculated
4371 from intake of drinking water. F0 dams were exposed to 250 µg/kg bw per day from 2 weeks before
4372 mating and through pregnancy and lactation. In male offspring, whole body fat (%) was increased at
4373 both weeks 3 and 24, whereas in female offspring, no effects were observed at either time point. In
4374 the Tier 2 rat study Santos-Silva et al. (2018) [RefID 13047], BPA was given by subcutaneous injections,
4375 thus, the doses were converted to oral doses. The offspring were exposed on PND3–PND15 through
4376 milk from F0 dams exposed to 1785 and 178500 µg/kg bw per day. At PND180, no effects on whole
4377 body fat (%) were found with either dose in either sex.

4378 In the Tier 2 study Veiga-Lopez et al. (2016) [RefID 7424], in their 'study 2', F0 sheep were exposed
4379 to 40500 µg/kg bw per day GD30–GD90 (term at ~147 days). In female offspring, no effects were
4380 found on subcutaneous, visceral or both (total) fat mass volume by whole-body scans at age 19 months
4381 on either normal diet or an overfed diet. Male offspring were not studied.

4382 There were two studies (both Tier 2) that showed no effects on body fat mass in two species and four
4383 studies (one Tier 1, two Tier 2, one Tier 3) that showed increasing body fat mass in two species.
4384 Apparently, the lower dose range (0.45–10000 µg/kg bw) had more effects than the higher dose range

4385 (500–178500 µg/kg bw), but these dose ranges overlapped. The CEP Panel judged this endpoint as
4386 ALAN.

4387 For the endpoint **visceral adipocyte size**, there were four studies in which the exposure of the animals
4388 was under the developmental until weaning period. One study was on mice, two studies were on rats
4389 and one study was on sheep.

4390 In the Tier 1 mouse study by van Esterik et al. (2014) [RefID 7393], the F1 female mice were exposed
4391 to HFD weeks 17–23, whereas the F1 males were given normal diet the whole time. Both sexes were
4392 exposed to 3, 10, 30, 100, 300, 1000 or 3000 µg/kg bw per day during gestation and lactation. In
4393 females, decreased adipocyte size was observed in perirenal WAT (–17%) (not known with which
4394 dose(s)), whereas no effects were seen in males with any dose. The effects in females were reported
4395 as MDR by the authors (data only shown with symbols).

4396 Both rat studies were Tier 1. In Lejonklou et al. (2017) [RefID 3975], no effects on gonadal white
4397 adipocyte size as number/area were observed in female or male rats exposed to either 0.5 or 50 µg/kg
4398 bw from GD3.5 to PND22. In the other rat study from Desai et al. (2018) [RefID 11817], BPA doses in
4399 µg/kg bw per day were calculated from intake of drinking water. The males were exposed to 250 µg/kg
4400 bw via their dams from 2 weeks before mating and through pregnancy and lactation and increased
4401 retroperitoneal adipocyte size as area was observed at week 3.

4402 In the Tier 2 sheep study Veiga-Lopez et al. (2016) [RefID 7424], the female sheep were given normal
4403 diet or were overfed from 14 weeks until termination at ~21.5 months of age. They were exposed to
4404 40500 µg/kg bw per day GD30–GD90 (term at ~147 days), and increased area and diameter of visceral
4405 adipocytes at termination at 21 months of age were found both when given normal diet or overfed.

4406 No effects on visceral adipocyte size were observed in one study (Tier 1, in rats), increased effects
4407 were seen in two studies (one Tier 1 in rats, one Tier 2 in sheep) and decreased effects were seen in
4408 one study (Tier 1 in mice). Based on this limited evidence, the CEP Panel judged this endpoint as ALAN.

4409 For the endpoint **non-visceral adipocyte size**, there were two rat studies in which the exposure of
4410 the animals was under the developmental until weaning period.

4411 In the Tier 1 study Lejonklou et al. (2017) [RefID 3975], the rats were exposed to 0.5 or 50 µg/kg bw
4412 from GD3.5 to PND22 and terminated at 5 weeks of age. In females, increased inguinal white adipocytes
4413 (iWAT) size was observed with 0.5 µg, but not with 50 µg, whereas in males, there were no effects
4414 with either dose, although the highest dose was significantly higher than the lowest dose. No effects
4415 were observed on interscapular WAT with either dose as number/area in either sex. In the other Tier
4416 1 study by Dunder et al. (2018) [RefID 11866], the rats were exposed to 0.5 or 50 µg/kg bw from
4417 GD3.5–PND22 and studied at 2 weeks and 52 weeks of age. No effects on iWAT size as number/area
4418 were observed with either dose at any time point with any sex.

4419 An increasing effect on non-visceral adipocyte size was observed in only one of the two available Tier
4420 1 rat studies, both studies used both sexes. The effect was observed in one of two adipose tissues in
4421 females only and only at the lowest of two doses tested. Based on this limited evidence, the CEP Panel
4422 judged this endpoint as Not Likely.

4423 The likelihood level ALAN was assigned for effects of BPA on body weight, Inadequate evidence for
4424 BMI, ALAN for effects on body fat mass, ALAN for visceral adipose size and Not Likely for non-visceral
4425 adipocyte size. Thus, in this exposure period, most, three of four, endpoints with available studies or
4426 adequate evidence were scored ALAN. The CEP Panel assigned a likelihood level of ALAN to the obesity
4427 effects of BPA in the exposure period developmental until weaning, thus, none of the endpoints included
4428 in this cluster was taken forward for BMD analysis. However, the ALAN endpoints were considered in
4429 the uncertainty analysis (see Appendix D).

4430 Developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood)

4431 For the endpoint body weight, there were 21 studies in which the exposure of the animals was under
4432 the developmental until adulthood period, 10 studies on mice and 11 studies on rats.

4433 There were seven studies that did not show any effects on body weight at all. Of these, there was one
4434 study on mice (Kasneci et al., 2017 [RefID 3399]), which was Tier 2. There were six studies on rats, of
4435 which there were three, two and one study, respectively, in Tier 1 (Ben-Jonathan, 2018 (NTP Grantee

4436 study) [RefID 13786]; Gonzalez-Cadavid, 2018 (NTP Grantee study) [RefID 13787] and Leung et al.,
4437 2020 [RefID 13789]), Tier 2 (Greenberg, 2018 (NTP Grantee study) [RefID 13785] and Auxietre et al.,
4438 2014 [RefID 305]) and Tier 3 (Wang J et al., 2014 [RefID 7640]). The doses that were found not to
4439 have an effect on body weight were in the range 0.5–25000 µg/kg bw per day.

4440 There were nine studies that showed increasing body weight after BPA exposure. Of these, there were
4441 six studies on mice, of which there were one, two and three studies, respectively, in Tier 1
4442 (Chatsantiprapa et al., 2016 [RefID 9822]), Tier 2 (Ke et al., 2016 [RefID 3447] and Patel et al., 2014
4443 [RefID 5695]) and Tier 3 (Biasiotto et al., 2016 [RefID 575]; Dobrzynska et al., 2018 [RefID 11837]
4444 and Patel et al., 2013 [RefID 5697]). There were three studies on rats, of which two, zero and one
4445 studies on Tier 1 (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370] and Boudalia et al.,
4446 2014 [RefID 670]), Tier 2 and Tier 3 (Jeong et al., 2017 [RefID 3133]), respectively. The doses that
4447 were found to increase the body weight were in the range 0.5–25000 µg/kg bw per day.

4448 The increasing effect observed in NTP Clarity Report (2018)/Camacho et al. (2019) [RefID 11370] was
4449 with 25000 µg/kg bw per day in the sensitivity analysis, i.e. above the cut-off value of 10000 µg/kg bw
4450 per day.

4451 There were five studies that showed decreased body weight after BPA exposure. Of these, there were
4452 three studies on mice, of which there was one in Tier 1 (Patel et al., 2015b [RefID 5698]), one in Tier
4453 2 (Patel et al., 2014 [RefID 5695]) and one in Tier 3 (Dobrzynska et al., 2018 [RefID 11837]). In Patel
4454 et al. (2015b) [RefID 5698], the reduced body weight was seen in F1 males on a HFD, but not on a
4455 normal diet. There were two studies on rats, which both were in Tier 1 (Boudalia et al., 2014 [RefID
4456 670] and Dere et al., 2018 [RefID 11815]). The doses that were found to decrease the body weight
4457 were in the range 0.6 + 1.1 (two periods) to 250000 µg/kg bw per day.

4458 The decreasing effect observed in Dobrzynska et al. (2018) [RefID 11837] was with 20000 µg/kg bw
4459 per day, i.e. above the cut-off value of 10000 µg/kg bw per day.

4460 There was approximately the same number of studies that did not find any effects of BPA on body
4461 weight (seven), (three Tier 1, three Tier 2, one Tier 3), as found an increasing body weight (i.e. an
4462 obesogenic effect) (nine) (three Tier 1, two Tier 2, four Tier 3), and somewhat lower number of studies
4463 that observed a decreased effect (i.e. potentially a toxic effect) (five), (three Tier 1, one Tier 2, one
4464 Tier 3). The dose ranges were more or less in the same orders between these three categories of
4465 effects. Thus, the CEP Panel judged this endpoint as ALAN.

4466 For the endpoint **BMI**, there was two studies on mice in which the exposure of the animals was under
4467 the developmental until adulthood period. In the Tier 2 study Patel et al. (2014) [RefID 5695], the mice
4468 were exposed to 5 µg/kg bw per day on GD11.5–PND21, and 0.6 and 0.7 µg/kg bw per day for F1
4469 males and females (doses converted from drinking water), respectively, on PND21–4 months, and were
4470 given either normal diet or a HFD from PND21–4 months. On a normal diet, no effects were observed
4471 either in females or males at weaning, or at week 4, 8 or 12. On an HFD, there were no effects in
4472 females or males at weeks four and eight, and in females, no effects at week 12. In males, BMI was
4473 decreased at week 12. In the Tier 3 study Patel et al. (2013) [RefID 5697], F0 dams were exposed
4474 from GD11.5 to pups' weaning (PND21) and F1 female and male offspring were exposed directly further
4475 from PND21 to 4 months of age to assumed doses of 0.5 or 5.0 µg/kg bw per day. At weaning, there
4476 were no effects on BMI for F1 females and males of either dose. At months 1–4, BMI was increased
4477 both for F1 females and males with 5.0 µg/kg bw per day at 4 months only. At termination, there were
4478 no effects on BMI either for F1 females or males.

4479 Based on this limited evidence, with one Tier 2 study finding decreased effects on BMI on a HFD and
4480 no effects on a normal diet, and one Tier 3 study finding increased effects on normal diet, the CEP
4481 Panel judged this endpoint as ALAN.

4482 For the endpoint **body fat mass**, there was only one Tier 3 study on mice (Biasiotto et al., 2016 [RefID
4483 575]) in which the exposure of the animals was under the developmental until adulthood period. In
4484 this study, increased body fat mass was found only with the dose 5 µg/kg bw in F1 males, and no
4485 effects were found with 0.5, 50 or 500 µg/kg bw after exposure on PND90–PND140. Females were not
4486 studied. The CEP Panel considered this evidence as Inadequate.

4487 For the endpoint **visceral adipocyte size**, there was one study in which the exposure of the animals
4488 was under the developmental until adulthood period. This was a study on rats (Ben-Jonathan, 2018
4489 (NTP Grantee study) [RefID 13786]). In this Tier 1 study, the rats were exposed to 2.5, 25, 250, 2500
4490 or 25000 µg/kg bw per day from GD6 until PND90 or PND180. At both PND90 and PND180, no effects
4491 were observed in either F1 females (in periovarian adipose tissue) or in F1 males (in epididymal adipose
4492 tissue) with any doses. Based on this limited evidence, the CEP Panel judges this endpoint as Not Likely.

4493 For the endpoint **non-visceral adipocyte size**, there was one study in which the exposure of the
4494 animals was under the developmental until adulthood period. This was a Tier 1 study on rats [Ben-
4495 Jonathan, 2018 (NTP Grantee study) [RefID 13786]]. The rats were exposed to 2.5, 25, 250, 2500 or
4496 25000 µg/kg bw per day from GD6 until PND90 or PND180. At PND90, no effects in either F1 females
4497 or males on inguinal adipocyte size were observed with any doses. At PND180 (6 months), the only
4498 effect was decreased size of inguinal adipocytes with the highest dose 25000 µg/kg bw in females.
4499 Based on this limited evidence, the CEP Panel judged this endpoint as Not Likely for an obesogenous
4500 effect of BPA.

4501 The likelihood level ALAN was assigned for effects of BPA on body weight, ALAN for effects on BMI,
4502 Inadequate evidence for effects on body fat mass and Not Likely for both visceral and non-visceral
4503 adipocyte size. Thus, two of four endpoints with adequate evidence were scored ALAN and two were
4504 scored Not Likely. There was approximately the same number of studies that did not find any effects
4505 of BPA on body weight (seven), as found an increasing body weight (10), and somewhat lower number
4506 of studies that observed a decreased effect (five), thus, the weight of the evidence was judged clearly
4507 as ALAN for the endpoint body weight. The same was the case for BMI, were the limited evidence
4508 pointed in opposite directions. For visceral and non-visceral adipocyte size, the evidence was less
4509 convincing since the likelihood of both endpoints were based on only one study. The CEP Panel assigned
4510 a likelihood level of ALAN to the obesity effects of BPA in the exposure period developmental until
4511 adulthood, thus, none of the endpoints included in this cluster was taken forward for BMD analysis.
4512 However, the ALAN endpoints were considered in the uncertainty analysis (see Appendix D).

4513 Growth phase/young age exposure

4514 For the endpoint body weight, there were 16 studies in which the exposure of the animals was under
4515 the growth phase, eight studies on mice and eight studies on rats.

4516 There were 11 studies that did not show any effects on body weight at all. Of these, there were four
4517 studies on mice, of which there was one study in Tier 1 (Cetkovic-Cvrlje et al., 2017 [RefID 916]), two
4518 studies in Tier 2 (Dong et al., 2013 [RefID 1676] and Ke et al., 2016 [RefID 3447]) and one study in
4519 Tier 3 (Dobrzynska et al., 2014 [RefID 1645]). In Dong et al. (2013) [RefID 1676], BPA doses in µg/kg
4520 bw per day were calculated from intake of drinking water. There were seven studies on rats, of which
4521 two studies were Tier 1 (Gurmeet et al., 2014 [RefID 2502] and Ullah et al., 2018a [RefID 13281]) and
4522 five studies were Tier 2 (Yang YJ et al., 2014 [RefID 10269]; Zhang J et al., 2017 [RefID 8770]; Müller
4523 et al., 2018 [RefID 12781]; Brouard et al., 2016 [RefID 734] and Zaid et al., 2014 [RefID 10261]). In
4524 Brouard et al. (2016) [RefID 734], BPA was given by subcutaneous injections, and the doses were
4525 converted to oral doses. The doses that were found not to have an effect on body weight were in the
4526 range 0.5–100000 µg/kg bw per day.

4527 There were five studies that showed increased body weight after BPA exposure. Of these, there were
4528 four studies on mice, of which there were one, two and one studies, respectively, in Tier 1 (Yang et al.,
4529 2016 [RefID 8375]), Tier 2 (Lin Y et al., 2017 [RefID 4338] and Wyatt et al., 2016 [RefID 8080]) and
4530 Tier 3 (Rubin et al., 2017 [RefID 6319]). In Rubin et al. (2017) [RefID 6319], BPA was given via
4531 subcutaneous osmotic minipumps, and the doses were converted to oral doses. There was one study
4532 on rats (Ullah et al., 2018b [RefID 13282]), which was Tier 3. In this study, the doses used were not
4533 clear (in most places the doses 5000, 25000 and 50000 µg/kg bw were mentioned, but also 5000,
4534 50000 and 500000 µg/kg bw were mentioned once), and the BPA doses in µg/kg bw per day were
4535 calculated from intake of drinking water. The doses that were found to increase the body weight were
4536 in the range 2.5–5000 µg/kg bw per day.

4537 There were approximately twice as many studies that observed no effects of BPA on body weight (11),
4538 (three Tier 1, seven Tier 2, one Tier 3), as found an increasing effect on body weight (five), (one Tier

4539 1, two Tier 2, two Tier 3). The dose ranges overlapped in both effect categories. Thus, the CEP Panel
4540 judged this endpoint as ALAN.

4541 For the endpoint **BMI**, there were no studies in which the exposure of the animals was under the
4542 growth phase.

4543 For the endpoint **body fat mass**, there were two mouse studies in which the exposure of the animals
4544 was under the growth phase.

4545 In the Tier 1 study by Yang et al. (2016) [RefID 8375], mice were given a normal diet or a high-fat diet
4546 (HFD) and exposed to 5, 50, 500 or 5000 µg/kg bw per day for 30 days from 5 weeks of age. In males
4547 and females, whole-body fat mass (% bw) was increased with all doses on normal diet. However, the
4548 same study found no effects in either sex with any dose on a HFD. In the Tier 3 mouse study by Rubin
4549 et al. (2017) [RefID 6319], BPA was given via subcutaneous osmotic minipumps and the doses were
4550 converted to oral doses. The F0 dams were exposed to 60, 600, 5600 or 55600 µg/kg bw per day from
4551 GD8 to LD16 and PND21–PND35. The overall treatment effect on whole-body fat mass (g) or with any
4552 individual doses was not significant in F1 males. In F1 females, the overall treatment effect on body fat
4553 mass (g) was increased, and the dose 600 µg/kg bw was increased vs. controls and increased vs. 60
4554 and 55600 µg/kg bw at PND141. The overall treatment effect on whole body fat (%) was increased in
4555 F1 males and females, but there were no significant differences between the doses in F1 males. For
4556 whole body fat (%) in F1 females, 600 µg/kg bw was significantly increased vs. 60 µg/kg bw, and
4557 nearly significantly increased vs. control and 55600 µg/kg bw with P adjusted for hyperactive females
4558 (P = 0.007).

4559 An increasing effect on body fat mass was demonstrated in parts only of two mouse studies. The
4560 increasing effect was seen at a large dose range on normal diet, but not on HFD, in both sexes in a
4561 Tier 1 mouse study. An increasing effect on body fat mass was only seen with a lower dose on only
4562 females in the other Tier 3 mouse study. Based on this limited evidence, the CEP Panel judged this
4563 endpoint as ALAN.

4564 For the endpoint **visceral adipocyte size**, there were two studies on mice in which the exposure of
4565 the animals was under the growth phase.

4566 In this Tier 2 study by Patel et al. (2014) [RefID 5695], the mice were given HFD from PND21–4 months
4567 and exposed to 5 µg/kg bw per day of BPA on GD11.5–PND21, and 0.6 and 0.7 µg/kg bw per day for
4568 F1 males and females (doses converted from drinking water), from PND21 to 4 months. There were no
4569 effects on mesenteric adipocyte area (% of vehicle) in either females or males. In the other Tier 2 study
4570 by Wyatt et al. (2016) [RefID 8080], two strains of male mice, C57BL/6J and DBA/2J, were exposed to
4571 28 µg/kg bw per day from 4 weeks to 11 weeks of age. Perigonadal adipocyte size as area was increased
4572 in both strains.

4573 No effects on **visceral adipocyte size** was demonstrated on either sex given a HFD in one Tier 2
4574 mouse study, whereas increasing effects were seen on two strains of male mice in another Tier 2 study.
4575 Based on this limited evidence, the CEP Panel judged this endpoint as ALAN.

4576 For the endpoint **non-visceral adipocyte size**, there was one study in which the exposure of the
4577 animals was under the growth phase. This was a Tier 3 study on mice (Yang et al., 2016 [RefID 8375]),
4578 in which male mice were exposed to 5, 50, 500 or 5000 µg/kg bw per day for 30 days from 5 weeks of
4579 age and given a normal diet. The diameter of iWAT was increased with all doses. Females and HFD
4580 were not studied. The CEP Panel considered this evidence as Inadequate.

4581 There were no studies on BMI in this exposure period. The likelihood level ALAN was assigned for
4582 effects of BPA on body weight, ALAN for effects on body fat mass and visceral adipocyte size and
4583 Inadequate evidence for non-visceral adipocyte size. In this exposure period, all three of the endpoints
4584 with available studies or adequate data were scored ALAN. The CEP Panel assigned a likelihood level
4585 of ALAN to the obesity effects of BPA in the exposure period growth phase, thus, none of the endpoints
4586 included in this cluster was taken forward for BMD analysis. However, the ALAN endpoints were
4587 considered in the uncertainty analysis (see Appendix D).

4588 Adult exposure (after puberty)

4589 For the endpoint body weight, there were 56 studies in which the exposure of the animals was as
4590 adults; 22 studies on mice, 31 studies on rats, one study on rabbits and two studies on monkeys (one
4591 study each on rhesus macaques and common marmosets, respectively). In Park et al. (2018) [RefID
4592 12869], it was not clear from the study if the mice were exposed to 10000 µg/kg bw per day by i.p.
4593 injection or by gavage, and, thus, the dose and administration route could not be taken into
4594 consideration in the WoE. In Rubin et al. (2017) [RefID 6319] and Kass et al. (2015) [RefID 3402], BPA
4595 was given via subcutaneous osmotic minipumps and the doses were converted to oral doses. In Santos-
4596 Silva et al. (2018) [RefID 13047] and Chouhan et al. (2015) [RefID 1216], BPA was given by
4597 subcutaneous or intraperitoneal injections, respectively, and the doses were converted to oral doses.
4598 In Meng Y et al. (2018) [RefID 12707], Desai et al. (2018) [RefID 11817], Dong et al. (2013) [RefID
4599 1676] and Ullah et al. (2018b) [RefID 13282], BPA doses in µg/kg bw per day were calculated from
4600 intake of drinking water.

4601 There were 43 studies that did not show any effects on body weight at all. Of these, there were 18
4602 studies on mice, of which there were seven, six and five studies, respectively, in Tier 1 (van Esterik et
4603 al., 2014 [RefID 7393]; Cetkovic-Cvrlje et al., 2017 [RefID 916]; MacKay et al., 2017 [RefID 4767];
4604 Chatsantiprapa et al., 2016 [RefID 9822]; Wang HF et al., 2016 [RefID 7618]; Xu XH et al., 2015 [RefID
4605 8232] and Meng Y et al., 2018 [RefID 12707]), Tier 2 (Dong et al., 2013 [RefID 1676]; Marmugi et al.,
4606 2014 [RefID 4884]; Lv Q et al., 2017 [RefID 4697]; Kim MJ et al., 2014 [RefID 3534]; Ma et al., 2018
4607 [RefID 12637]; Hu et al., 2018 [RefID 11119]) and Tier 3 (Liang et al., 2018 [RefID 12508]; Rubin et
4608 al., 2017 [RefID 6319]; Yuan et al., 2018 [RefID 13593]; Chouhan et al., 2015 [RefID 1216] and
4609 Dobrzynska et al., 2014 [RefID 1645]). There were 23 studies on rats, of which there were 15, seven
4610 and one studies, respectively, in Tier 1 (Vahdati Hassani et al., 2017 [RefID 2614]; Cao et al., 2015
4611 [RefID 831]; Ding et al., 2014 [RefID 1620]; Altamirano et al., 2015 [RefID 155]; NTP Clarity Report,
4612 2018/Camacho et al., 2019 [RefID 11370]; Desai et al., 2018 [RefID 11817]; Song SZ et al., 2014
4613 [RefID 6829]; Jiang et al., 2014 [RefID 3190]; Xia et al., 2014 [RefID 8103]; Poormoosavi et al., 2018
4614 [RefID 12913]; Lejonklou et al., 2017 [RefID 3975]; Bernardo et al., 2015 [RefID 533]; Boudalia et al.,
4615 2014 [RefID 670]; Olukole et al., 2018 [RefID 12841] and Brandt et al., 2014 [RefID 700]), Tier 2 (Ding
4616 et al., 2016 [RefID 1621]; Santos-Silva et al., 2018 [RefID 13047]; Zhang J et al., 2017 [RefID 8770];
4617 Kass et al., 2015 [RefID 3402]; Jiang et al., 2016 [RefID 3179]; Huang DY et al., 2018 [RefID 12167]
4618 and Santamaria et al., 2016 [RefID 6448]) and Tier 3 (Wu et al., 2016 [RefID 8036]). The study Kass
4619 et al. (2015) [RefID 3402] contained two experiments. In addition, there was one study on rabbits
4620 (Fang et al., 2014 [RefID 1914]), which was Tier 2, and one study on monkeys (rhesus macaques),
4621 which was Tier 2. The doses that were found not to have an effect on body weight were in the range
4622 0.03–178500 µg/kg bw per day.

4623 There were seven studies that showed increasing body weight after BPA exposure. Of these, there
4624 were three studies on mice (Lin Y et al., 2017 [RefID 4338]; Wyatt et al., 2016 [RefID 8080] and Park
4625 et al., 2018 [RefID 12869]), all three studies were in Tier 2. There were four studies on rats, of which
4626 one study was in Tier 1 (Thilagavathi et al., 2017 [RefID 9247]), two studies were in Tier 2 (Mahmoudi
4627 et al., 2018 [RefID 12656] and Amraoui et al., 2018 [RefID 11503]) and one study was in Tier 3 (Ullah
4628 et al., 2018b [RefID 13282]). The doses that were found to increase the body weight were in the range
4629 2.5–10000 µg/kg bw per day.

4630 There were six studies that showed decreasing body weight after BPA exposure. Of these, there was
4631 one study on mice (Sivashanmugan et al., 2017 [RefID 6773]), which was in Tier 2. There were four
4632 studies on rats, of which one and three studies were in Tier 1 (Thilagavathi et al., 2017 [RefID 9247])
4633 and Tier 2 (Abdel-Rahman et al., 2018 [RefID 11426]; Kazemi et al., 2017 [RefID 3441] and Ola-Davies
4634 and Olukole, 2018 [RefID 12837]), respectively. In addition, one study on monkeys (common
4635 marmosets) (Vijaykumar et al., 2017 [RefID 7477]) was in Tier 3. The doses that were found to
4636 decrease the body weight were in the range 2.5–400000 µg/kg bw per day.

4637 The decreasing effects observed in Sivashanmugan et al. (2017) [RefID 6773] were with 100000 and
4638 400000 µg/kg bw per day, i.e. above the cut-off value of 10000 µg/kg bw per day.

4639 The large majority of the studies showed no effects on body weight (43 studies), (22 Tier 1, 15 Tier 2,
4640 six Tier 3), whereas approximately the same number of studies showed increased effect (i.e. an
4641 obesogenic effect) (seven studies), (one Tier 1, five Tier 2, one Tier 3) and a decreased effect (i.e.

4642 potentially a toxic effect) (six studies) (one Tier 1, four Tier 2, one Tier 3). The dose ranges overlapped
4643 for these three categories of effects. Thus, the CEP Panel judged this endpoint as Not Likely.

4644 For the endpoint **BMI**, there was one study in which the exposure of the animals was as adults. In this
4645 Tier 2 rat study Mahmoudi et al. (2018) [RefID 12656], it was not explained how BMI was measured,
4646 except that it was given as g/cm². In this study Mahmoudi et al. (2018) [RefID 12656], male rats were
4647 exposed to 10000 µg/kg bw from 7 weeks to ~15.5 weeks of age. BMI was increased (23 ± 0.02%).
4648 Females were not studied. The CEP Panel considered this evidence as Inadequate.

4649 For the endpoint **body fat mass**, there were two studies in which the exposure of the animals was as
4650 adults, one on mice and one on rats.

4651 In the Tier 2 study by Kim MJ et al. (2014) [RefID 3534], the apolipoprotein E knockout (ApoE^{-/-})
4652 mouse model for atherosclerosis was used. Male ApoE^{-/-} mice were exposed to 50 µg/kg bw per day
4653 of BPA from 8 weeks of age for 12 weeks on a high-fat/high cholesterol diet. No effects were observed
4654 on whole body fat %. Females were not studied.

4655 In the Tier 1 study by Desai et al. (2018) [RefID 11817], BPA doses in µg/kg bw per day were calculated
4656 from intake of drinking water. The F0 rat dams were exposed from 2 weeks before mating and through
4657 pregnancy and lactation with 250 µg/kg bw per day. No effect on whole body fat weight was observed
4658 in these F0 dams.

4659 No effects of BPA of body fat mass were observed in the two available studies (one Tier 1 study on rats
4660 and one Tier 2 study on mice). Based on this limited evidence, the CEP Panel judged this endpoint as
4661 Not Likely.

4662 For the endpoint **visceral adipocyte size**, there were three studies in which the exposure of the
4663 animals was as adults. One study was on rats, one study on mice and one study on rabbits.

4664 In the Tier 2 study by Wyatt et al. (2016) [RefID 8080], two strains of male mice, C57BL/6J and DBA/2J,
4665 were exposed to 28 µg/kg bw per day from 4 weeks to 11 weeks of age. Perigonadal adipocyte size as
4666 area was increased in both strains. Females were not studied.

4667 In the Tier 1 study by Desai et al. (2018) [RefID 11817], BPA doses in µg/kg bw per day were calculated
4668 from intake of drinking water. The F0 rat dams were exposed to 250 µg/kg bw per day from 2 weeks
4669 before mating and through pregnancy and lactation. No effect on retroperitoneal adipocyte size as area
4670 was found.

4671 In the Tier 2 study by Fang et al. (2014) [RefID 1914], male rabbits were exposed to 400 µg/kg bw
4672 per day 12 weeks from 14 weeks of age. Visceral adipocyte diameter was non-significantly increased
4673 (+11%). The visceral adipocyte size distribution (%) was shifted towards larger cells and there were
4674 significant differences in some of the cell size categories. Females were not studied.

4675 No effects on visceral adipocyte size were observed in one Tier 1 study on female rats, whereas
4676 increasing effects were seen in two Tier 2 studies; on two strains of male mice and on male rabbits.
4677 Based on this limited evidence, the CEP Panel considered this endpoint ALAN.

4678 For the endpoint **non-visceral adipocyte size**, there were two studies in which the exposure of the
4679 animals was as adults. One study was on rats and one study on rabbits.

4680 In the Tier 2 study by Mahmoudi et al. (2018) [RefID 12656], male rats were exposed to 10000 µg/kg
4681 bw per day from seven to ~15.5 weeks of age. Increased adipocyte size (not stated which adipocytes)
4682 as increased adipocyte surface area and as decreased number/area were observed. Females were not
4683 studied.

4684 In the Tier 2 study by Fang et al. (2014) [RefID 1914], male rabbits were exposed to 400 µg/kg bw
4685 per day 12 weeks from 14 weeks of age. Increased diameter of subcutaneous adipocytes was observed.
4686 Subcutaneous adipocyte size distribution (%) curves were shifted towards larger cells, with mostly
4687 significant cell size differences. Females were not studied.

4688 Increasing effects on non-visceral adipocyte size were demonstrated in two Tier 2 studies with single
4689 doses on male rats and rabbits. Based on this limited evidence, the CEP Panel judged this endpoint as
4690 ALAN.

4691 The likelihood level Not Likely was assigned for effects of BPA on body weight, Inadequate evidence
4692 for BMI, Not Likely for effects on body fat mass and ALAN for both visceral adipocyte size and non-
4693 visceral adipocyte size. In this exposure period, the large majority of the studies (43) on body weight
4694 showed no effects, whereas approximately the same number of studies (seven) showed increasing
4695 effect and decreasing effect (six), thus, the weight of the evidence was considered clearly Not Likely
4696 for the endpoint body weight. The same was the case for body fat mass, where the only two studies
4697 available found no effects. For visceral adipocyte size, the effects were not consistent and for non-
4698 visceral adipocyte size the evidence was less convincing. The CEP Panel assigned a likelihood level of
4699 Not Likely to the obesity effects of BPA during adult exposure, thus, none of the endpoints included in
4700 this cluster was taken forward for BMD analysis.

4701 Indirect (germline) exposure

4702 For the endpoint **body weight**, there were six studies in which the exposure of the animals was via
4703 the germline, two studies on mice and four studies on rats.

4704 There were five studies that did not show any effects on body weight at all. Of these, there were two
4705 studies on mice (Susiarjo et al., 2015 [RefID 7022] and Ziv-Gal et al., 2015 [RefID 9143]), in Tier 2
4706 and 3, respectively. There were three studies on rats (Li GQ et al., 2014 [RefID 4039]; Mao et al., 2015
4707 [RefID 4864] and Auxietre et al., 2014 [RefID 305]), which all were Tier 2. The doses that were found
4708 not to have an effect on body weight were in the range 0.5–10000 µg/kg bw per day.

4709 There was one Tier 1 rat study that showed increased body weight of F2 offspring after BPA exposure
4710 (Altamirano et al., 2015 [RefID 155]). The dose 2.6 µg/kg bw per day had increasing effect when given
4711 during the lactation period, whereas 0.03 µg/kg bw per day had no effect.

4712 One Tier 1 rat study showed an increasing effect on body weight in F2 offspring, whereas five studies
4713 found no effects (four Tier 2 studies; one on mice and three on rats, and one Tier 3 study on mice).
4714 Thus, the CEP Panel judged this endpoint as Not Likely.

4715 For the endpoint **BMI**, there were no studies in which the exposure of the animals was via the germline.

4716 For the endpoint **body fat mass**, there was one study in which the exposure of the animals was via
4717 the germline. In this Tier 2 mouse study Susiarjo et al. (2015) [RefID 7022], F0 dams were exposed to
4718 10 or 10000 µg/kg bw per day from 2 weeks before mating and through pregnancy and lactation. At
4719 PND98–117, whole body fat % was increased in F2 males only with highest dose, 10000 µg/kg bw. F2
4720 females were not studied.

4721 Increasing effects on body fat mass was only seen in F2 males with one dose in one Tier 2 mouse
4722 study. Based on this limited evidence, the CEP Panel considered this endpoint ALAN.

4723 For the endpoints **visceral adipocyte size and non-visceral adipocyte size**, there were no studies
4724 in which the exposure of the animals was via the germline.

4725 There were no studies on BMI, visceral adipocyte size and non-visceral adipocyte size in this exposure
4726 period. The likelihood level Not Likely was assigned for effects of BPA on body weight and ALAN for
4727 effects on body fat mass. In this exposure period, the evidence for the endpoint body weight was
4728 considered more convincing than the evidence for body fat mass, since for body weight an increasing
4729 effect was found for a single dose in only one study and five studies found no effects, whereas for body
4730 fat mass an increasing effect was found only in males in one study. The CEP Panel assigned a likelihood
4731 level of Not Likely to the obesity effects of BPA in the exposure period germline exposure, thus, none
4732 of the endpoints included in this cluster was taken forward for BMD analysis.

4733 Overall cluster selection of the endpoints/studies for BMD analysis for obesity

4734 Overall, the CEP Panel assigned a likelihood level to the obesity effects of BPA of ALAN in the exposure
4735 periods developmental until weaning, developmental until adulthood and growth phase and Not Likely
4736 after after exposure as adults and exposure via the germline. The overall likelihood across all exposure
4737 periods, i.e. the highest likelihood given in the cluster obesity, was ALAN.

4738 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
4739 Likely effect of BPA on the cluster obesity in any exposure period, therefore, none of the endpoints
4740 included in this cluster was taken forward for BMD analysis.

4741 **Fat deposition in the liver**

4742 The specific endpoints that were included for the effects of BPA in the cluster fat deposition in the liver
4743 were liver cholesterol and liver triglycerides.

4744 In this assessment, there are in total 12 unique studies that reported data included in the cluster fat
4745 deposition in the liver. Among these studies, there were 12 results on liver cholesterol and 17 results
4746 on liver triglycerides, in total 29 results.

4747 For all endpoints within the cluster fat deposition in the liver, 18 results were on mice, of which five
4748 had exposure during the developmental until weaning period, two had exposure during the
4749 developmental until adulthood period, five had exposure during the growth phase, six were exposed as
4750 adults and none had exposure via the germline. Of the 11 results on rats, three had exposure during
4751 the developmental until weaning period, none had exposure during the developmental until adulthood
4752 period, none had exposure during the growth phase, eight were exposed as adults and none had
4753 exposure via the germline.

4754 Developmental exposure (pre-natal and/or post-natal until weaning)

4755 For the endpoint **liver cholesterol**, there were three studies in which the exposure of the animals was
4756 under the developmental until weaning period. Two studies were on mice and one study was on rats.

4757 Both mouse studies were Tier 2. In one of the mouse studies (Ke et al., 2016 [RefID 3447]), males
4758 were exposed to 0.5 µg/kg bw per day from birth to 8 weeks of age. Increased liver cholesterol was
4759 observed at 8 weeks of age. Females were not studied. In the other mouse study Meng Z et al. (2018)
4760 [RefID 12708], females were exposed to 100 µg/kg bw per day from GD7 to PND21 and terminated at
4761 5 weeks of age. No effects on liver cholesterol were observed. Males were not studied.

4762 In the Tier 2 rat study Santos-Silva et al. (2018) [RefID 13047], BPA was given by subcutaneous
4763 injections to the dams and the doses were converted to oral doses. The offspring were exposed on
4764 PND3–PND15 through milk from F0 dams exposed to 1785 and 178500 µg/kg bw per day. No effects
4765 of either dose were seen in either sex at PND15 or PND21. At PND180, decreased liver cholesterol was
4766 observed in females only with 1785 µg/kg bw, whereas in males, no effects were observed with either
4767 dose.

4768 The three Tier 2 studies had either no effects (on female mice, males not tested, single dose), increasing
4769 effects (on male mice, females not tested, single dose) or decreasing effects (effect only in one of two
4770 doses on female rats, no effects of any doses on males; two other timepoints, no effects in either sex
4771 of either doses) on liver cholesterol. Based on this limited evidence, the CEP Panel judged the effects
4772 on this endpoint as Not Likely.

4773 For the endpoint **liver triglycerides**, there were five studies in which the exposure of the animals was
4774 under the developmental until weaning period. Three studies were on mice and two studies were on
4775 rats.

4776 Two Tier 2 mouse studies using single dose found no effects with 0.5 µg/kg bw per day (Ke et al., 2016
4777 [RefID 3447]) and with 100 µg/kg bw per day (Meng Z et al., 2018 [RefID 12708]). The third mouse
4778 study by Rubin et al. (2017) [RefID 6319] was Tier 3 and on females only. BPA was given via
4779 subcutaneous osmotic minipumps and the doses were converted to oral doses. The mice were exposed
4780 in two different periods, either on GD8 to LD16, or GD8 to LD16 and PND21–PND35. They did not find
4781 any effects after exposure from GD8 to LD16, but after exposure to 600, 5600 or 55600 µg/kg bw from
4782 GD8 to LD16 and PND21–PND35, increased liver triglycerides were found with 5600 and 55600 µg/kg
4783 bw, but not with 600 µg/kg bw.

4784 Of the two rat studies, one was Tier 1 (Jiang et al., 2014 [RefID 3190]) and found increased liver
4785 triglycerides in male rats at 15 and 26 weeks, but not at 3 weeks, after exposure to 40 µg/kg bw per
4786 day from GD0 to weaning PND21. Females were not studied. The other rat study Santos-Silva et al.
4787 (2018) [RefID 13047] was Tier 2. BPA was given by subcutaneous injections and the doses were
4788 converted to oral doses. No effects were found of either dose at PND15 or PND21 in male or female
4789 offspring after exposure on PND3–PND15 through milk from F0 dams exposed to 1785 and 178500
4790 µg/kg bw.

4791 Approximately the same number of studies had no effect on liver triglycerides (three studies), all Tier
4792 2 (two on mice, both single dose, either males or females; one on rats, both sexes, two doses, two
4793 time points), and had an increasing effect (two studies), one Tier 1 (male rats, single dose, at two of
4794 three timepoints), one Tier 3 (mouse, females only, effects of two of three doses after the longest
4795 exposure period). The dose ranges overlapped in the three effect categories. The CEP Panel judged the
4796 likelihood of this endpoint as ALAN.

4797 The likelihood level Not Likely was assigned for effects of BPA on liver cholesterol and ALAN for effects
4798 on liver triglycerides. In this exposure period, the evidence for liver triglycerides was considered more
4799 convincing than the evidence for liver cholesterol. The CEP Panel assigned a likelihood level of ALAN to
4800 the fat deposition in the liver effects of BPA in the exposure period developmental until weaning, thus,
4801 none of the endpoints included in this cluster was taken forward for BMD analysis. However, the ALAN
4802 endpoints were considered in the uncertainty analysis (see Appendix D).

4803 Developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood)

4804 For the endpoint **liver cholesterol**, there was one study in which the exposure of the animals was
4805 under the developmental until adulthood period. This was a Tier 2 study on mice (Ke et al., 2016 [RefID
4806 3447]), in which male mice were exposed to 0.5 µg/kg bw per day from birth to 10 months of age (40
4807 weeks). Liver cholesterol was increased at 10 months. Females were not studied. The CEP Panel
4808 considered this evidence as Inadequate.

4809 For the endpoint **liver triglycerides**, there was one study in which the exposure of the animals was
4810 under the developmental until adulthood period. This Tier 2 study was on male mice (Ke et al., 2016
4811 [RefID 3447]). After exposure to 0.5 µg/kg bw per day of BPA from birth to 10 months of age (40
4812 weeks), increased liver triglycerides were found at 10 months. Females were not studied. The CEP
4813 Panel considered this evidence as Inadequate.

4814 The CEP Panel considered that there was Inadequate evidence on the effects of BPA on fat deposition
4815 in the liver in the exposure period developmental until adulthood, thus, none of the endpoints included
4816 in this cluster was taken forward for BMD analysis.

4817 Growth phase/young age exposure

4818 For the endpoint **liver cholesterol**, there were two studies on mice in which the exposure of the
4819 animals was under the growth phase.

4820 In the Tier 2 study by Ke et al. (2016) [RefID 3447], male mice were exposed to 0.5 µg/kg bw per day
4821 from birth to 8 weeks of age. Liver cholesterol was increased at 8 weeks. Females were not studied. In
4822 the other Tier 2 study Lin Y et al. (2017) [RefID 4338], male mice were exposed to 50 µg/kg bw per
4823 day from 3 weeks of age for 90 days until ~16 weeks. No effect on liver cholesterol was observed.
4824 Females were not studied.

4825 The two Tier 2 studies found either no effects or increasing effects on liver cholesterol, both studying
4826 only a single dose, only in males. Based on this limited evidence, the CEP Panel judged this endpoint
4827 as ALAN.

4828 For the endpoint liver triglycerides, there were three studies in which the exposure of the animals was
4829 under the growth phase, all on mice.

4830 In one Tier 2 study (Ke et al., 2016 [RefID 3447]), in males exposed to 0.5 µg/kg bw per day from
4831 birth to 8 weeks of age, no effects on liver triglycerides were observed at 8 weeks. Females were not
4832 studied. In another Tier 2 study on males (Lin Y et al., 2017 [RefID 4338]), increased liver triglycerides
4833 were observed after exposure to 50 µg/kg bw per day from 3 weeks of age for 90 days until ~16 weeks.
4834 Females were not studied. The third study was Tier 3 (Rubin et al., 2017 [RefID 6319]), in which BPA
4835 was given via subcutaneous osmotic minipumps and the doses converted to oral doses. After exposure
4836 to 600, 5600 or 55600 µg/kg bw from GD8 to LD16 and PND21–PND35, increased liver triglycerides
4837 were found with 5600 and 55600 µg/kg bw, but not with 600 µg/kg bw, in females. Males were not
4838 studied. The dose 55600 µg/kg bw per day showing increasing effect in Rubin et al. (2017) [RefID
4839 6319] was above the cut-off value of 10000 µg/kg bw per day.

4840 One study found no effects on liver triglycerides (Tier 2, male mice, single dose) and two studies
4841 demonstrated increasing effects (one Tier 2, male mice, single dose, and one Tier 3, female mice,

4842 effects with two of three doses, however, one of the doses was above the oral cut-off). Based on this
4843 limited evidence, the CEP Panel judged this endpoint as ALAN.

4844 The likelihood level ALAN was assigned for the effects of BPA on liver cholesterol and ALAN for effects
4845 on liver triglycerides. The CEP Panel assigned a likelihood level of ALAN to the fat deposition in the liver
4846 effects of BPA in the exposure period growth phase, thus, none of the endpoints included in this cluster
4847 was taken forward for BMD analysis. However, the ALAN endpoints were considered in the uncertainty
4848 analysis (see Appendix D).

4849 Adult exposure (after puberty)

4850 For the endpoint **liver cholesterol**, there were six studies in which the exposure of the animals was
4851 as adults. Two studies were on mice and four studies were on rats.

4852 One Tier 2 mouse study (Lin Y et al., 2017 [RefID 4338]), in which males were exposed to 50 µg/kg
4853 bw per day from 3 weeks of age for 90 days until ~16 weeks, found no effects. Females were not
4854 studied. The other Tier 2 mouse study Marmugi et al. (2014) [RefID 4884], in which males were
4855 exposed to 5, 50, 500 or 5000 µg/kg bw per day from 6 weeks of age for 8 months found increased
4856 liver cholesterol only with 5000 µg/kg bw and no effects with the other doses. Females were not studied.

4857 Among the studies on rats, two studies used only a single dose (both 50 µg/kg bw) and were Tier 1
4858 (Ding et al., 2014 [RefID 1620]) or Tier 2 (Ding et al., 2016 [RefID 1621]) and showed no effects on
4859 males either on a normal diet or a HFD. The third rat study using a single dose was Tier 2 (Mahmoudi
4860 et al., 2018 [RefID 12656]) and found increased liver cholesterol after exposure of males to 10000
4861 µg/kg bw per day from seven to ~15.5 weeks of age. Females were not studied. The fourth rat study
4862 Santos-Silva et al. (2018) [RefID 13047] was Tier 2. BPA was given by subcutaneous injections and the
4863 doses were converted to oral doses. In F0 dams exposed to 1785 and 178500 µg/kg bw per day, no
4864 effects of either dose were found on PND21 of their offspring.

4865 Among the Tier 2 studies, it seemed that longer exposure (8 months or 8½ weeks) to higher doses of
4866 BPA (5000 or 10000 µg/kg bw) increased liver cholesterol in male mice and rats, respectively. However,
4867 one other mouse study (Tier 2, males only, single dose) and three other rat studies (one Tier 1 and
4868 one Tier 2, both single dose, males only, on two diets, and one Tier 2, females only, with two doses)
4869 failed to show any effects after exposure for similar time periods. Thus, most studies showed no effects
4870 and the CEP Panel judged this endpoint as Not Likely.

4871 For the endpoint **liver triglycerides**, there were eight studies in which the exposure of the animals
4872 was as adults. Four studies were on mice and four studies were on rats.

4873 All four studies on mice were Tier 2 and only performed in males. In one of these studies Marmugi et
4874 al. (2014) [RefID 4884], no effects with any dose were observed on males after exposure to 5, 50, 500
4875 or 5000 µg/kg bw per day from 6 weeks of age for 8 months. Another study Lv Q et al. (2017) [RefID
4876 4697], found increased liver triglycerides after exposure of males to all doses (5, 50 or 500 µg/kg bw
4877 per day) from 6 weeks of age for 8 weeks. A third study on males (Lin Y et al., 2017 [RefID 4338]),
4878 observed increased liver triglycerides after exposure to 50 µg/kg bw per day from 3 weeks of age for
4879 90 days until ~16 weeks. The fourth study performed on males (Yang SM et al., 2017 [RefID 8398])
4880 observed increased triglyceride content/protein in the liver after exposure to 5, 50 or 500 µg/kg bw per
4881 day from 6 weeks of age for 8 weeks. The increase was +58, +69 and +83% for the three doses,
4882 respectively, and showed dose–response.

4883 Among the rat studies, one Tier 1 study (Ding et al., 2014 [RefID 1620]) and one Tier 2 study (Ding et
4884 al., 2016 [RefID 1621]) found no effects after a single dose (50 µg/kg bw) on either a normal diet or a
4885 HFD. In another Tier 2 rat study (Santos-Silva et al., 2018 [RefID 13047]), BPA was given by
4886 subcutaneous injections and the doses were converted to oral doses. No effects were found on F0 dams
4887 exposed on their offspring's PND3–PND15 to 1785 and 178500 µg/kg bw on PND21 of their offspring.
4888 The fourth rat study, Tier 2 (Mahmoudi et al., 2018 [RefID 12656]) found increased liver triglycerides
4889 after exposure of males to 10000 µg/kg bw per day from seven to ~15.5 weeks of age.

4890 The same number of studies showed no effects on **liver triglycerides** (four studies), one Tier 1 study
4891 and one Tier 2 study on rats (both males only, single dose, on two diets), two Tier 2 studies (one in
4892 male mice and one in female rats, three or two doses), or increasing effects (four studies), all Tier 2

4893 (three on male mice, one on male rats, two single dose, two with two or three doses). The dose ranges
4894 overlapped in the three effect categories. The CEP Panel judged this endpoint as ALAN.

4895 The likelihood level Not Likely was assigned for the effects of BPA on liver cholesterol and ALAN for
4896 effects on liver triglycerides. In this exposure period, the evidence for liver triglycerides was considered
4897 more convincing than the evidence for liver cholesterol (more studies, more of them with two or three
4898 doses vs. one dose). The CEP Panel assigned a likelihood level of ALAN to the fat deposition in the liver
4899 effects of BPA in the exposure period adult exposure, thus, none of the endpoints included in this cluster
4900 was taken forward for BMD analysis. However, the ALAN endpoints were considered in the uncertainty
4901 analysis (see Appendix D).

4902 Indirect (germline) exposure

4903 For the endpoints, **liver cholesterol and liver triglyceride**, there were no studies in which the
4904 exposure of the animals was via the germline.

4905 Overall cluster selection of the endpoints/studies for BMD analysis for fat deposition in the liver

4906 Overall, the CEP Panel assigned a level to the fat deposition in the liver effects of BPA of ALAN in the
4907 exposure periods developmental until weaning, growth phase and adulthood, and there was Inadequate
4908 Evidence to conclude on the likelihood of effects in the exposure period developmental until adulthood.
4909 There were no studies with germline exposure. The overall likelihood across all exposure periods, i.e.
4910 the highest likelihood given in the cluster fat deposition in the liver, was ALAN.

4911 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
4912 Likely effect of BPA on the cluster fat deposition in the liver in any exposure period, therefore, none of
4913 the endpoints was taken forward for BMD analysis.

4914 **Glucose regulation**

4915 The specific endpoints that were included for the effects of BPA in the cluster glucose regulation were
4916 glucose level, insulin level, intraperitoneal (i.p.) GTT (ipGTT), intravenous (i.v.) GTT (ivGTT), oral GTT
4917 (oGTT) or GTT where the route of administration was not stated in the studies, i.p. insulin tolerance
4918 test (ipITT), pancreas weight, β -cell morphometry and α -cell morphometry.

4919 Glucose level was measured in full blood, serum or plasma in animals that were fasting or fed, or this
4920 information was not stated in the publications. Insulin level was measured in serum or plasma in fasting
4921 or fed animals or this information was not stated. In addition, functional tests were performed. In GTT,
4922 glucose was administered via various routes (i.p., i.v. or oral) or the route was not stated in the
4923 publications, and changes in glucose levels over time were measured. In insulin tolerance test (ITT),
4924 insulin was injected via the i.p. route, and changes in glucose levels over time were measured. In some
4925 studies, also insulin levels were measured in the ipGTT tests. Since insulin is important to regulate the
4926 blood glucose levels, the relationship between glucose and insulin is tight and effects on both should
4927 be evaluated together. Pancreas weight is also a metabolic endpoint, since insulin and glucagon are
4928 produced in the β -cells and α -cells in the pancreas, respectively. The endpoint β -cell morphometry was
4929 given in the studies as insulin-stained β -cells as ratio of β -cells/total pancreas, β -cell mass, β -cell fraction
4930 (%) or as insulin-reactive cells in pancreas (%). The endpoint α -cell morphometry was given as
4931 glucagon-stained α -cells as ratio of α -cells/total pancreas or as α -cell mass.

4932 In this assessment, there were in total 38 unique studies that reported data included in the cluster
4933 glucose regulation. Among these studies, there were 40 results on glucose level, 29 on insulin level, 16
4934 on ipGTT, one on ivGTT, two on oGTT, one on GTT where the administration route was not stated,
4935 seven on ipITT, five on pancreas weight, eight on β -cell morphometry and two on α -cell morphometry,
4936 in total 111 results.

4937 For all endpoints within the cluster glucose regulation, 54 results were on mice, of which 16 studies
4938 had exposure during the developmental until weaning period, five had exposure during the
4939 developmental until adulthood period, 13 had exposure during the growth phase, 16 were exposed as
4940 adults and four had exposure via the germline. Of the 54 studies on rats, 25 studies had exposure
4941 during the developmental until weaning period, seven had exposure during the developmental until
4942 adulthood period, two had exposure during the growth phase, 11 were exposed as adults and nine had
4943 exposure via the germline. In addition, one publication with two experiments from sheep had exposure

4944 during the developmental until weaning period, one study on rabbits and one on monkeys (common
4945 marmosets) had both exposure as adults.

4946 Developmental exposure (pre-natal and/or post-natal until weaning)

4947 For the endpoint **glucose level**, there were 12 studies in which the exposure of the animals was under
4948 the developmental until weaning period. Three studies were on mice, nine studies were on rats.

4949 Of the mouse studies, two were in Tier 2 and showed no effects of BPA. In one Tier 2 study (Susiarjo
4950 et al., 2015 [RefID 7022]), the F0 dams were exposed to 10 µg/kg bw or 10000 µg/kg bw per day from
4951 2 weeks before mating and through pregnancy and lactation. In the male offspring, no effects were
4952 observed with any dose. Female offspring were not studied. In the other Tier 2 study (Ke et al., 2016
4953 [RefID 3447]), only male mice were exposed to 0.5 µg/kg bw per day from birth to 8 weeks of age. No
4954 effect was observed on the glucose level at 8 weeks.

4955 One mouse study was Tier 3 and showed increasing effect. In this study by Rubin et al. (2017) [RefID
4956 6319], BPA was given via subcutaneous osmotic minipumps and the doses were converted to oral
4957 doses. The mice were exposed in two different periods, either on GD8 to LD16, or GD8 to LD16 and
4958 PND21–PND35. On female offspring of F0 dams exposed to 600 or 5600 µg/kg bw per day from GD8
4959 to LD16, there were no effects at 28 or 34 weeks. When the dams were exposed to the same doses
4960 from GD8 to LD16 and PND21–PND35, the glucose level was increased with 600 µg/kg bw at 34 weeks,
4961 but not at 28 weeks in the female offspring. No effects were seen with 5600 µg/kg bw at either time
4962 point. Male offspring were not studied.

4963 Five of the rat studies found no effects on the glucose level. Two of the Tier 1 rat studies (Altamirano
4964 et al., 2015 [RefID 155] and NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) found no
4965 effects of BPA on glucose levels, of which one used two doses (Altamirano et al., 2015 [RefID 155])
4966 and one used six doses (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). In the third
4967 Tier 1 rat study Ding et al. (2014) [RefID 1620], F0 sires were exposed to 50 µg/kg bw for 35 weeks
4968 on a normal or a HFD. In male or female offspring, no effects were observed at 10 weeks of age with
4969 either diet. Two Tier 2 rat studies found no effects (Ma et al., 2013 [RefID 4748] and Greenberg, 2018
4970 (NTP Grantee study) [RefID 13785]), using a single dose or five doses, respectively.

4971 Three rat studies found an increasing effect on the glucose level. One Tier 1 study (Song SZ et al.,
4972 2014 [RefID 6829]) exposed only male rats to 80 or 758 µg/kg bw from GD6 through lactation and
4973 terminated them at PND100. With the 758 µg/kg bw dose only, the glucose level was increased at the
4974 puberty stage (PND50), whereas it was increased with both doses at the adult stage (PND100). Females
4975 were not studied. In the Tier 2 study Mao et al. (2017) [RefID 4865], the rats were exposed to 40
4976 µg/kg bw GD0–PND21. In male offspring, the glucose level was increased at 3 weeks, but not at 9
4977 weeks, whereas in female offspring, no effects were observed at 3 weeks or 9 weeks. In the Tier 2
4978 study Zhang et al. (2013) [RefID 8798], only female rats were exposed on GD6–PND21 to 33 or 310
4979 µg/dam during gestation, 80 or 758 µg/kg bw per day at the end of gestation. In F1 females, the
4980 glucose level was increased with 80 µg, but not with 758 µg at 7 weeks of age. Males were not studied.

4981 In the Tier 2 study by Santos-Silva et al. (2018) [RefID 13047], BPA was given by subcutaneous
4982 injections and the doses were converted to oral doses. The F0 rat dams were exposed on PND3–PND15
4983 to 1785 and 178500 µg/kg bw per day. At PND15 and PND21, no effects of either dose were found on
4984 either sex exposed through milk from the dams. At PND180, decreased glucose levels were found with
4985 178500 µg/kg bw in females, whereas no effects were found on males with either dose.

4986 The dose 178500 µg/kg bw inducing a decreasing effect in Santos-Silva et al. (2018) [RefID 13047]
4987 was above the cut-off value of 10000 µg/kg bw per day.

4988 Most the studies (seven) showed no effects on the glucose level (three Tier 1 and four Tier 2), only
4989 four studies found an increasing effect demonstrated in one sex only (one Tier 1, two Tier 2 and one
4990 Tier 3) and one Tier 2 study found a decreasing effect. The CEP Panel judged this endpoint as Not
4991 Likely.

4992 For the endpoint insulin level, 11 studies in which the exposure of the animals was under the
4993 developmental until weaning period. Four studies were on mice and seven studies were on rats.

4994 Among the mouse studies, there was one Tier 1 study (van Esterik et al., 2014 [RefID 7393]), two Tier
4995 2 studies (Susiarjo et al., 2015 [RefID 7022] and Ke et al., 2016 [RefID 3447]) and one Tier 3 study
4996 (Rubin et al., 2017 [RefID 6319]). In van Esterik et al. (2014) [RefID 7393], the F1 female mice were
4997 given a HFD weeks 17–23, whereas the F1 males were given a normal diet the whole time. Two of
4998 these studies found increasing effects of BPA on insulin levels (Susiarjo et al., 2015 [RefID 7022] and
4999 Rubin et al., 2017 [RefID 6319]). In Susiarjo et al. (2015) [RefID 7022], the dams were exposed to 10
5000 or 10000 µg/kg bw per day from 2 weeks before mating and through pregnancy and lactation. In F1
5001 males, increased insulin level was found with 10 µg/kg bw, but not with 10000 µg/kg bw (i.e. it was
5002 nearly significant, $p = 0.08$). F1 females were not studied. In Rubin et al. (2017) [RefID 6319], BPA
5003 was given via subcutaneous osmotic minipumps and the doses converted to oral doses. The mice were
5004 exposed in two different periods, either on GD8 to LD16, or GD8 to LD16 and PND21–PND35. When
5005 the dams were exposed to 600 or 5600 µg/kg bw for day from GD8 to LD16, no effects on insulin level
5006 were observed at 28 or 34 weeks in F1 females. When the same exposure was on GD8 to LD16 and
5007 PND21–PND35, the insulin level was increased in F1 females with 600 µg/kg bw both at 28 and 34
5008 weeks, but not with 5600 µg/kg bw at either time point. F1 males were not studied.

5009 Among the rat studies, three were Tier 1 (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
5010 11370]; Song SZ et al., 2014 [RefID 6829] and Ding et al., 2014 [RefID 1620]), of which one used a
5011 single dose (Ding et al., 2014 [RefID 1620]), one used two doses (Song SZ et al., 2014 [RefID 6829])
5012 and one used six doses (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). In Ding et al.
5013 (2014) [RefID 1620], the rats were given a normal diet or a HFD. Only one of the Tier 1 studies found
5014 any effects of BPA on insulin levels. In Song SZ et al. (2014) [RefID 6829], male rats were exposed to
5015 80 or 758 µg/kg bw from GD6 through lactation and terminated at PND100. The insulin level was
5016 increased only with the dose 758 µg/kg bw at the puberty stage (PND50) and with both doses at the
5017 adult stage (PND100). There were four Tier 2 studies on rats, of which two studies found increasing
5018 effects on the insulin level. In Ma et al. (2013) [RefID 4748], F0 dams were exposed to 50 µg/kg bw
5019 through gestation and lactation, and increased insulin level was found both at three and 21 weeks in
5020 F1 males. F1 females were not studied. In Zhang et al. (2013) [RefID 8798], F0 dams were exposed
5021 to 33 or 310 µg/dam during gestation, 80 or 758 µg/kg bw per day at the end of gestation, on GD6–
5022 PND21. In female offspring, the insulin level was increased with both doses. Male offspring were not
5023 studied.

5024 In the Tier 2 study by Santos-Silva et al. (2018) [RefID 13047], BPA was given by subcutaneous
5025 injections and the doses were converted to oral doses. The F0 dams were exposed on PND3–PND15 to
5026 1785 and 178500 µg/kg bw per day. At PND15 and PND21, on F1 females exposed through milk, no
5027 effects were found of either dose, in F1 males, the insulin level decreased with both doses at PND15,
5028 but no effects were seen on PND21. At PND180, the insulin levels were decreased with both doses in
5029 F1 females, whereas no effects were seen on F1 males with either dose.

5030 The dose 178500 µg/kg bw inducing decreasing effect in Santos-Silva et al. (2018) [RefID 13047] was
5031 above the cut-off value of 10000 µg/kg bw per day.

5032 The same number of studies had no effects on the insulin level (five studies; three Tier 1, two Tier 2),
5033 or increasing effects (five studies; one Tier 1, three Tier 2, one Tier 3), and one study had a decreasing
5034 effect (Tier 2). The dose ranges overlapped in the three effect categories. Thus, the CEP Panel judged
5035 this endpoint as ALAN.

5036 For the endpoint ipGTT, there were six studies in which the exposure of the animals was under the
5037 developmental until weaning period. Four studies were on mice, and two studies were on rats.

5038 The one Tier 1 single-dose study on mice (van Esterik et al., 2014 [RefID 7393]) found no effects of
5039 BPA on the AUC for glucose in ipGTT. The same was the result in the Tier 2 single-dose study on mice
5040 (Wang D et al., 2018 [RefID 13341]). In this study, BPA was given by subcutaneous injections and the
5041 doses were converted to oral doses. The same result of no effects was also found in the Tier 3 study
5042 on mice using two doses (Rubin et al., 2017 [RefID 6319]). In this study, BPA was given via
5043 subcutaneous osmotic minipumps and the doses were converted to oral doses and the exposure period
5044 was GD8 to LD16 and PND21–PND35. The only mouse study finding an effect, was in the Tier 2 study
5045 Susiarjo et al. (2015) [RefID 7022], in which the F0 dams were exposed to 10 or 10000 µg/kg bw per
5046 day from 2 weeks before mating and through pregnancy and lactation. In F1 male offspring, the 10000

5047 µg/kg bw dose increased glucose AUC at PND98–PND117, whereas the increased AUC by 10 µg/kg bw
5048 nearly reached significance ($p = 0.06$). In F1 female offspring, no effects were found with either dose.

5049 Both rat studies found increasing effects of BPA in the ipGTT. In the Tier 2 study by Chang et al. (2016)
5050 [RefID 965], the F0 dams were exposed to 10 µg/kg bw through gestation and lactation. The AUC for
5051 glucose was not increased for F1 males and females combined at 4 weeks and 8 weeks, whereas it was
5052 increased at 20 weeks. For both male and female offspring separately, there were no effects at 4 weeks
5053 and 8 weeks, but glucose AUC was increased at 20 weeks in both sexes. In the Tier 2 study by Mao et
5054 al. (2017) [RefID 4865], the rats were exposed to 40 µg/kg bw on GD0–PND21. Both male and female
5055 offspring had increased glucose AUC at 9 weeks, but none had increased AUC at 3 weeks.

5056 The same number of studies had no effects in ipGTT (three studies; one in each Tier) or showed
5057 increasing effects (three studies; all Tier 2). The dose ranges overlapped in the three effect categories.
5058 Thus, the CEP Panel judged this endpoint as ALAN.

5059 For the endpoint ivGTT, there was only one study in which the exposure of the animals was under the
5060 developmental until weaning period. Two experiments, called study 1 and study 2, were reported in
5061 sheep in this Tier 2 study Veiga-Lopez et al. (2016) [RefID 7424]. In study 1, female F0 sheep were
5062 exposed to 4050, 40500 or 405000 µg/kg bw per day at GD30–GD90 (term at ~147 days). Glucose
5063 and insulin were measured after 24 hours fasting at the pre-pubertal stage (age ~6 weeks) and after
5064 48 hours fasting at the post-pubertal stage (age ~13 months) in female F1 offspring on a normal diet.
5065 In study 2, female F0 sheep were exposed to 40500 µg/kg bw per day at GD30–GD90 (term at ~147
5066 days). Glucose and insulin were measured after 48 hours fasting at ~15 months of age in female F1
5067 offspring on either a normal diet or on an overfed diet. In study 1, on normal diet, only the dose 4050
5068 µg increased glucose at 6 weeks of age (pre-pubertal stage). At 13 months (post-pubertal stage), there
5069 were no effects on glucose of any doses. In the same study, at 6 weeks of age (pre-pubertal stage)
5070 there were no effects on insulin of any dose in female sheep. At 13 months (post-pubertal stage), the
5071 dose 40500 µg/kg bw increased significantly ($p < 0.05$) mean cumulative insulin response at 20 minutes
5072 and nearly significantly at 180 minutes ($p = 0.054$), increased significantly mean cumulative
5073 insulin/glucose ratio response at both 20 and 180 minutes, and increased acute insulin response (AIR)
5074 at five minutes. The other doses had no effects on insulin levels in this study. In study 2, at 15 months
5075 (post-pubertal stage), there were no effects on glucose on either diet (normal or overfed). In the same
5076 study, no differences were observed in insulin responses (acute response, AUC, cumulative response
5077 over 180 minutes, insulin sensitivity index or insulin/glucose ratio) on either diet. Male F1 offspring
5078 were not studied in either study 1 or 2.

5079 One Tier 2 study with two experiments in female sheep showed mixed results of no effects or increased
5080 effects in **ivGTT** in various parts of the experiments. Based on this limited evidence, the CEP Panel
5081 judged this endpoint as ALAN.

5082 For the endpoint **oGTT**, there was one study in which the exposure of the animals was under the
5083 developmental until weaning period. This Tier 2 study was on mice (Malaisé et al., 2017 [RefID 4815]).
5084 The F0 dams were exposed to 50 µg/kg bw on GD15 to PND21. In male offspring, glucose AUC was
5085 increased at PND35. Female offspring were not studied. The CEP Panel considered this evidence as
5086 Inadequate.

5087 For the endpoint **GTT without know administration route**, there were no studies in which the
5088 exposure of the animals was under the developmental until weaning period.

5089 For the endpoint **ipITT**, there were five studies in which the exposure of the animals was under the
5090 developmental until weaning period. Three studies were on mice and two studies were on rats.

5091 Of the three mouse studies, two were Tier 2 (Malaisé et al., 2017 [RefID 4815] and Wang D et al.,
5092 2018 [RefID 13341]) and one was Tier 3 (Rubin et al., 2017 [RefID 6319]). The single-dose study with
5093 2222 µg/kg bw (Wang D et al., 2018 [RefID 13341]), in which BPA was given by subcutaneous
5094 injections and the doses converted to oral doses, found no effects on glucose AUC in males. In Malaisé
5095 et al. (2017) [RefID 4815], F0 dams were exposed to 50 µg/kg on bw GD15 to PND21. In F1 males,
5096 glucose AUC was increased at PND125. F1 females were not studied. In Rubin et al. (2017) [RefID
5097 6319], BPA was given via subcutaneous osmotic minipumps and the doses were converted to oral
5098 doses. The mice were exposed in two different periods, either on GD8 to LD16, or GD8 to LD16 and
5099 PND21–PND35. When F0 dams were exposed to 600 or 5600 µg/kg bw per day on GD8–LD16, no

5100 effects were found on the glucose level in female offspring with either dose at 40 weeks. Male offspring
5101 were not studied. When F0 dams were exposed to the same doses on GD8–LD16 and PND21–PND35,
5102 female offspring showed an overall increased effect of BPA on the glucose level, a nearly significant
5103 increase ($P = 0.07$) with 600 $\mu\text{g}/\text{kg}$ bw for overall comparison and increased glucose levels with 600
5104 and 5600 $\mu\text{g}/\text{kg}$ bw at 45 minutes, at 40 weeks. Male offspring were not studied.

5105 The two rat studies were both Tier 2 using a single dose. In Mao et al. (2017) [RefID 4865], F0 dams
5106 were exposed to 40 $\mu\text{g}/\text{kg}$ bw on GD0–PND21. In F1 males, at 9 weeks, glucose levels (as % of 0
5107 value) were increased at 15, 45 and 60 minutes, and at 3 weeks at 10 and 60 minutes. In F1 females,
5108 glucose levels were increased at 9 weeks at 30, 45 and 60 minutes, but not at 3 weeks for any measured
5109 time points. In Chang et al. (2016) [RefID 965], the F0 dams were exposed to 10 $\mu\text{g}/\text{kg}$ bw through
5110 gestation and lactation. In F1 males and females combined, at 4 weeks and 8 weeks, there were no
5111 effects on glucose AUC, whereas it was increased at 20 weeks. In F1 males separately, at 4 weeks,
5112 there were no effects, whereas at 8 weeks and 20 weeks, glucose AUC was increased. In F1 females
5113 separately, there were no effects on glucose AUC at either 4, 8 or 20 weeks.

5114 Three Tier 2 and one Tier 3 studies in two species (mouse, rat) found increasing effects and only one
5115 Tier 2 study did not find an effect in ipITT. However, all studies except the Tier 3 study were with only
5116 one dose and mostly studied or demonstrating effects in only one sex. The CEP Panel judged this
5117 endpoint as ALAN.

5118 For the endpoint pancreas weight, there were two studies, both on rats, in which the exposure of the
5119 animals was under the developmental until weaning period.

5120 In the Tier 2 study by Greenberg (2018) (NTP Grantee study) [RefID 13785], the rats were exposed to
5121 2.5, 25, 250, 2500 or 25000 $\mu\text{g}/\text{kg}$ bw from GD6 until PND21 (stop-dose study) and kept unexposed to
5122 1 year. No effects of any doses were observed in either sex. In the other Tier 2 study Chang et al.
5123 (2016) [RefID 965], the rats were exposed to 10 $\mu\text{g}/\text{kg}$ bw through gestation and lactation. For males
5124 and females combined, no effects on pancreas to body weight ratio were observed on GD15.5.

5125 No effects were demonstrated on pancreas weight in the two available Tier 2 studies, using both sexes
5126 of rats with one or five doses. Based on this limited evidence, the CEP Panel judged this endpoint as
5127 Not Likely.

5128 For the endpoint β -cell morphometry, there were three studies in which the exposure of the animals
5129 was under the developmental until weaning period. One study was on mice and two studies were on
5130 rats.

5131 In the Tier 1 study on male mice by Bansal et al. (2017) [RefID 9499], F0 dams were exposed to 10 or
5132 10000 $\mu\text{g}/\text{kg}$ bw per day from 2 weeks before mating until PND21. In male offspring, β -cell mass/body
5133 weight was decreased with 10 $\mu\text{g}/\text{kg}$ bw, but not with 10000 $\mu\text{g}/\text{kg}$ bw. Females were not studied.

5134 In the Tier 2 rat study by Chang et al. (2016) [RefID 965], they were exposed to 10 $\mu\text{g}/\text{kg}$ bw through
5135 gestation and lactation and examined at birth. For female and male offspring combined, absolute β -cell
5136 mass at birth was decreased. In the other Tier 2 study (Greenberg, 2018 (NTP Grantee study) [RefID
5137 13785]), the rats were exposed to 2.5, 25, 250, 2500 or 25000 $\mu\text{g}/\text{kg}$ bw from GD6 until PND21 (stop-
5138 dose study) and kept unexposed to 1 year. Insulin-stained β -cells as ratio of β -cells/total pancreas was
5139 decreased in females with 25000 μg , whereas no effects were observed with the other doses. In males,
5140 no effects were observed of any doses. For β -cell mass, there were no effects of any doses in either
5141 sex.

5142 The dose 25000 $\mu\text{g}/\text{kg}$ bw inducing decreasing effect in (Greenberg, 2018 (NTP Grantee study) [RefID
5143 13785]) was above the cut-off value of 10000 $\mu\text{g}/\text{kg}$ bw per day.

5144 Decreasing effects were observed in three studies (one Tier 1 mouse study and two Tier 2 rat studies).
5145 However, the decreasing effects were only studied or observed on combined sexes at birth and on one
5146 sex of adults, did not show consistent results between sexes, showed effects with very varying doses
5147 (10 $\mu\text{g}/\text{kg}$ bw, and 25000 $\mu\text{g}/\text{kg}$ bw, above the oral cut-off) and not consistent results for related
5148 parameters. Based on this limited and inconsistent evidence, the CEP Panel judged this endpoint as
5149 ALAN.

5150 For the endpoint α -cell morphometry, there was one study in which the exposure of the animals was
5151 under the developmental until weaning period. This Tier 2 study was on rats (Greenberg, 2018 (NTP
5152 Grantee study) [RefID 13785]). They were exposed to 2.5, 25, 250, 2500 or 25000 $\mu\text{g}/\text{kg}$ bw from GD6
5153 until PND21 (stop-dose study) and kept unexposed to 1 year. Glucagon-stained α -cells as ratio of α -
5154 cells/total pancreas was increased in males with 2.5 and 25 $\mu\text{g}/\text{kg}$ bw, but no effects were observed
5155 with the other doses. In the females, no effects were observed with any doses. For α -cell mass, no
5156 effects of any doses were observed in either sex.

5157 Increasing effects on α -cell morphometry were seen in one Tier 2 rat study in only one sex and on one
5158 of two related parameters. Based on this limited evidence, the CEP Panel judged this endpoint as ALAN.

5159 There were no studies in which the administration route for GTT was not mentioned in this exposure
5160 period. The likelihood level Not Likely was assigned for effects of BPA on glucose level, ALAN for effects
5161 on insulin level, ALAN for ipGTT, ALAN for ivGTT, Inadequate evidence for oGTT, ALAN for ipITT, Not
5162 Likely for pancreas weight and ALAN for both β -cell morphometry and α -cell morphometry. In this
5163 exposure period, the majority, six of eight, endpoints for which there were available studies or adequate
5164 evidence were scored ALAN.

5165 The CEP Panel assigned a likelihood level of ALAN to the glucose regulation effects of BPA in the
5166 exposure period developmental until weaning, thus, none of the endpoints included in this cluster was
5167 taken forward for BMD analysis. However, the ALAN endpoints were considered in the uncertainty
5168 analysis (see Appendix D).

5169 Developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood)

5170 For the endpoint **glucose level**, there were four studies in which the exposure of the animals was
5171 under the developmental until adulthood period. Two studies were on mice and two studies were on
5172 rats.

5173 In the Tier 2 study by Ke et al. (2016) [RefID 3447], male mice were exposed to 0.5 $\mu\text{g}/\text{kg}$ bw per day
5174 from birth to 10 months of age (40 weeks). The glucose level was increased at 10 months. Females
5175 were not studied. The other Tier 2 study on mice of both sexes using a single dose and a normal diet
5176 or a HFD (Patel et al., 2014 [RefID 5695]), found no effects of BPA on the glucose level in either sex
5177 on either diet.

5178 One Tier 1 study on rats using six doses of BPA (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
5179 11370]) and one Tier 2 study on rats using five doses (Greenberg, 2018 (NTP Grantee study) [RefID
5180 13785]), did not find any effects on glucose level with any doses in either sex.

5181 No effects on glucose level were observed in one Tier 1 and two Tier 2 studies, all on both sexes and
5182 two of them with a large dose range, whereas an increasing effect was seen only in one Tier 2 study
5183 using a single dose on one sex. Based on this limited evidence, the CEP Panel judged this endpoint as
5184 Not Likely.

5185 For the endpoint insulin level, there were three studies in which the exposure of the animals was under
5186 the developmental until adulthood period. One study was on mice and two studies were on rats.

5187 The only study that found an effect was the Tier 2 mouse study Ke et al. (2016) [RefID 3447], in which
5188 male mice were exposed to 0.5 $\mu\text{g}/\text{kg}$ bw per day from birth to 10 months of age (40 weeks). The
5189 insulin level was increased at 10 months. Females were not studied.

5190 One rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) was Tier 1, and one rat
5191 study (Greenberg, 2018 (NTP Grantee study) [RefID 13785]) was Tier 2, using six and five doses,
5192 respectively, and none of these studies found any effects of BPA on the insulin level.

5193 No effects on insulin level were observed in one Tier 1 and one Tier 2 study, both studies on both sexes
5194 and with a large dose range, whereas increasing effects were seen only in one Tier 2 study using a
5195 single dose on one sex. Based on this limited evidence, the CEP Panel judged this endpoint as Not
5196 Likely.

5197 For the endpoint ipGTT, there was only one study in which the exposure of the animals was under the
5198 developmental until adulthood period. This Tier 2 study Patel et al. (2014) [RefID 5695] was performed
5199 on mice, which were given either a normal diet or a HFD. The mice were exposed to 5 $\mu\text{g}/\text{kg}$ bw per

5200 day on GD11.5–PND21, and 0.6 and 0.7 µg/kg bw per day for F1 males and females (doses converted
5201 from drinking water), from PND21–4 months. Neither male nor female F1 offspring showed an effect
5202 on glucose AUC when the mice were given a normal diet. In the same experiment, when the mice were
5203 given a HFD, there was no effect in male offspring, and in female offspring, glucose AUC was increased
5204 only at the time point 120 minutes. The CEP Panel considered this evidence as Inadequate.

5205 For the endpoints ivGTT, oGTT, glucose GTT without know administration route, ipITT and pancreas
5206 weight there were no studies in which the exposure of the animals was under the developmental until
5207 adulthood period.

5208 In the Tier 3 study on mice (Biasiotto et al., 2016 [RefID 575]), males were exposed to 0.5, 5, 50 or
5209 500 µg/kg bw per day from week 2 of pregnancy to birth and then from weaning for 140 days. Increased
5210 pancreas weight as fold increase was observed with 500 µg/kg bw, but no effects were found with the
5211 other doses. Females were not studied.

5212 In the Tier 2 study by Greenberg (2018) (NTP Grantee study) [RefID 13785], the rats were exposed to
5213 2.5, 25, 250, 2500 or 25000 µg/kg bw from GD6 until PND21 and further until 12 months continuously.
5214 No effects of any doses were observed in either sex.

5215 No effects on **pancreas weight** were observed in a Tier 2 study on either sex with a large dose range,
5216 whereas an increasing effect was seen only in one Tier 3 study, studying only one sex. Based on this
5217 limited evidence, the CEP Panel judged this endpoint as Not Likely.

5218 For the endpoint β-cell morphometry, there was one study in which the exposure of the animals was
5219 under the developmental until adulthood period. This Tier 2 study Greenberg (2018) (NTP Grantee
5220 study) [RefID 13785] was on rats. The rats were exposed to 2.5, 25, 250, 2500 or 25000 µg/kg bw
5221 from GD6 until PND21 and further until 12 months continuously. For insulin-stained β-cells as ratio of
5222 β-cells/total pancreas, no effects of any doses were seen in either sex. For β-cell mass, no effects were
5223 seen of any dose in either sex.

5224 No effects were demonstrated on two parameters for β-cell morphometry in the only available Tier 2
5225 study on either sex with a large dose range. Based on this limited evidence, the CEP Panel judged this
5226 endpoint as Not Likely.

5227 For the endpoint α-cell morphometry, there was one study in which the exposure of the animals was
5228 under the developmental until adulthood period. This Tier 2 study was on rats (Greenberg, 2018 (NTP
5229 Grantee study) [RefID 13785]). The rats were exposed to 2.5, 25, 250, 2500 or 25000 µg/kg bw from
5230 GD6 until PND21 and further until 12 months continuously. For glucagon-stained α-cells as ratio of α-
5231 cells/total pancreas, no effects of any doses were observed in either sex. However, α-cell mass was
5232 increased in females with 2.5, 250 and 25000 µg/kg bw, with no effects of the other doses. In males,
5233 there were no effects of any doses.

5234 The dose 25,000 µg/kg bw inducing increasing effect in Greenberg (2018) (NTP Grantee study) [RefID
5235 13785] was above the cut-off value of 10000 µg/kg bw per day.

5236 Increasing effects were demonstrated on only one of two parameters for α-cell morphometry on only
5237 one of the sexes in only one Tier 2 study. Based on this limited evidence, the CEP Panel judged this
5238 endpoint as ALAN.

5239 There were no studies on ivGTT, oGTT, in which the administration route for GTT was not mentioned
5240 and ipITT in this exposure period. The likelihood level Not Likely was assigned for effects of BPA on
5241 glucose level, Not Likely for effects on insulin level, Inadequate evidence for ipGTT, Not Likely for
5242 pancreas weight, Not Likely for β-cell morphometry and ALAN for α-cell morphometry. In this exposure
5243 period, the majority, four of five, of the endpoints for which there were available studies or adequate
5244 evidence were scored Not Likely. The CEP Panel assigned a likelihood level of Not Likely to the glucose
5245 regulation effects of BPA in the exposure period developmental until adulthood, thus, none of the
5246 endpoints included in this cluster was taken forward for BMD analysis. However, the ALAN endpoints
5247 were considered in the uncertainty analysis (see Appendix D).

5248 Growth phase/young age exposure

5249 For the endpoint glucose level, there were five studies, all on mice, in which the exposure of the animals
5250 was under the growth phase.

5251 Of the five studies on mice, three studies were Tier 2 (Ke et al., 2016 [RefID 3447]; Lin Y et al. (2017)
5252 [RefID 4338] and Wyatt et al., 2016 [RefID 8080]), of which only the last study had three doses,
5253 whereas the other two used a single dose. None of these three the studies found any effects of BPA on
5254 the glucose level. One Tier 1 study with two doses (Cetkovic-Cvrlje et al., 2017 [RefID 916]) did not
5255 find any effects either. The only study that found any effects was a Tier 3 study (Rubin et al., 2017
5256 [RefID 6319]), in which BPA was given via subcutaneous osmotic minipumps and the doses were
5257 converted to oral doses. The F0 dams were exposed to 600 or 5600 µg/kg bw per day on GD8–LD16
5258 and PND21–PND35. The female offspring showed increased glucose level with 600 µg/kg bw at 34
5259 weeks, but not at 28 weeks. No effects were seen with 5600 µg/kg bw at any time point. The male
5260 offspring were not studied.

5261 Four mouse studies (one Tier 1 and three Tier 2) found no effects on glucose level and only one Tier 3
5262 mouse study found increasing effects when tested only on one sex. Thus, the CEP Panel judged this
5263 endpoint as Not Likely.

5264 For the endpoint **insulin level**, there were five the studies in which the exposure of the animals was
5265 under the growth phase. Four the studies were on mice and one study was on rats.

5266 Of the four mouse the studies, three found effects on the insulin level, of these two were Tier 2 (Lin Y
5267 et al., 2017 [RefID 4338] and Wyatt et al., 2016 [RefID 8080]) and one was Tier 3 (Rubin et al., 2017
5268 [RefID 6319]). In Lin Y et al. (2017) [RefID 4338], male mice were exposed to 0.5 µg/kg bw per day
5269 from 3 weeks of age for 90 days until ~16 weeks, and increased insulin level was found. Females were
5270 not studied. In Wyatt et al. (2016) [RefID 8080], two strains of mice (C57BL/6J and DBA/2J) were
5271 exposed to 2.8, 28 or 280 µg/kg bw per day from 4 weeks to 11 weeks of age. In males, no effects on
5272 the insulin level were found of any dose in either strain. In females, the insulin level was increased in
5273 C57BL/6J mice only with the doses 2.8 and 280 µg/kg bw, and in DBA/2J mice with the doses 2.8, 28
5274 and 280 µg/kg bw. In Lin Y et al. (2017) [RefID 4338], the insulin level was increased with 0.5 µg/kg
5275 bw per day in males. Females were not studied. In Rubin et al. (2017) [RefID 6319], BPA was given
5276 via subcutaneous osmotic minipumps and the doses were converted to oral doses. F0 dams were
5277 exposed to 600 or 5600 µg/kg bw per day on GD8–LD16 and PND21–PND35. In female offspring, the
5278 insulin level was increased with 600 µg/kg bw both at 28 and 34 weeks, whereas there were no effects
5279 with 5600 µg. Male offspring were not studied. In the Tier 2 study by Ke et al. (2016) [RefID 3447],
5280 no effects were found in male mice exposed to 0.5 µg/kg bw per day from birth to 8 weeks of age.
5281 Females were not studied.

5282 In the only rat study by Yang YJ et al. (2014) [RefID 10269], which was Tier 2, rats (sex not specified)
5283 were exposed to 1 or 100 µg/kg bw per day from 5 weeks to 10 weeks of age and terminated 1 week
5284 later. The insulin level was increased with both doses.

5285 Four of five studies observed increasing effects on insulin level in two species (three Tier 2 studies; two
5286 studies on mice, including one on two strains, and one study on rats, and one Tier 3 mouse study).
5287 Only one Tier 2 study found no effects when tested only on male mice with a single dose. The CEP
5288 Panel considered this endpoint Likely.

5289 For the endpoint ipGTT, there were two studies, both on mice, in which the exposure of the animals
5290 was under the growth phase.

5291 In the Tier 1 study by Yang et al. (2016) [RefID 8375] with four doses (5, 50, 500 and 5000 µg/kg bw
5292 per day), no effects were found on males either on a normal diet or a HFD. In the other Tier 3 mouse
5293 study (Rubin et al., 2017 [RefID 6319]), BPA was given via subcutaneous osmotic minipumps and the
5294 doses were converted to oral doses. F0 dams were exposed to 600 or 5,600 µg/kg bw per day on GD8–
5295 LD16 and PND21–PND35. No effects were found on female offspring in ipGTT. Male offspring were not
5296 studied.

5297 Two mouse studies, one Tier 1 and one Tier 3, observed no effects on ipGTT. Based on this limited
5298 evidence, the CEP Panel judged this endpoint as Not Likely.

5299 For the endpoint ivGTT, there were no studies in which the exposure of the animals was during the
5300 growth phase.

5301 For the endpoint oGTT, there was one study in which the exposure of the animals was under the growth
5302 phase. In this Tier 2 study by Yang YJ et al. (2014) [RefID 10269], the rats (sex not specified) were

5303 exposed to 1 or 100 µg/kg bw per day from 5 weeks to 10 weeks of age and terminated 1 week later.
5304 The glucose level was increased with 100 µg/kg bw at 60, 90 and 120 minutes, whereas no effects
5305 were observed with 1 µg/kg bw.

5306 Increasing effects on oGTT were observed on unspecified sex(s) in only one Tier 2 rat study. Based on
5307 this limited evidence, the CEP Panel considered this endpoint ALAN.

5308 For the endpoint GTT without known administration route, there was one study in which the exposure
5309 of the animals was under the growth phase. In this Tier 2 study by Wyatt et al. (2016) [RefID 8080],
5310 two strains of mice (C57BL/6J and DBA/2J) were exposed to 2.8, 28 or 280 µg/kg bw per day from 4
5311 weeks to 11 weeks of age. No effects on glucose AUC were observed in males or females with any dose
5312 in either strain.

5313 No effects on GTT without known administration route were observed on either sex in two strains of
5314 mice in one Tier 2 study. Based on this limited evidence, the CEP Panel judged this endpoint as Not
5315 Likely.

5316 For the endpoint ipITT, there was one study in which the exposure of the animals was under the growth
5317 phase. In this Tier 3 study Rubin et al. (2017) [RefID 6319], BPA was given via subcutaneous osmotic
5318 minipumps and the doses were converted to oral doses. F0 dams were exposed to 600 or 5600 µg/kg
5319 bw per day on GD8–LD16 and PND21–PND35. The F1 females showed an overall increased effect of
5320 treatment on the glucose level, a nearly significant increase ($P = 0.07$) with 600 µg/kg bw for overall
5321 comparison and increased glucose levels with 600 and 5600 µg/kg bw at 45 minutes, at 40 weeks. F1
5322 males were not studied. The CEP Panel considered this evidence as Inadequate.

5323 For the endpoints pancreas weight, β -cell morphometry and α -cell morphometry there were no studies
5324 in which the exposure of the animals was during the growth phase.

5325 There were no studies on ivGTT, pancreas weight, β -cell morphometry and α -cell morphometry in this
5326 exposure period. The likelihood level Not Likely was assigned for effects of BPA on glucose level, Likely
5327 for effects on insulin level, Not Likely for ipGTT, ALAN for oGTT, Not Likely for GTT with unknown
5328 administration route and Inadequate evidence for ipITT. In this exposure period, the majority, three of
5329 five, of the endpoints for which there were available studies or adequate evidence were scored Not
5330 Likely. The CEP Panel assigned a likelihood level of Not Likely to the glucose regulation effects of BPA
5331 in the exposure period growth phase, thus, none of the endpoints included in this cluster was taken
5332 forward for BMD analysis. The Likely and ALAN endpoints were considered in uncertainty analysis
5333 (Appendix D)

5334 Adult exposure (after puberty)

5335 For the endpoint glucose level, there were 16 studies in which the exposure of the animals was as
5336 adults. Nine studies were on mice and five studies were on rats. There was also one study on rabbits
5337 and one study on monkeys (common marmosets).

5338 Only one study on mice was Tier 1 (Cetkovic-Cvrlje et al., 2017 [RefID 916]), which did not find any
5339 effects of two doses (160 and 1600 µg/kg bw) in males. The other eight mouse studies were Tier 2. Of
5340 these, four studies (Wyatt et al., 2016 [RefID 8080]; Kim MJ et al., 2014 [RefID 3534]; Ma et al., 2018
5341 [RefID 12637] and Lin Y et al., 2017 [RefID 4338]) found no effects of BPA on the glucose level, of
5342 which two studies used a single dose (Kim MJ et al., 2014 [RefID 3534] and Lin Y et al., 2017 [RefID
5343 4338]), and two studies used three doses (Wyatt et al., 2016 [RefID 8080] and Ma et al., 2018 [RefID
5344 12637]). Kim MJ et al. (2014) [RefID 3534] used the apolipoprotein E knockout ($ApoE^{-/-}$) mouse model
5345 for atherosclerosis.

5346 Four mouse studies found increasing effects of BPA on the glucose level. In Marmugi et al. (2014)
5347 [RefID 4884], male mice were exposed to 5, 50, 500 or 5000 µg/kg bw per day from 6 weeks of age
5348 for 8 months. The glucose level was increased with 500 and 5000 µg/kg bw, but not with the other
5349 doses. In Park et al. (2018) [RefID 12869], male mice were exposed to 10000 µg/kg bw by i.p. or
5350 gavage? (not clear in the study) from age 9 weeks to 21 weeks and terminated 24 hours later. The
5351 glucose level was increased. In Ahn et al. (2018) [RefID 11452], male mice were exposed to 5000
5352 µg/kg bw for 5 days at 7 weeks of age and terminated 22 days after being given STZ to induce insulin
5353 deficiency. The glucose level was increased both in mice with and without treatment with STZ. In
5354 Sivashanmugan et al. (2017) [RefID 6773], male mice were exposed to 10000, 100000 or 400000

- 5355 $\mu\text{g}/\text{kg}$ per day for 30 days from 12–14 weeks of age. The glucose levels were increased with 100000
5356 and 400000 $\mu\text{g}/\text{kg}$ bw, but not with 10000 μg .
- 5357 The doses 100000 and 400000 $\mu\text{g}/\text{kg}$ bw per day inducing increasing effects in Sivashanmugan et al.
5358 (2017) [RefID 6773] were above the cut-off value of 10000 $\mu\text{g}/\text{kg}$ bw per day.
- 5359 Among the rat studies, two were in Tier 1 (Ding et al., 2014 [RefID 1620] and Vahdati Hassani et al.,
5360 2017 [RefID 2614]). In Ding et al. (2014) [RefID 1620], no effects at 0 or 21 weeks were found in the
5361 male offspring when the F0 sires were exposed to 50 $\mu\text{g}/\text{kg}$ bw for 35 weeks and given a normal diet,
5362 whereas when the F0 sires were given a HFD, at 0 and 21 weeks, there were no effects in the male
5363 offspring, but at 35 weeks, the glucose level was increased. Female offspring were not studied. In
5364 Vahdati Hassani et al. (2017) [RefID 2614], when males were exposed to 500, 5000 or 50000 $\mu\text{g}/\text{kg}$
5365 bw per day for 30 days from 7 weeks of age and terminated 1 day later, there were no effects of any
5366 dose. Females were not studied. There were three Tier 2 studies on rats (Santos-Silva et al., 2018
5367 [RefID 13047]; Amraoui et al., 2018 [RefID 11503] and Özaydin et al., 2018b [RefID 12854]). In
5368 Santos-Silva et al. (2018) [RefID 13047], BPA was given by subcutaneous injections and the doses
5369 were converted to oral doses. Of these studies, only Amraoui et al. (2018) [RefID 11503] found any
5370 effects of BPA on the glucose level. In male mice exposed to 10000 $\mu\text{g}/\text{kg}$ bw per day for 3 weeks and
5371 terminated 24 hours later, the glucose level was increased. Females were not studied.
- 5372 In the Tier 2 study on rabbits by Fang et al. (2014) [RefID 1914], no effects were found at 0–12 weeks
5373 when only males were exposed to 400 $\mu\text{g}/\text{kg}$ bw for 12 weeks from 14 weeks of age.
- 5374 In the Tier 2 study on monkeys (common marmosets) by Vijaykumar et al. (2017) [RefID 7477], no
5375 effects were found with any doses when only adult males were exposed to 2.5, 12.5 or 25 $\mu\text{g}/\text{kg}$ bw
5376 per day for 70 days.
- 5377 Ten studies observed no effects on glucose level (two Tier 1, eight Tier 2, in four species), whereas
5378 only six studies observed increasing effects (one Tier 1, five Tier 2, all six studies only in males and
5379 four of these only using a single dose). The CEP Panel considered this endpoint Not Likely.
- 5380 For the endpoint insulin level, there were seven studies in which the exposure of the animals was as
5381 adults. Four studies were on mice and three studies were on rats.
- 5382 All four studies on mice were in Tier 2. Three of the mouse studies found an effect of BPA on insulin
5383 level (Wyatt et al., 2016 [RefID 8080]; Ahn et al., 2018 [RefID 11452] and Lin Y et al., 2017 [RefID
5384 4338]). In Wyatt et al. (2016) [RefID 8080], two strains of mice (C57BL/6J and DBA/2J) were exposed
5385 to 2.8, 28 or 280 $\mu\text{g}/\text{kg}$ bw per day from 4 weeks to 11 weeks of age. In males, no effects on the
5386 insulin level were found of any dose in either strain. In females, the insulin level was increased in
5387 C57BL/6J mice only with the doses 2.8 and 280 $\mu\text{g}/\text{kg}$ bw, and in DBA/2J mice with the doses 2.8, 28
5388 and 280 $\mu\text{g}/\text{kg}$ bw. In Ahn et al. (2018) [RefID 11452], male mice were exposed to 5000 $\mu\text{g}/\text{kg}$ bw for
5389 5 days at 7 weeks of age and terminated 22 days after being given STZ to induce insulin deficiency.
5390 The insulin level was increased both in mice with and without treatment with STZ. Females were not
5391 studied. In Lin Y et al. (2017) [RefID 4338], male mice were exposed to 50 $\mu\text{g}/\text{kg}$ bw per day from 3
5392 weeks of age for 90 days until \sim 16 weeks, and increased insulin level was observed. Females were not
5393 studied.
- 5394 Of the three rat studies, one was Tier 1 (Ding et al., 2014 [RefID 1620]) and two were Tier 2 (Özaydin
5395 et al., 2018b [RefID 12854] and Santos-Silva et al., 2018 [RefID 13047]). In Santos-Silva et al. (2018)
5396 [RefID 13047], BPA was given by subcutaneous injections and the doses were converted to oral doses.
5397 Only one rat study found an effect on insulin level by BPA. In this Tier 1 study Ding et al. (2014) [RefID
5398 1620], the F0 rat sires were exposed to 50 $\mu\text{g}/\text{kg}$ bw for 35 weeks. On a normal diet, there were no
5399 effects on the insulin level at 21 weeks, whereas this was increased at 35 weeks. On a HFD, no effects
5400 were observed at either 21 or 35 weeks.
- 5401 Approximately the same number of studies (three) found no effects on insulin level (three Tier 2 studies,
5402 one on mice and two on rats) or found increasing effects (four studies; one Tier 1 on rats and three
5403 Tier 2 on mice). The dose ranges overlapped in the two effect categories. Thus, the CEP Panel judged
5404 this endpoint as ALAN.
- 5405 For the endpoint ipGTT, there were four studies in which the exposure of the animals was as adults.
5406 Three studies were on mice and one study was on rats.

5407 All the three mouse studies were Tier 2. Kim MJ et al. (2014) [RefID 3534] used the apolipoprotein E
5408 knockout (ApoE^{-/-}) mouse model for atherosclerosis and found no effects of 50 µg/kg bw per day of
5409 BPA on a high-fat/high cholesterol diet. Two mouse studies found an effect in the ipGTT. In Marmugi
5410 et al. (2014) [RefID 4884], the mice were exposed to 5, 50, 500 or 5000 µg/kg bw per day from 6
5411 weeks of age for 8 months. In males, at both 2 and 4½ months, glucose AUC was increased with 5000
5412 µg, whereas there were no effects of the other doses. Females were not studied. In Susiarjo et al.
5413 (2015) [RefID 7022], the effects of BPA were studied both in F0 and F1 generation dams. F0 dams
5414 were exposed to 10 or 10000 µg/kg bw per day from 2 weeks before mating and through pregnancy
5415 and lactation. In F0 dams studied between E16.5-E17.5, glucose AUC was increased with both doses.
5416 When F1 dams were tested with only the dose 10000 µg/kg bw between E16.5-E17.5, glucose AUC
5417 was not affected.

5418 In the Tier 1 rat study Ding et al. (2014) [RefID 1620], in F0 sires exposed to 50 µg/kg bw for 35 weeks
5419 on a normal diet, there was no effect on glucose AUC. However, on a HFD, glucose AUC was increased
5420 in the F0 sires.

5421 Three studies of four found increasing effects on ipGTT (one Tier 1 study on rats and two Tier 2 studies
5422 on mice). However, all studies tested only females and the Tier 1 study found effect on HFD, but not
5423 on normal diet, and two of these studies used only one dose. Based on this limited evidence, the CEP
5424 Panel judged this endpoint as ALAN.

5425 For the endpoints ivGTT, oGTT, GTT without known administration route, ipITT and pancreas weight
5426 there were no studies in which the exposure of the animals was as adults.

5427 For the endpoint β-cell morphometry, there were two studies in which the exposure of the animals was
5428 as adults. Both studies were on rats.

5429 In the Tier 1 study on male rats Ding et al. (2014) [RefID 1620], F0 sires were exposed to 50 µg/kg
5430 bw for 35 weeks and were found to have increased β-cell mass on a normal diet. On a HFD, no effects
5431 on β-cell mass were observed. In the Tier 2 study on rats by Özaydin et al. (2018b) [RefID 12854],
5432 males were exposed to 5, 50 or 500 µg/kg bw per day for 8 weeks from 8 weeks of age. For area of
5433 islet of Langerhans in pancreas, no effects of any doses were observed. Percentage of insulin-reactive
5434 cells in pancreas was increased with 500 µg, but no effects of the other two doses were observed. The
5435 H-score (taking degree of staining into account) was decreased with 5 and 50 µg, but no effects were
5436 seen of 500 µg/kg bw. Females were not studied.

5437 Increasing effects were observed on β-cell morphometry in one Tier 1 and one Tier 2 study, both only
5438 in one sex of rats. In the Tier 2 study, the results were not consistent regarding the dose(s) having
5439 effects and the direction of the effects in related parameters. Based on this limited evidence, the CEP
5440 Panel judged this endpoint as ALAN.

5441 For the endpoint α-cell morphometry, there were no studies in which the exposure of the animals was
5442 as adults.

5443 There were no studies on ivGTT, oGTT, GTT with unknown administration route, ipITT, pancreas weight
5444 and α-cell morphometry. The likelihood level Not Likely was assigned for effects on glucose level, ALAN
5445 for insulin level, ALAN for ipGTT and ALAN for β-cell morphometry. In this exposure period, the majority,
5446 three of four, of the endpoints for which there were available studies were scored ALAN. The CEP Panel
5447 assigned a likelihood level of ALAN to the glucose regulation effects of BPA in the exposure period adult
5448 exposure, thus, none of the endpoints included in this cluster was taken forward for BMD analysis.
5449 However, the ALAN endpoints were considered in the uncertainty analysis (see Appendix D).

5450 Indirect (germline) exposure

5451 For the endpoint glucose level, there were three studies in which the exposure of the animals was via
5452 the germline. One study was on F2 mice and two studies were on F2 rats.

5453 One Tier 2 study on male F2 mice with two doses (10 or 10000 µg/kg bw) (Susiarjo et al., 2015 [RefID
5454 7022]) did not find any effects of either dose. F2 females were not studied.

5455 Two Tier 2 rat studies using a single dose (both 40 µg/kg bw) (Li GQ et al., 2014 [RefID 4039] and
5456 Mao et al., 2015 [RefID 4864]) did not find any effects of BPA on the glucose level in the F2 offspring,
5457 sex not stated or in either sex, respectively.

5458 None of the three available studies (all Tier 2, one on mice and two on rats) found any effects on
5459 glucose level. Based on this limited evidence, the CEP Panel considered this endpoint Not Likely.

5460 For the endpoint insulin level, there were three studies in which the exposure of the animals was via
5461 the germline. One study was on mice and two studies were on rats, all three studies were Tier 2.

5462 In the Tier 2 mouse study Susiarjo et al. (2015) [RefID 7022], F0 dams were exposed to 10 or 10000
5463 µg/kg bw per day from 2 weeks before mating and through pregnancy and lactation. In F2 males, no
5464 effects were observed with either dose. F2 females were not studied.

5465 The two rat studies found effects of BPA on insulin levels. In Li GQ et al. (2014) [RefID 4039], F0 dams
5466 were exposed to 40 µg/kg bw on GD0–PND21. In the F2 offspring (sex not stated) at 9 weeks, there
5467 were no effects, whereas at 20 weeks, the insulin level was increased. In Mao et al. (2015) [RefID
5468 4864], F0 dams were exposed to 40 µg/kg bw on GD0–PND21. In the F2 females, no effects were
5469 observed on insulin level either when fasted or fed at 3 weeks or 21 weeks. In F2 males, the insulin
5470 level was increased when fasted or fed at 21 weeks, but not in either at 3 weeks.

5471 Two of three Tier 2 studies found increasing effects on insulin level in rats, but only one study was
5472 tested on both sexes and found effects only on males, and both studies used only one dose. The third
5473 study showed no effects. Based on this limited evidence, the CEP Panel judged this endpoint as ALAN.

5474 For the endpoint ipGTT, there were three studies in which the exposure of the animals was via the
5475 germline. One study was on mice and two studies were on rats.

5476 In the Tier 2 mouse study by Susiarjo et al. (2015) [RefID 7022], F0 dams were exposed to 10 or 10000
5477 µg/kg bw per day from 2 weeks before mating and through pregnancy and lactation. In F2 males,
5478 10000 µg/kg bw increased glucose AUC at PND98–PND117, whereas 10 µg/kg bw did not. F2 females
5479 were not studied.

5480 The two rat studies were Tier 2. In Li GQ et al. (2014) [RefID 4039], F0 dams were exposed to 40
5481 µg/kg bw on GD0–PND21. In F2 offspring (sex not stated), glucose AUC was increased at 20 weeks,
5482 but not at 9 weeks. In Mao et al. (2015) [RefID 4864], F0 dams were exposed to 40 µg/kg bw on GD0–
5483 PND21. In F2 males and females, there were no effects on glucose AUC at 3 weeks, but glucose AUC
5484 was increased at 21 weeks in both sexes.

5485 Three Tier 2 studies found increasing effects on the functional test ipGTT in two species. However, only
5486 one study tested both sexes and only one study used more than one dose. Based on this limited
5487 evidence, the CEP Panel judged this endpoint as Likely.

5488 For the endpoints ivGTT, oGTT and GTT without know administration route there were no studies in
5489 which the exposure of the animals was via the germline.

5490 For the endpoint ipITT, there was one study in which the exposure of the animals was via the germline.
5491 In this Tier 2 study on rats by Li GQ et al. (2014) [RefID 4039], F0 dams were exposed to 40 µg/kg bw
5492 on GD0–PND21. In F2 offspring (sex not stated), the glucose level was increased at 20 weeks at 15,
5493 30 and 60 minutes, at 9 weeks, there were no effects. The CEP Panel considered this as Inadequate
5494 evidence.

5495 For the endpoint pancreas weight, there was one study in which the exposure of the animals was via
5496 the germline. This Tier 2 single-dose study was on rats (Mao et al., 2015 [RefID 4864]). F0 dams were
5497 exposed to 40 µg/kg bw GD0–PND21. Increased pancreas weight to body weight ratio (%) at birth
5498 (both sexes together) and at 3 weeks and 21 weeks (both sexes separately) was observed in the F2
5499 offspring. The CEP Panel considered this as Inadequate evidence.

5500 For the endpoint β-cell morphometry, there were two studies in which the exposure of the animals was
5501 via the germline. One study was on mice and one study was on rats.

5502 In the Tier 1 study on male mice by Bansal et al. (2017) [RefID 9499], the F0 dams were exposed to
5503 10 or 10000 µg/kg bw per day from 2 weeks before mating until PND21. In the F2 males, relative β-
5504 cell mass was decreased with 10 µg, but not with 10000 µg. F2 females were not studied.

5505 In the Tier 2 study on rats by Mao et al. (2015) [RefID 4864], F0 dams were exposed to 40 µg/kg bw
5506 on GD0–PND21. Absolute β-cell mass (mg) was decreased in F2 males, whereas in F2 females, no

5507 effects were observed at 21 weeks. Also, the β -cell fraction (%) was decreased in F2 males, whereas
5508 in F2 females, no effects were observed at 21 weeks.

5509 Two studies, one Tier 1 on mice and one Tier 2 on rats, found decreasing effects on one or two
5510 parameters for β -cell morphometry in males. However, only one study was tested in both sexes and
5511 only one study used more than one dose. Based on this limited evidence, the CEP Panel judged this
5512 endpoint as Likely.

5513 For the endpoint α -cell morphometry, there were no studies in which the exposure was of the germline.
5514 There were no studies on ivGTT, oGTT, GTT with unknown administration route and α -cell
5515 morphometry. The likelihood level Not Likely was assigned for effects on glucose level, ALAN for insulin
5516 level, Likely for ipGTT, Inadequate evidence for ipITT and pancreas weight and Likely for β -cell
5517 morphometry. In this exposure period, the majority, two of four, of the few endpoints for which there
5518 were available studies or adequate evidence were scored Likely. In addition, there was one endpoint
5519 scored Not Likely and one endpoint scored ALAN.

5520 The CEP Panel assigned a likelihood level of ALAN to the glucose regulation effects of BPA in the
5521 exposure period germline exposure, thus, none of the endpoints included in this cluster was taken
5522 forward for BMD analysis. However, the Likely and ALAN endpoints were considered in the uncertainty
5523 analysis (Appendix D).

5524 Overall cluster selection of the endpoints/studies for BMD analysis for glucose regulation

5525 The endpoint insulin levels was scored Likely in the exposure period growth phase. The endpoints ipGTT
5526 and β -cell morphometry were both scored Likely in the germline exposure period. However, these
5527 effects were not observed in the exposure period adult exposure, in which they were all scored ALAN
5528 as endpoint and were all in a cluster scored ALAN. Thus, the increasing effects of BPA on insulin levels
5529 and ipGTT and the decreasing effect of BPA on β -cell morphometry that were indicated during the
5530 growth phase and the germline exposure periods, respectively, did not appear to be permanent effects
5531 into adult age.

5532 Overall, the CEP Panel assigned a likelihood level to the Glucose regulation cluster of effects of BPA of
5533 ALAN in the exposure periods developmental until weaning, adulthood and germline exposure, and of
5534 Not Likely in the exposure periods developmental until adulthood and growth phase. The overall
5535 likelihood across all exposure periods, i.e. the highest likelihood given in the cluster glucose regulation,
5536 was ALAN.

5537 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
5538 Likely effect of BPA on the cluster glucose regulation in any exposure period, therefore, none of the
5539 endpoints was taken forward for BMD analysis.

5540

5541 **Blood lipids**

5542 The endpoints indicating a possible effect of BPA on metabolism of lipids were cholesterol, HDL
5543 cholesterol, LDL cholesterol, triglycerides and FFA. HDL cholesterol and LDL cholesterol should be
5544 assessed together as increased HDL-cholesterol levels are beneficial effects whereas increased LDL
5545 cholesterol levels are adverse effects and vice versa.

5546 Within the cluster blood lipids, 27 studies were assessed. From these 27 studies, 92 results were
5547 obtained in total; 27 in mice, of which six results were obtained when exposure was during
5548 development, 9 in development and adult age, 3 in growth and young age, and 9 with exposure during
5549 adulthood. Of the 57 results in rats, 20 results were obtained when exposure was during development,
5550 2 in development and adult age, 2 in growth and young age and 33 with exposure during adulthood.
5551 Four results were obtained in rabbits and four in monkeys in the exposure period adulthood.

5552 Results of several of the endpoints were obtained from blood samples taken in the same study so that
5553 the number of studies is lower than the numbers of results. For example, the four results in rabbits and
5554 in monkeys were obtained in blood taken from the one study performed in this species.

5555 Developmental exposure (pre-natal and/or post-natal until weaning)

5556 For the endpoint cholesterol, in this exposure period, seven studies, five in rats (Lejonklou et al., 2017
5557 [RefID 3975]; Dunder et al., 2018 [RefID 11866]; Altamirano et al., 2015 [RefID 155]; NTP Clarity
5558 Report, 2018/Camacho et al., 2019 [RefID 11370]; Santos-Silva et al., 2018 [RefID 13047]) and two
5559 in mice (van Esterik et al., 2014 [RefID 7393]; Rubin et al., 2017 [RefID 6319]) were identified. In the
5560 rat studies, doses between 0.5 and 1111000 µg/kg bw per day were tested with exposures from GD3
5561 to PND21. In four of the rat studies, all studies in Tier 1 (Lejonklou et al., 2017 [RefID 3975]; Dunder
5562 et al., 2018 [RefID 11866]; Altamirano et al., 2015 [RefID 155]; NTP Clarity Report, 2018/Camacho et
5563 al., 2019 [RefID 11370]), no effect was observed with measurements taken on PND10, PND35 and at
5564 1 year. In one study (Altamirano et al., 2015 [RefID 155]), females only were tested; in the three other
5565 studies both males and females were studied. In one study (Tier 2, Santos-Silva et al., 2018 [RefID
5566 13047]) decreases of cholesterol levels were observed at 111100 µg/kg bw per day at PND180, in
5567 females only. In the mouse studies, doses between 0.5 and 55550 µg/kg bw per day were tested with
5568 exposure from pre-mating until end of lactation. No effect was observed in the Tier 1 study with six
5569 doses (van Esterik et al., 2014 [RefID 7393]), while a decrease in the Tier 2 study with only one dose
5570 (0.5 mg/kg bw per day) (Santos-Silva et al., 2018 [RefID 13047]) was observed. The CEP Panel judged
5571 this endpoint as Not Likely.

5572 For the endpoint HDL cholesterol, in this exposure period, four studies, three in rats (Lejonklou et al.,
5573 2017 [RefID 3975]; Dunder et al., 2018 [RefID 11866]; Santos-Silva et al., 2018 [RefID 13047]) and
5574 one in mice (van Esterik et al., 2014 [RefID 7393]), were identified. In the rat studies, doses between
5575 0.5 and 1111000 µg/kg bw per day were tested with exposures from GD3 to PND21. In all rat studies,
5576 two in Tier 1 (Lejonklou et al., 2017 [RefID 3975]; Dunder et al., 2018 [RefID 11866]) and one in Tier
5577 2 (Santos-Silva et al., 2018 [RefID 13047]), no effect has been observed with measurements taken on
5578 PND35 and 180 days and at 1 year (Tier 2, Santos-Silva et al., 2018 [RefID 13047]). Observations were
5579 made in both sexes (Lejonklou et al., 2017 [RefID 3975]; Dunder et al., 2018 [RefID 11866]) and in
5580 females only (Santos-Silva et al., 2018 [RefID 13047]). In the mouse study (van Esterik et al., 2014
5581 [RefID 7393]), the doses of 3, 10, 30, 100, 300, 1000, 3000 µg/kg bw per day were tested with
5582 exposure from pre-mating until end of lactation and no effect was observed. The CEP Panel judged this
5583 endpoint as Not Likely.

5584 For the endpoint LDL cholesterol, in this exposure period, three studies, all in rats (Lejonklou et al.,
5585 2017 [RefID 3975]; Dunder et al., 2018 [RefID 11866]; Santos-Silva et al., 2018 [RefID 13047]), were
5586 identified. In the studies, doses between 0.5 and 1111000 µg/kg bw per day were tested with exposures
5587 from GD3 to PND21. In all studies, two in Tier 1 (Lejonklou et al., 2017 [RefID 3975]; Dunder et al.,
5588 2018 [RefID 11866]), one in Tier 2 (Santos-Silva et al., 2018 [RefID 13047]), no effect has been
5589 observed with measurements taken on PND35 and 180 day and at 1 year (Tier 2, Santos-Silva et al.,
5590 2018 [RefID 13047]). Observations were made in both sexes (Lejonklou et al., 2017 [RefID 3975];
5591 Dunder et al., 2018 [RefID 11866]) and in females only (Santos-Silva et al., 2018 [RefID 13047]). The
5592 CEP Panel judged this endpoint as Not Likely.

5593 For the endpoint triglycerides, in this exposure period, nine studies, seven in rats (Altamirano et al.,
5594 2015 [RefID 155]; Lejonklou et al., 2017 [RefID 3975]; Dunder et al., 2018 [RefID 11866]; NTP Clarity
5595 Report, 2018/Camacho et al., 2019 [RefID 11370]; Jiang et al., 2014 [RefID 3190]; Ding et al., 2014
5596 [RefID 1620], experiments 1 and 2 and Santos-Silva et al., 2018 [RefID 13047]) and two in mice (van
5597 Esterik et al., 2014 [RefID 7393] and Rubin et al., 2017 [RefID 6319]), were identified. In the rat
5598 studies, doses between 0.5 and 25000 µg/kg bw per day were tested with exposures from GD3 to
5599 PND21. In five of the rat studies, all in Tier 1 (Altamirano et al., 2015 [RefID 155]; Dunder et al., 2018
5600 [RefID 11866]; NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]; Ding et al., 2014 [RefID
5601 1620], experiments 1 and 2), no effect has been observed with measurements taken on PND10, PND35
5602 and at 1 year. In Altamirano et al. (2015) [RefID 155], females only were tested, the other studies
5603 were in males and females. In study Lejonklou et al. (2017) [RefID 3975] (Tier 1) with two doses (0.5
5604 and 50 µg/kg bw per day), at 5 µg/kg bw per day no effect was seen in females but an increase in
5605 males, whereas an increase was seen with 50 µg/kg bw per day in females but no effect in males. In
5606 the study by Jiang et al. (2014) [RefID 3190] (Tier 1), with a single dose of 40 µg/kg bw per day, no
5607 effect was observed at 3 weeks, however, the level was increased at 15 and 26 weeks. In Santos-Silva
5608 et al. (2018) [RefID 13047] (Tier 2), for the measurement taken at 180 days, a decrease of triglycerides
5609 levels at 1110 µg/kg bw per day in females, but not in males, was observed; no effect was observed
5610 with the higher dose of 11110 µg/kg bw per day. In the mouse studies, doses between 3 and 55550

5611 µg/kg bw per day were tested with exposure from pre-mating until end of lactation. No effect was
5612 observed in the one Tier 1 study (van Esterik et al., 2014 [RefID 7393]) with six doses in males, but a
5613 decrease was seen in females. No effect was seen in a Tier 3 study (Rubin et al., 2017 [RefID 6319])
5614 with doses between 55.5 and 555555 µg/kg bw per day in females only, males were not tested. The
5615 CEP Panel judged this endpoint as Not Likely.

5616 For the endpoint FFA, in this exposure period, three studies, one in rats (Jiang et al., 2014 [RefID
5617 3190]) and two in mice (van Esterik et al., 2014 [RefID 7393] and Rubin et al., 2017 [RefID 6319]),
5618 were identified. In these studies, doses between 3 and 55550 µg/kg bw per day were tested with
5619 exposures from GD0 to PND21. In the rat study (Jiang et al., 2014 [RefID 3190]) (Tier 1), with a single
5620 dose of 40 µg/kg bw per day, no effect has been observed at 3 weeks, and an elevated level of FFA at
5621 15 and 26 weeks. In mice, from van Esterik et al. (2014) [RefID 7393] (Tier 1), no effect in males and
5622 a not adverse decrease in females were observed; measurements were taken at 23 weeks. No effect
5623 was observed in Rubin et al. (2017) [RefID 6319] (Tier 3), in both sexes. The CEP Panel judged this
5624 endpoint as Not Likely.

5625 All endpoints in this cluster were considered Not Likely. The CEP Panel assigned a likelihood level of
5626 Not Likely to the cluster of blood lipids in Developmental (pre-natal and/or post-natal until weaning),
5627 thus, none of the endpoints included in this cluster was taken forward for BMD analysis.

5628 Developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood)

5629 For the endpoint cholesterol, in this exposure period, four studies, one in rats (NTP Clarity Report,
5630 2018/Camacho et al., 2019 [RefID 11370]) and three in mice (Ke et al., 2016 [RefID 3447]; Patel et
5631 al., 2014 [RefID 5695], experiments 1 and 2 were identified. In the rat study (NTP Clarity Report,
5632 2018/Camacho et al., 2019 [RefID 11370]), doses between 2.5 and 25000 µg/kg bw per day were
5633 tested with exposure from GD6 until 1 year. No effect has been observed in males and females with
5634 measurements taken at 1 year. In the mouse studies, doses between 0.5 and 55,550 µg/kg bw per day
5635 were tested with exposure from pre-mating until 43 weeks. No effect was observed in one Tier 2 study
5636 (Patel et al., 2014 [RefID 5695], experiment 2, high-fat diet) with one dose level (5 µg/kg bw per day
5637 during development and afterwards 0.6 µg/kg bw per day). In the Tier 2 study (Patel et al., 2014 [RefID
5638 5695] experiment 1, normal diet) with one dose level (5 µg/kg bw per day during development and
5639 afterwards 0.6 µg/kg bw per day), a decrease in females and an increase in males was observed. In
5640 the single-dose study (Ke et al., 2016 [RefID 3447], Tier 2) with 0.5 µg/kg bw per day, an increase in
5641 males was seen. Since the Tier 1 study with five doses, one Tier 2 study and one Tier 3 study with four
5642 doses showed no effects, and in addition, one study gave divergent results with unexplained
5643 inconsistency and only one single-dose study was available within the dose range below the doses
5644 tested in the other studies, the CEP Panel judged this endpoint as Not Likely.

5645 For the endpoint HDL cholesterol, in this exposure period, one study in mice (Ke et al., 2016 [RefID
5646 3447]) was identified. In this study, a dose of 0.5 µg/kg bw per day was tested with exposure from
5647 PND1 to PND21 and afterwards until sacrifice at 8 weeks or 10 months. In this Tier 2 study, no effect
5648 has been observed at 8 weeks and a decrease at 10 months. Only males were tested. The CEP Panel
5649 judged this endpoint as ALAN.

5650 For the endpoint LDL cholesterol, in this exposure period, one study in mice (Ke et al., 2016 [RefID
5651 3447]) was identified. In this study, a dose of 0.5 µg/kg bw per day was tested with exposure from
5652 PND1 to PND21 and afterwards until sacrifice at 8 weeks or 10 months. In this Tier 2 study, no effect
5653 has been observed at 8 weeks and an increase at 10 months. Only males were tested. The CEP Panel
5654 judged this endpoint as ALAN.

5655 For the endpoint triglycerides, in this exposure period, three studies, one in rats (NTP Clarity Report,
5656 2018/Camacho et al., 2019 [RefID 11370]) and two in mice (Patel et al., 2014 [RefID 5695],
5657 experiments 1 and 2 and Ke et al., 2016 [RefID 3447]), were identified. In the rat study (NTP Clarity
5658 Report, 2018/Camacho et al., 2019 [RefID 11370], Tier 1), doses between 2.5 and 25000 µg/kg bw
5659 per day were tested in males and females. In the two mouse studies, both in Tier 2 (Patel et al., 2014
5660 [RefID 5695], experiments 1 and 2), single doses were tested, 0.5 µg/kg bw per day with exposure
5661 from PND1 to PND21 and afterwards until sacrifice at 8 weeks or 10 months, 5 µg/kg bw per day during
5662 development and 0.6 µg/kg bw per day afterwards. In Ke et al. (2016) [RefID 3447], the dose 0.5
5663 µg/kg bw per day was administered for the same period as Patel et al. (2014) [RefID 5695]. No effect

5664 has been observed in the study from Patel et al. (2014) [RefID 5695] experiments 1 and 2 and an
5665 increase was observed in the study from Ke et al. (2016) [RefID 3447]. The effect in this endpoint was
5666 judged as Not Likely.

5667 All endpoints in this cluster were considered as Not Likely. The CEP Panel assigned a likelihood level of
5668 Not Likely to the cluster of blood lipids in the developmental and adult (pre-natal and/or post-natal in
5669 pups until adulthood) period, thus, none of the endpoints included in this cluster was taken forward for
5670 BMD analysis. However, the ALAN endpoints were considered in the uncertainty analysis (Appendix D).

5671 Growth phase/young age exposure

5672 For the endpoint cholesterol, in this exposure period, two studies, one in rats (Yang YJ et al., 2014
5673 [RefID 10269]) and one in mice (Lin Y et al., 2017 [RefID 4338]), were identified. In the rat study
5674 (Yang YJ et al., 2014 [RefID 10269]), two doses (1 and 100 µg/kg bw per day) were tested with
5675 exposure from week 5 until week 10. No effect has been observed in males and females with
5676 measurements taken at 10 weeks. In the Tier 2 mouse study (Lin Y et al., 2017 [RefID 4338]), a dose
5677 of 50 µg/kg bw per day was tested with exposure for 90 days. No effect was observed in this study.
5678 The effect in this endpoint was judged as Not Likely.

5679 For this exposure period (growth phase/young age), no studies were identified testing the endpoints
5680 HDL and LDL cholesterol.

5681 For the endpoint triglycerides, in this exposure period, three studies, one in rats (Yang YJ et al., 2014
5682 [RefID 10269]) and two in mice (Lin Y et al., 2017 [RefID 4338] and Rubin et al., 2017 [RefID 6319]),
5683 were identified. In the rat study (Yang YJ et al., 2014 [RefID 10269], Tier 2), two doses (1 and 100
5684 µg/kg bw per day) were tested with exposure from week 5 until week 10. No effect has been observed
5685 in males and females with measurements taken at 10 weeks. In the study in mice (Rubin et al., 2017
5686 [RefID 6319], Tier 3), doses of 55.5, 555.5, 5555 and 55550 µg/kg bw per day were given throughout
5687 the development and afterwards until week 43. No effect was reported in females (males were not
5688 studied). In the other mouse study (Lin Y et al., 2017 [RefID 4338], Tier 2), a dose of 50 µg/kg bw per
5689 day was tested with exposure for 90 days. An increase of triglycerides was observed in this study,
5690 where only males were tested. The CEP Panel considered this endpoint as Not Likely, because
5691 one study (Yang YJ et al., 2014 [RefID 10269]) showed no effect with two doses encompassing the
5692 dose of the study from Lin Y et al. (2017) [RefID 4338], which showed an increase in triglycerides.
5693 Moreover, the second study without effect (Rubin et al., 2017 [RefID 6319]) included four doses, and
5694 the lowest dose was nearly identical with the dose of the study showing an effect.

5695 For the endpoint FFA, in this exposure period, no studies on BPA effect were identified.

5696 The CEP Panel assigned a likelihood level of Not Likely to the cluster of blood Lipids in the growth
5697 phase/young age period, thus, none of the endpoints included in this cluster was taken forward for
5698 BMD analysis.

5699 Adult exposure (after puberty)

5700 For the endpoint cholesterol, in this exposure period, 13 studies, one in monkeys (Vijakumar et al.,
5701 2017 [RefID 7477]), one in rabbits (Fang et al., 2014 [RefID 1914]), eight in rats (Özaydin et al., 2018b
5702 [RefID 12854]; Thilagavathi et al., 2017 [RefID 9247]; Ding et al., 2014 [RefID 1620], experiments 1
5703 and 2; Ding et al., 2016 [RefID 1621], experiments 1 and 2; Vahdati Hassani et al., 2017 [RefID 2614];
5704 Amraoui et al., 2018 [RefID 11503]; Mahmoudi et al., 2018 [RefID 12656]; Abdel-Rahman et al., 2018
5705 [RefID 11426]), and three in mice (Marmugi et al., 2014 [RefID 4884]; Lin Y et al., 2017 [RefID 4338];
5706 Kim MJ et al., 2014 [RefID 3534]) were identified. In the monkey study (Vijakumar et al., 2017 [RefID
5707 7477]), doses between 2.4 and 125 µg/kg bw per day were administered for 70 days and no effect
5708 observed in males, the only sex tested. In the rabbit study (Fang et al., 2014 [RefID 1914]), a dose of
5709 400 µg/kg bw per day was administered for 2 weeks and no effect was observed in males, the only sex
5710 tested. In six rat studies, one Tier 1 (Vahdati Hassani et al., 2017 [RefID 2614]), the others Tier 2
5711 (Özaydin et al., 2018b [RefID 12854]; Thilagavathi et al., 2017 [RefID 9247]; Ding et al., 2014 [RefID
5712 1620], experiments 1 and 2; Ding et al., 2016 [RefID 1621], experiments 1 and 2; Abdel-Rahman et
5713 al., 2018 [RefID 11426]), no effect was observed, the tested doses ranging between 5 and 50000 µg/kg
5714 bw per day encompassing exposure periods from 3 weeks to 35 weeks. In two rat studies (Amraoui et
5715 al., 2018 [RefID 11503] and Mahmoudi et al., 2018 [RefID 12656]), both Tier 2, the cholesterol levels

5716 were increased in males, the only sex tested, at the only dose tested of 10000 µg/kg bw per day. In
5717 one of the mouse studies (Lin Y et al., 2017 [RefID 4338]), no effect was observed with single doses
5718 of 50 µg/kg bw per day and in two of the mouse studies (Marmugi et al., 2014 [RefID 4884] and Kim
5719 MJ et al., 2014 [RefID 3534]) increased cholesterol levels were noted. In Marmugi et al. (2014) [RefID
5720 4884] (Tier 2), elevated levels were seen at all doses tested, between 5 and 5000 µg/kg bw per day,
5721 without a clear dose–response. In Lin Y et al. (2017) [RefID 4338], increased levels were seen in males,
5722 the only sex tested, at the only dose tested of 10000 µg/kg bw per day. This endpoint was scored as
5723 ALAN because only four out of the 13 studies showed an increase at doses which were tested also in
5724 the studies not showing an effect, which were the majority.

5725 For the endpoint HDL cholesterol, in this exposure period, nine studies, one in monkeys (Vijaykumar et
5726 al., 2017 [RefID 7477]), one in rabbits (Fang et al., 2014 [RefID 1914]), six in rats (Özaydin et al.,
5727 2018b [RefID 12854]; Thilagavathi et al., 2017 [RefID 9247]; Vahdati Hassani et al., 2017 [RefID
5728 2614]; Mahmoudi et al., 2018 [RefID 12656]; Abdel-Rahman et al., 2018 [RefID 11426]; Amraoui et
5729 al., 2018 [RefID 11503]) and one in mice (Kim MJ et al., 2014 [RefID 3534]), were identified. In the
5730 monkey study (Vijaykumar et al., 2017 [RefID 7477]), doses between 2.4 and 125 µg/kg bw per day
5731 were administered for 70 days and no effect was observed in males, the only sex tested. In the rabbit
5732 study (Fang et al., 2014 [RefID 1914]), a dose of 400 µg/kg bw per day was administered for 2 weeks
5733 and no effect was observed in males, the only sex tested. In four rat studies, one Tier 1 (Vahdati
5734 Hassani et al., 2017 [RefID 2614]), the others Tier 2 (Özaydin et al., 2018b [RefID 12854]; Mahmoudi
5735 et al., 2018 [RefID 12656]; Abdel-Rahman et al., 2018 [RefID 11426]), no effect was observed, the
5736 tested doses ranging between 5 and 50000 µg/kg bw per day encompassing exposure periods from 3
5737 weeks to 35 weeks. In two rat studies, the HDL-cholesterol levels were decreased in males and
5738 unchanged in females (Amraoui et al., 2018 [RefID 11503], Tier 2) at the only dose tested of 10000
5739 µg/kg bw per day, or marginally decreased in females, the only sex tested (Thilagavathi et al., 2017
5740 [RefID 9247], Tier 1). In one of the mouse studies (Kim MJ et al., 2014 [RefID 3534], Tier 2), increased
5741 levels were observed with single doses of 50 µg/kg bw per day in male animals, the only sex tested. In
5742 Thilagavathi et al. (2017) [RefID 9247] (Tier2), decreased levels were seen at all doses tested, between
5743 5 and 5000 µg/kg bw per day, without a clear dose–response. This endpoint was considered as Not
5744 Likely because only in two studies a decrease was shown (in one study in females, in one study only in
5745 males and not in females) at doses which were tested also in the studies without an effect, whereas in
5746 six studies no effect and in one study even an increase was observed.

5747 For the endpoint HDL cholesterol, in this exposure period, 10 studies, one in monkeys (Vijakumar et
5748 al., 2017 [RefID 7477]), one in rabbits (Fang et al., 2014 [RefID 1914]), six in rats (Özaydin et al.,
5749 2018b [RefID 12854]; Vahdati Hassani et al., 2017 [RefID 2614]; Thilagavathi et al., 2017 [RefID
5750 9247]; Amraoui et al., 2018 [RefID 11503]; Mahmoudi et al., 2018 [RefID 12656]; Abdel-Rahman et
5751 al., 2018 [RefID 11426]) and two in mice (Kim MJ et al., 2014 [RefID 3534] and Marmugi et al., 2014
5752 [RefID 4884]), were identified. In the monkey study (Vijakumar et al., 2017 [RefID 7477]), doses
5753 between 2.4 and 125 µg/kg bw per day were administered for 70 days and an increase at 25 µg/kg bw
5754 per day was observed in males, the only sex tested. In the rabbit study (Fang et al., 2014 [RefID
5755 1914]), a dose of 400 µg/kg bw per day was administered for 2 weeks and no effect was observed in
5756 males, the only sex tested. In two Tier 2 rat studies (Özaydin et al., 2018b [RefID 12854] and Vahdati
5757 Hassani et al., 2017 [RefID 2614]), no effect was observed and the tested doses (ranging between 5
5758 and 50000 µg/kg bw per day) encompassed exposure periods from 3 weeks to 35 weeks. In four rat
5759 studies, the LDL cholesterol levels were increased in males and unchanged in females (Abdel-Rahman
5760 et al., 2018 [RefID 11426], Tier 2) at the only dose tested of 10000 µg/kg bw per day, marginally
5761 increased in females, the only sex tested (Thilagavathi et al., 2017 [RefID 9247], Tier 1), or increased
5762 at the only dose tested of 10000 µg/kg bw per day (Mahmoudi et al., 2018 [RefID 12656], Tier 2, and
5763 Amraoui et al., 2018 [RefID 11503], Tier 2). In one of the mouse studies (Kim MJ et al., 2014 [RefID
5764 3534], Tier 2), increased levels were observed with single doses of 50 µg/kg bw per day in male
5765 animals, the only sex tested. In Thilagavathi et al. (2017) [RefID 9247] (Tier 2), increased levels were
5766 seen at all doses tested, between 5 and 5,000 µg/kg bw per day, without a clear dose–response.

5767 Overall, in three out of 10 studies no effect was observed. In one study, only marginal effects were
5768 seen, in one study unexplained sex differences were observed and in two studies no clear dose–
5769 responses were seen. In three studies, only one dose was tested, of these in two studies the only dose
5770 tested was the dose of the cut-off value. Hence, the effect in this endpoint was judged as ALAN.

5771 For the endpoint triglycerides, in this exposure period, 14 studies, one in monkeys (Vijakumar et al.,
5772 2017 [RefID 7477]), one in rabbits (Fang et al., 2014 [RefID 1914]), nine in rats (Özaydin et al., 2018b
5773 [RefID 12854]; Thilagavathi et al., 2017 [RefID 9247]; Ding et al., 2014 [RefID 1620], experiments 1
5774 and 2; Lejonklou et al., 2017 [RefID 3975]; Ding et al., 2016 [RefID 1621], experiments 1 and 2;
5775 Vahdati Hassani et al., 2017 [RefID 2614]; Amraoui et al., 2018 [RefID 11503]; Mahmoudi et al., 2018
5776 [RefID 12656]; Abdel-Rahman et al., 2018 [RefID 11426]) and three in mice (Kim MJ et al., 2014 [RefID
5777 3534]; Lin Y et al., 2017 [RefID 4338]; Ma et al., 2018 [RefID 12637]), were identified. In the monkey
5778 study (Vijakumar et al., 2017 [RefID 7477]), doses between 2.4 and 125 µg/kg bw per day were
5779 administered for 70 days and no effect was observed in males, the only sex tested. In the rabbit study
5780 (Fang et al., 2014 [RefID 1914]), a dose of 400 µg/kg bw per day was administered for 2 weeks and
5781 no effect was observed in males, the only sex tested. In the rat studies, doses between 0.5 and 50000
5782 µg/kg bw per day were tested. In four rat studies, two Tier 1 (Ding et al., 2014 [RefID 1620],
5783 experiments 1 and 2; Lejonklou et al., 2017 [RefID 3975]) and two Tier 2 (Özaydin et al., 2018b [RefID
5784 12854] and Ding et al., 2016 [RefID 1621], experiments 1 and 2), no effect was observed, and the
5785 tested doses ranging between 5 and 500 µg/kg bw per day. In four rat studies, the triglyceride level
5786 was increased in females (Abdel-Rahman et al., 2018 [RefID 11426], Tier 2) at the only dose tested of
5787 10000 µg/kg bw per day, marginally increased in females, the only sex tested (Thilagavathi et al., 2017
5788 [RefID 9247], Tier 1), or increased at the only dose tested of 10000 µg/kg bw per day (Mahmoudi et
5789 al., 2018 [RefID 12656], Tier 2 and Amraoui et al., 2018 [RefID 11503], Tier 2). Inconclusive results
5790 with increased levels without a dose–response were seen in Vahdati Hassani et al. (2017) [RefID 2614].
5791 In all mouse studies (Kim MJ et al., 2014 [RefID 3534]; Lin Y et al., 2017 [RefID 4338]; Ma et al., 2018
5792 [RefID 12637], all Tier 2), no effect was observed with single doses of 50 µg/kg bw per day (two
5793 studies) and 5 to 500 µg/kg bw per day (one study).

5794 Overall, from 14 studies, nine studies showed no effect, in one study the effect was marginal, in one
5795 study the effects were inconsistent, in three studies the increase was seen at the dose of 10000 µg/kg
5796 bw per day. Hence, this endpoint was judged as ALAN.

5797 For the endpoint FFA, in this exposure period, no studies on BPA effects were identified.

5798 The CEP Panel assigned a likelihood level of ALAN to the cluster of blood Lipids in the adult exposure
5799 period, thus, none of the endpoints included in this cluster was taken forward for BMD analysis.
5800 However, the ALAN endpoints were considered in the uncertainty analysis (see Appendix D).

5801 Indirect (germline) exposure

5802 For the endpoint cholesterol, in this exposure period, one Tier 1 study in rats with two arms, high-fat
5803 diet and normal diet, was performed. The paternal animals were treated with 50 µg/kg bw per day for
5804 21 weeks before mating and then for further 14 weeks; treatment was 35 weeks in total. Maternal
5805 animals and offspring were not treated. Neither in F1 nor in F2 animals, BPA had an effect on cholesterol
5806 levels.

5807 For this exposure period, no studies were identified testing the endpoints HDL cholesterol and LDL
5808 cholesterol, as well as FFA and triglycerides.

5809 The CEP Panel assigned a likelihood level of Not Likely to the cluster of blood Lipids in the indirect
5810 (germline) exposure period, thus, none of the endpoints included in this cluster was taken forward for
5811 BMD analysis.

5812 Overall cluster selection of the endpoints/studies for BMD analysis for blood lipids

5813 Overall, the CEP Panel assigned a likelihood level to effects of BPA on the blood lipids of Not Likely in
5814 the exposure periods developmental until weaning, developmental until adulthood, growth
5815 phase/young age and indirect (germline), and of ALAN in the adult exposure.

5816 The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster blood
5817 lipids, was ALAN.

5818 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
5819 Likely effect of BPA in any exposure period, therefore, none of the endpoints was taken forward for
5820 BMD analysis.

5821 **Uric acid**

5822 The effects of BPA on uric acid in serum, urine or liver were assessed as a measure of the influence of
5823 BPA on purine metabolism. Within the cluster uric acid, two studies were performed in mice, of which
5824 one study had exposure during development and one had exposure during adulthood. The only study
5825 in rats was performed during the adult phase.

5826 Developmental exposure (pre-natal and/or post-natal until weaning)

5827 For this exposure period, one study in mice (Bodin et al., 2014 [RefID 623]) was identified. In this
5828 study, a dose of 397.5 µg/kg bw per day was given once. No effect has been observed on uric acid
5829 concentration in serum and urine.

5830 The CEP Panel considered that there was Inadequate Evidence on the effect of BPA on uric acid in the
5831 developmental exposure period and, thus, this endpoint was not taken forward for BMD analysis.

5832 Adult exposure (after puberty)

5833 For this exposure period, one study in two strains of male mice, CD-1 and C57BL/6 (Ma et al., 2018
5834 [RefID 12637] (experiment 1), Tier 2), was identified. In this study, doses of 5, 50 and 500 µg/kg bw
5835 per day were given for 8 weeks from week 6. A dose-dependent increase in serum concentration of
5836 uric acid was observed in both strains and the increases were statistically significant at 50 and 500
5837 µg/kg bw per day. A dose-dependent increase in liver concentration of uric acid was observed in CD1
5838 mice, the increases were statistically significant at 50 and 500 µg/kg bw per day.

5839 Doses of 5, 50 and 500 µg/kg bw per day were given for 8 weeks from week 6 in rats (Ma et al., 2018
5840 [RefID 12637] (experiment 2), Tier 2). A statistically significant increase of uric acid was shown at 500
5841 µg/kg bw per day. No results for 5 and 50 µg/kg bw per day were presented.

5842 The CEP Panel assigned a likelihood level of Likely to the uric Acid effect of BPA in the adult exposure
5843 period. Since the likelihood level for this cluster is Likely for the endpoints uric acid concentration in
5844 serum and in liver in mice strain CD-1, and for uric acid concentration in serum in mice strain C57BL/6
5845 (Ma et al., 2018 [RefID 12637]), these endpoints were taken forward for BMD analysis (see Chapter
5846 3.2.1) and uncertainty analysis (see Appendix D).

5847 Developmental and adult, growth phase/young age and indirect (germline) exposure periods

5848 In these exposure periods, no studies on BPA effects on uric acid were identified.

5849 Overall cluster selection of the endpoints/studies for BMD analysis for uric acid

5850 No effect was seen in one mouse study with a single-dose of BPA in the developmental exposure period.
5851 A dose-dependent increase of uric acid concentration in serum in two mice strains and in liver in one
5852 mice strain was observed after 8 weeks exposure in the adult age. In one rat study, uric acid
5853 concentration in serum was also statistical significantly increased at the highest dose of 500 µg/kg bw
5854 per day after 8 weeks exposure in the adult age.

5855 Overall, the CEP panel considered that there was Inadequate Evidence for concluding on the likelihood
5856 of effects of BPA on Uric acid in the developmental until weaning exposure period, whereas the CEP
5857 Panel assigned a likelihood level of Likely to effects of BPA on Uric Acid in the adult exposure period.

5858 The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster uric
5859 acid, was Likely.

5860 The CEP Panel considered that the evidence from the studies available showed a Likely effect of BPA
5861 in the exposure period adult for the endpoint uric acid (Ma et al., 2018 [RefID 12637]), therefore, this
5862 endpoint was taken forward for BMD analysis (see Chapter 3.2.1).

5863 **Type 1 diabetes mellitus (T1DM)**

5864 The incidence of T1DM was studied in a mouse model which spontaneously develops TD1M (the NOD
5865 mouse) (Bodin et al., 2014 [RefID 623]) and in an experimental model in which β-cells of the pancreas
5866 were destroyed by a β-cell-specific toxin (streptozotocin) (Cetkovic-Cvrlje et al., 2017 [RefID 916]).
5867 Both are mouse models commonly used in experimental animal studies of T1DM.

5868 Within the cluster T1DM, two studies were performed in mice, of which one study had exposure during
5869 development, and one had exposure both during growth phase/young age and during adulthood. No
5870 studies in other species were available.

5871 Developmental exposure (pre-natal and/or post-natal until weaning)

5872 For this exposure period, one study in mice was identified (Bodin et al., 2014 [RefID 623], Tier 3). This
5873 study used a special mouse strain (diabetes type 1 prone NOD) and BPA doses of 30, 300 and 3,000
5874 µg/kg bw per day were given from mating until weaning. In the F1 generation, no effect was seen at
5875 7 weeks. Above 20 weeks, the incidence of diabetes increased at the dose of 3,000 µg/kg bw per day
5876 in females only. Because an effect was seen only in the highest dose tested and only in females in a
5877 Tier 3 study, the CEP considered that there was Inadequate Evidence for concluding on the likelihood
5878 of effects of BPA on the incidence of T1DM in this special mouse strain, in the developmental exposure
5879 period and, thus, none of the endpoints included in this cluster was taken forward for BMD analysis.
5880 However, the ALAN endpoints were considered in the uncertainty analysis (see Appendix D).

5881 Developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood)

5882 In this exposure period, no studies on effects of BPA on T1DM were identified.

5883 Growth phase/young age exposure

5884 In this exposure period, one study in mice (Cetkovic-Cvrlje et al., 2017 [RefID 916], Tier 1) was
5885 identified. In this study, only male animals were tested at BPA doses of 160 and 1600 µg/kg bw per
5886 day, starting at 4 weeks. T1DM was initiated in the mice by five streptozotocin i.p. injections at 9 weeks.
5887 Both doses increased the T1DM incidence statistically significantly compared with the control but
5888 without a statistically significant difference between the two dose groups. The higher dose showed a
5889 trend towards higher glycaemic levels, even if not statistically significant.

5890 Because an effect was seen at both dose levels without a dose–response relationship and only in males,
5891 the CEP Panel assigned a likelihood of ALAN to the effects of BPA on the incidence of T1DM in a
5892 streptozotocin model in the growth phase/young age exposure period and, thus, none of the endpoints
5893 included in this cluster was taken forward for BMD analysis.

5894 Adult exposure (after puberty)

5895 In this exposure period, one study in mice (Cetkovic-Cvrlje et al., 2017 [RefID 916], Tier 1) was
5896 identified, which is the same study as described under Growth phase/young age.

5897 In the mouse study, only male animals were tested, at doses of 160 and 1600 µg/kg bw per day,
5898 starting at 4 weeks. T1DM was initiated in the mice by five streptozotocin i.p. injections at 9 weeks.
5899 Both doses increased the T1DM incidence statistically significantly compared with the control, but
5900 without statistically significant difference between the two dose groups. The higher dose showed a
5901 trend towards higher glycaemic levels, even if not statistically significant.

5902 Because an effect was seen in both dose levels without a dose–response relationship and only in males,
5903 the CEP Panel assigned a likelihood of ALAN to effects of BPA on the incidence of T1DM in the adult
5904 exposure period and, thus, none of the endpoints included in this cluster was taken forward for BMD
5905 analysis.

5906 Indirect (germline) exposure

5907 No studies were available on BPA effects on T1DM in this exposure period.

5908 Overall cluster selection of the endpoints/studies for BMD analysis for type I diabetes mellitus (T1DM)

5909 Two models of initiation of T1DM in mice were used to study the influence of BPA on the development
5910 of T1DM. In the mouse model which spontaneously develops TD1M (the NOD mouse), an increase in
5911 the incidence of T1DM was seen only in the highest dose tested and only in females in a Tier 3 study
5912 with developmental exposure. No effect was observed with BPA exposure during development and in
5913 adulthood. Overall, the BPA effect on the incidence of T1DM was judged as ALAN.

5914 In the experimental model in which β-cells of the pancreas were destroyed by streptozotocin, a β-cell-
5915 specific toxin, one study with exposure encompassing growth phase/young age and adulthood was

5916 performed only in males. A higher incidence of T1DM was seen at both dose levels without a dose–
5917 response relationship and the effect was judged as ALAN.

5918 The CEP Panel considered that there was Inadequate Evidence for concluding on the likelihood of BPA
5919 effects on T1DM in the developmental exposure period, while assigned a likelihood level of ALAN in the
5920 growth phase/young age and adult exposure periods.

5921 The overall likelihood across all exposure periods, i.e. the highest likelihood given in T1DM, was ALAN.

5922 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
5923 Likely effect of BPA in any exposure period, therefore, none of the endpoints was taken forward for
5924 BMD analysis.

5925 **Other metabolic hormones**

5926 Adiponectin, leptin, glucagon and resistin in plasma were included as metabolic endpoints because they
5927 are hormones related to obesity, glucose regulation and lipid metabolism. These endpoints therefore
5928 formed the cluster other metabolic hormones.

5929 Within this cluster, the hormones were measured in five mouse studies, two of them with exposure
5930 during development until weaning period, one during development and adulthood, three during growth
5931 phase/young age, two during adulthood, whereas no studies were available with a germline exposure.
5932 In 10 studies, the metabolic hormone measurements were performed in rats, seven of them when
5933 exposure was during development until weaning, two during developmental and adulthood, one during
5934 growth phase/young age, one during adulthood and one with a germline exposure.

5935 Developmental exposure (pre-natal and/or post-natal until weaning)

5936 For the endpoint adiponectin, in this exposure period, six studies, five in rats (Dunder et al., 2018
5937 [RefID 11866]; Lejonklou et al., 2017 [RefID 3975]; Zhang et al., 2013 [RefID 8798]; Song SZ et al.,
5938 2014 [RefID 6829]; Leung et al., 2017 [RefID 3990]), and one in mice (van Esterik et al., 2014 [RefID
5939 7393]), were identified.

5940 In the rat studies, doses between 0.5 and 2500 µg/kg bw per day were tested. In three of the rat
5941 studies (two studies Tier 1, Dunder et al., 2018 [RefID 11866] and Lejonklou et al., 2017 [RefID 3975]
5942 and one in Tier 3, Leung et al., 2017 [RefID 3990]), no effect has been observed with measurements
5943 taken on PND21, PND35, PND50 and at 1 year. In two studies (Song SZ et al., 2014 [RefID 6829], Tier
5944 1 and Zhang et al., 2013 [RefID 8798], Tier 2), decreases of adiponectin concentrations were observed
5945 without dose–response at doses between 90 and 900 µg/kg bw per day, one study in males only and
5946 the other study in females only.

5947 In the mouse study, with doses between 2 and 3000 µg/kg bw per day, no effect has been observed
5948 at week 23 in males and no dose–response was seen in females.

5949 For the endpoint leptin, in this exposure period, six studies, five in rats (Dunder et al., 2018 [RefID
5950 11866]; Lejonklou et al., 2017 [RefID 3975]; NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
5951 11370]; Santos-Silva et al., 2018 [RefID 13047]; Leung et al., 2017 [RefID 3990], experiments 1 and
5952 2) and one in mice (van Esterik et al., 2014 [RefID 7393]), were identified.

5953 In the rat studies, doses between 0.5 and 2,500 µg/kg bw per day were tested. In four of the rat
5954 studies (three studies Tier 1, Dunder et al., 2018 [RefID 11866]; Lejonklou et al., 2017 [RefID 3975];
5955 NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) and one in Tier 3, Leung et al., 2017
5956 [RefID 3990], experiments 1 and 2), no effect has been observed with measurements taken on PND15,
5957 PND21, PND50 and at 1 year. In one study (Santos-Silva et al., 2018 [RefID 13047], Tier 2), no effects
5958 were seen in females, whereas in males, increase on PND15 and decrease on PND21 at 1,785 µg/kg
5959 bw per day was observed and a decrease was seen also at 178,500 µg/kg bw per day on PND21.

5960 In the mouse study, with doses between 2 and 3000 µg/kg bw per day no effect has been observed at
5961 week 23 in males and no dose–response was seen in females.

5962 For the endpoint glucagon, in this exposure period, one study in mice (van Esterik et al., 2014 [RefID
5963 7393], Tier 1) was identified. Six doses between 2 and 3000 µg/kg bw per day were tested with
5964 exposure between GD0 and PND22 and showed no effect.

5965 In this exposure period, 14 studies in total were available, in rats 11 studies and in mice three studies.
5966 Adiponectin was measured in six studies, leptin in seven studies and glucagon in one study, resistin
5967 was not measured. Most of the results showed no effect.

5968 Since most of the studies showed no effect, the CEP Panel assigned a likelihood level of Not Likely to
5969 the effect of BPA during developmental until weaning exposure period on adiponectin, leptin and
5970 glucagon, thus, none of these endpoints was taken forward for BMD analysis.

5971 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

5972 For the endpoint adiponectin, in this exposure period, two studies, one in rats (Ben-Jonathan et al.,
5973 2018 (NTP Grantee study) [RefID 13786]) and one in mice (Patel et al., 2014 [RefID 5695], experiments
5974 1 and 2), were identified.

5975 In the rat study (Ben-Jonathan et al., 2018 (NTP Grantee study) [RefID 13786], Tier 1), doses between
5976 0.05 and 25000 µg/kg bw per day were tested and no effect has been observed with measurements
5977 taken at 1 year.

5978 In the mouse study (Patel et al., 2014 [RefID 5695], experiments 1 and 2, Tier 2), a dose of 5 µg/kg
5979 bw per day was tested during gestation and afterwards a dose of 0.6 µg/kg bw per day until four
5980 months with and without a high-fat diet. No effect has been observed at four months.

5981 For the endpoint leptin, in this exposure period, three studies, two in rats (Ben-Jonathan et al., 2018
5982 (NTP Grantee study) [RefID 13786] and NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370])
5983 and one in mice (Patel et al., 2014 [RefID 5695], experiments 1 and 2), were identified.

5984 In the rat studies (both Tier 1), doses between 0.05 and 25,000 µg/kg bw per day were tested and no
5985 effect has been observed with measurements taken at 1 year.

5986 In the mouse study (Patel et al., 2014 [RefID 5695], experiments 1 and 2, Tier 2), a dose of 5 µg/kg
5987 bw per day was tested during gestation and afterwards a dose of 0.6 µg/kg bw per day was given until
5988 four months with and without a high-fat diet. No effect has been observed at four months in females
5989 and a decrease was observed in males.

5990 In this exposure period, three studies in total were available, in rats two studies and in mice one study.
5991 Adiponectin was measured in two studies, leptin in three studies, whereas glucagon and resistin were
5992 not measured. Most of the studies showed no effect.

5993 The CEP Panel assigned a likelihood level of Not Likely to the effect of BPA exposure during development
5994 and in adulthood on adiponectin (because all studies showed no effect) and on leptin (because both
5995 Tier 1 studies showed no effect and in the mouse study with and without high-fat diet an unexplained
5996 sex difference was observed) and, thus, none of these endpoints was taken forward for BMD analysis.

5997 Growth phase/young age exposure

5998 For the endpoint adiponectin, in this exposure period, two studies, one in rats (Yang YJ et al., 2014
5999 [RefID 10269]) and one in mice (Wyatt et al., 2016 [RefID 8080], experiments 1 and 2), were identified.

6000 In the rat study (Ben-Jonathan et al., 2018 (NTP Grantee study) [RefID 13786], Tier 2), doses between
6001 1 and 100 µg/kg bw per day were tested and no effect was observed with measurements taken at
6002 week 10.

6003 In the mouse study (Wyatt et al., 2016 [RefID 8080], experiments 1 and 2, Tier 2), doses of 1.71 to
6004 1171 µg/kg bw per day were tested from 4 weeks to 11 weeks in two strains. A decrease was observed
6005 at 17.1 µg/kg bw per day only in one strain.

6006 For the endpoint leptin, in this exposure period, two studies, one in rats (Yang YJ et al., 2014 [RefID
6007 10269]) and one in mice (Wyatt et al., 2016 [RefID 8080], experiments 1 and 2), were identified.

6008 In the rat study (Ben-Jonathan et al., 2018 (NTP Grantee study) [RefID 13786], Tier 2), doses between
6009 1 and 100 µg/kg bw per day were tested and no effect has been observed with measurements taken
6010 at week 10.

6011 In the mouse study (Wyatt et al., 2016 [RefID 8080], experiments 1 and 2, Tier 2), doses of 1.71 to
6012 1171 µg/kg bw per day were tested from 4 weeks to 11 weeks in two strains. A decrease was observed
6013 at 17.1 µg/kg bw per day only in one strain.

6014 For the endpoint resistin, in this exposure period, two studies in mice (Wyatt et al., 2016 [RefID 8080],
6015 experiments 1 and 2 and Yang et al., 2016 [RefID 8375], experiments 1 and 2), were identified.

6016 In the mouse study from Yang et al. (2016) [RefID 8375], experiments 1 and 2, Tier 1, doses between
6017 5 and 5000 µg/kg bw per day were tested. In the group with a high-fat diet, no adverse effect has been
6018 observed in males and females, whereas in the group with normal diet, increased levels were seen at
6019 both 50 and 500 µg/kg bw per day both in males and females. In another mouse study (Wyatt et al.,
6020 2016 [RefID 8080], experiments 1 and 2, Tier 2), doses of 1.71 to 1,171 µg/kg bw per day were tested
6021 from 4 weeks to 11 weeks in two strains and no effect was seen in males and females.

6022 In this exposure period, 4 studies in total were available, in rats one study and in mice three studies.
6023 Adiponectin was measured in two studies, leptin in three studies and resistin in two studies, whereas
6024 glucagon was not measured. Most of the results showed no effect.

6025 The CEP Panel assigned a likelihood level of Not Likely to the effect of BPA exposure during growth
6026 phase/young age on adiponectin and leptin (because only in one strain at one dose an effect was
6027 observed) and on resistin (because only in one mouse study with normal diet an effect was observed
6028 without dose–response and in the other three studies no effect was observed) and, thus, none of these
6029 endpoints was taken forward for BMD analysis.

6030 Adult exposure (after puberty)

6031 For the endpoint adiponectin, in this exposure period, one Tier 2 study in mice (Wyatt et al., 2016
6032 [RefID 8080]) was identified. Doses between 1.71 and 1171 µg/kg bw per day were tested and no
6033 adverse effect has been observed at week 11.

6034 For the endpoint leptin, in this exposure period, three studies, one in rats (Santos-Silva et al., 2018
6035 [RefID 13047]) and two in mice (Wyatt et al., 2016 [RefID 8080], experiments 1 and 2 and Yang et
6036 al., 2016 [RefID 8375], experiments 1 and 2), were identified.

6037 In the rat study (Santos-Silva et al., 2018 [RefID 13047], Tier 2), doses of 1785 and 178,500 were
6038 tested and no effects were observed in males and females.

6039 In the mouse study from Wyatt et al. (2016) [RefID 8080], experiments 1 and 2, Tier 2, doses between
6040 1.71 and 1171 µg/kg bw per day were tested and no adverse effect has been observed with
6041 measurements taken at week 11 in males and females in the two strains tested. In the mouse study
6042 from Yang et al. (2016) [RefID 8375], experiments 1 and 2, Tier 1, doses between 5 and 5000 µg/kg
6043 bw per day were tested. In the group with a high-fat diet, no adverse effect has been observed in males
6044 and females, whereas in the group with normal diet, increased levels were seen at all four doses tested
6045 in males and females, without dose–response.

6046 For the endpoint resistin, in this exposure period, two studies in mice (Wyatt et al., 2016 [RefID 8080],
6047 experiments 1 and 2 and Yang et al., 2016 [RefID 8375], experiments 1 and 2), were identified.

6048 In the mouse study from Yang et al. (2016) [RefID 8375], experiments 1 and 2, Tier 1, doses between
6049 5 and 5000 µg/kg bw per day were tested. In the group with a high-fat diet, no adverse effect has
6050 been observed in males and females, whereas in the group with normal diet increased levels were seen
6051 at both 50 and 500 µg/kg bw per day both in males and females. In the mouse study from Wyatt et al.
6052 (2016) [RefID 8080], experiments 1 and 2, Tier 2, doses of 1.71 to 1171 µg/kg bw per day were tested
6053 from 4 weeks to 11 weeks in two strains and no effect was seen in males and females.

6054 In this exposure period, 10 studies in total were available, in rats one study and in mice two studies.
6055 Adiponectin was measured in one study, leptin in three studies and resistin in one study, whereas
6056 glucagon was not measured. Most of the results showed no effect.

6057 The CEP Panel assigned a likelihood level of Not Likely to the effect of BPA during adult exposure period
6058 on adiponectin and leptin (because only in one mouse study with normal diet an effect was observed
6059 at all dose levels without dose–response and in the other four studies no effect was observed) and on
6060 resistin (because only in one mouse study with normal diet an effect was observed without dose–

6061 response and in the other three studies no effect was observed) and, thus, none of these endpoints
6062 was taken forward for BMD analysis.

6063 Indirect (germline) exposure

6064 For the endpoint adiponectin, in this exposure period, one rat study (Li GQ et al., 2014 [RefID 4039],
6065 Tier 2) with exposure during gestation (GD0) and lactation (PND21), at 40 µg/kg bw per day and
6066 measurements in the F2 generation at 20 weeks was identified. No effects in the pups or adult rats
6067 were observed.

6068 For the endpoint leptin, in this exposure period, one rat study (Li GQ et al., 2014 [RefID 4039], Tier 2)
6069 with exposure during gestation (GD0) and lactation (PND21), at 40 µg/kg bw per day and
6070 measurements taken in the F2 generation at 20 weeks was identified. No effects in the pups or adult
6071 rats were observed.

6072 The CEP Panel considered that there was Inadequate Evidence for concluding on the likelihood of
6073 effects of BPA on adiponectin and leptin during the indirect (germline) exposure period and, thus, none
6074 of these endpoints was taken forward for BMD analysis.

6075 Overall cluster selection of the endpoints/studies for BMD analysis for other metabolic hormones

6076 Overall, the CEP Panel assigned a likelihood level of Not Likely to the effect of BPA exposure on other
6077 metabolic hormones (adiponectin, leptin, resistin and glucagon), during the developmental until
6078 weaning, developmental until adulthood, growth phase/young age and adult exposure periods. The
6079 CEP Panel considered that there was Inadequate Evidence for concluding on the likelihood of effects of
6080 BPA during indirect (germline) exposure period.

6081 The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster other
6082 metabolic hormones, was Not Likely.

6083 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
6084 Likely effect of BPA in any exposure period, therefore, none of the endpoints was taken forward for
6085 BMD analysis.

6086 **Thyroid hormones**

6087 Hormone measurements (T₃, TT₃, FT₃, T₄, TT₄, FT₄ and reverse T₃/TT₄ (rT₃/TT₄)) were available for the
6088 assessment of the function of the thyroid.

6089 Thyroid hormones were measured in studies with exposure during development until weaning period
6090 (one Tier 1 study in sheep (Guignard et al., 2017 [RefID 2451]), two Tier 1 studies in rats (NTP Clarity
6091 Report, 2018/Camacho et al., 2019 [RefID 11370] and Bansal and Zoeller, 2019 [RefID 13783]) and
6092 one Tier 3 study in mice (Bodin et al., 2014 [RefID 623]), during developmental and adulthood (one
6093 Tier 1 study in rats (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) and during
6094 adulthood (one Tier 1 study in sheep (Guignard et al., 2017 [RefID 2451]) and one Tier 2 study in rats
6095 (Zhang J et al., 2017 [RefID 8770])).

6096 Developmental exposure (pre-natal and/or post-natal until weaning)

6097 For the endpoints T₃, TT₃, FT₃, in a rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
6098 11370]), doses between 2.5 µg/kg bw per day and 25000 µg/kg bw per day were tested in males and
6099 females, with exposure from GD6 until PND21. Measurements were done at 1 year. No effects were
6100 seen. In sheep (Guignard et al., 2017 [RefID 2451]), exposure was from GD28 until GD132 (134) at
6101 doses between 185 µg/kg bw per day and 625,000 µg/kg bw per day and measurement was done on
6102 PND132 (134) in males and females. No effects were seen.

6103 For the endpoints T₄, TT₄, FT₄, in a rat study, doses between 2.5 µg/kg bw per day and 25000 µg/kg
6104 bw per day were tested in males and females with exposure from GD6 until PND15 (or PND21).
6105 Measurements were done at PND15 (Bansal and Zoeller, 2019 [RefID 13783]) or 1 year (NTP Clarity
6106 Report, 2018/Camacho et al., 2019 [RefID 11370]). No effects were seen in any of the studies. In
6107 sheep (Guignard et al., 2017 [RefID 2451]), exposure was from GD28 until GD132 (134) and
6108 measurement was done on PND132 (134) in males and females. No effects were seen.

6109 The CEP Panel assigned a likelihood level of Not Likely to the effect of BPA in the developmental
6110 exposure period on the function of thyroid, thus, none of the endpoints was taken forward for BMD
6111 analysis.

6112 Developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood)

6113 For the endpoints T₃, total T₃ (TT₃), free T₃ (FT₃), in this exposure period one Tier 1 rat study (NTP
6114 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) was identified. No effect was seen on T₃ in
6115 this study. Male and female rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day.

6116 For the endpoints T₄, TT₄, FT₄, in this exposure period, one Tier 1 rat study (NTP Clarity Report,
6117 2018/Camacho et al., 2019 [RefID 11370]) was identified. Rats were dosed with 2.5, 25, 250, 2500 or
6118 25000 µg/kg bw per day; No effect was seen on T₄ in this study in females. No significant changes at
6119 any dose tested in males; Trend analysis by the authors is significant but the nature of the trend is not
6120 evident from inspection of the data.

6121 The CEP Panel assigned a likelihood level of Not Likely to the effect of BPA exposure in the
6122 developmental phase until adulthood on the function of thyroid, thus, none of the endpoints was taken
6123 forward for BMD analysis.

6124 Growth phase/young age exposure

6125 No studies were available on BPA effects on thyroid hormones for this exposure period.

6126 Adult exposure (after puberty)

6127 For the endpoints T₃, TT₃, FT₃, in a rat study (Zhang J et al., 2017 [RefID 8770]), doses between 250
6128 µg/kg bw per day and 1000 µg/kg bw per day were tested in female rats. Measurements of FT₃ were
6129 done. No effects were seen.

6130 In sheep (Guignard et al., 2017 [RefID 2451]), exposure was with 5, 50 and 5000 µg/kg bw per day
6131 and from GD28 to GD132(134). Measurement (T₃) was done on GD132 (134) in females. Reduced TT₃
6132 was observed only at 50 µg/kg bw per day. The ratio reverse T₃/TT₄ was increased, but this endpoint
6133 does not contribute to the assessment of the thyroid function (Smith and Wassner, 2020).

6134 For the endpoints T₄, TT₄, FT₄, in a rat study (Zhang J et al., 2017 [RefID 8770]), doses between 250
6135 µg/kg bw per day and 1000 µg/kg bw per day were tested in female rats. Measurements of FT₄ were
6136 done. No effects were seen.

6137 In sheep (Guignard et al., 2017 [RefID 2451]), exposure was with 5, 50 and 5000 µg/kg bw per day
6138 and from GD28 to GD132 (134). Measurement (FT₄) was done on PND132 (134) in females. Reduced
6139 FT₄ was not dose dependent.

6140 The CEP Panel assigned a likelihood level of Not Likely to the effect of BPA exposure in adulthood on
6141 the function of thyroid, thus, none of the endpoints was taken forward for BMD analysis.

6142 Indirect (germline) exposure

6143 No studies were available on BPA effects on thyroid hormones for this exposure period.

6144 Overall cluster selection of the endpoints/studies for BMD analysis for thyroid hormones

6145 Overall, the CEP Panel assigned a likelihood level of Not Likely to the effect of BPA exposure on the
6146 function of thyroid, in the developmental, developmental and adult, and adult exposure periods.

6147 The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster thyroid
6148 hormones effects, was Not Likely.

6149 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
6150 Likely effect of BPA on thyroid hormones in any exposure period, therefore, none of the endpoints was
6151 taken forward for BMD analysis.

6152 **3.1.4.3. Integration of likelihoods from human and animal studies**

6153 Table 10 presents the overall likelihood per cluster, across all exposure periods, from the human and
 6154 animal studies separately, as well as the integration of the likelihood per cluster from both the human
 6155 and animal studies when available, for the Metabolic HOCs.

6156 The division into clusters was done according to what was considered the best way of clustering the
 6157 endpoints from each of the two streams of evidence (human and animal). There was partial overlap in
 6158 the endpoints included in some of the human and animal clusters. Some endpoints were relevant in
 6159 more than one HOC, such as both for obesity and cardiovascular effects. For instance, levels of leptin
 6160 and adiponectin hormones were included in the human cluster obesity and in the animal cluster other
 6161 metabolic hormones. Other endpoints in the human cluster cardiometabolic effects (cholesterol (total),
 6162 HDL cholesterol, LDL cholesterol and triglycerides) were included in the animal cluster blood Lipids. The
 6163 reasoning behind the inclusion of the endpoints in the specific clusters can be found in Chapter 2.2.2.
 6164 for human evidence and in Chapter 3.1.4.2 for the animal evidence.

6165 **Table 10:** Integration of the human and animal studies for Metabolic effects.

Human stream		Animal stream		Integrated likelihood
Cluster: Obesity		Cluster: Obesity		
Exposure during Pregnancy	Not Likely	Developmental (pre-natal and/or post-natal until weaning)	ALAN	
Exposure during Childhood	Not Likely	Developmental and adult (pre-natal and/or postnatal in pups until adulthood)	ALAN	
Exposure during adulthood	ALAN	Growth phase/young age	ALAN	
		Adult exposure (after puberty)	Not Likely	
		Indirect (germline) exposure	Not Likely	
<i>Overall likelihood: ALAN</i>		<i>Overall likelihood: ALAN</i>		<i>ALAN</i>
Cluster: Thyroid effects		Cluster: Thyroid hormones		
Exposure during Pregnancy	Not Likely	Developmental (pre-natal and/or post-natal until weaning)	Not Likely	
		Developmental and adult (pre-natal and/or postnatal in pups until adulthood)	Not Likely	
		Adult exposure (after puberty)	Not Likely	
<i>Overall likelihood: Not Likely</i>		<i>Overall likelihood: Not Likely</i>		<i>Not Likely</i>
Cluster: Cardiometabolic effects				
Exposure during pregnancy	Not Likely	Not applicable		
<i>Overall likelihood: Not Likely</i>				<i>Not Likely</i>
Cluster: Type 2 Diabetes Mellitus				
Exposure during Adulthood	ALAN	Not applicable		
<i>Overall likelihood: ALAN</i>				<i>ALAN</i>
Cluster: Gestational Diabetes Mellitus				
Exposure during Adulthood	Not Likely	Not applicable		
<i>Overall likelihood: Not Likely</i>				<i>Not Likely</i>
Cluster:		Uric Acid		
Not applicable		Developmental (pre-natal and/or post-natal until weaning)	Inadequate evidence	

	Adult exposure (after puberty)	Likely	
	<i>Overall likelihood:</i>	<i>Likely</i>	<i>Likely</i>
Cluster:	Type 1 Diabetes Mellitus		
Not applicable	Developmental (pre-natal and/or post-natal until weaning)	Inadequate evidence	
	Growth phase/ young age	ALAN	
	Adult exposure (after puberty)	ALAN	
	<i>Overall likelihood:</i>	<i>ALAN</i>	
Cluster:	Fat deposition in the liver		
Not applicable	Developmental (pre-natal and/or post-natal until weaning)	ALAN	
	Developmental and adult (pre-natal and/or postnatal in pups until adulthood)	Inadequate evidence	
	Growth phase/ young age	ALAN	
	Adult exposure (after puberty)	ALAN	
	<i>Overall likelihood:</i>	<i>ALAN</i>	
Cluster:	Glucose regulation		
Not applicable	Developmental (pre-natal and/or post-natal until weaning)	ALAN	
	Developmental and adult (pre-natal and/or postnatal in pups until adulthood)	Not Likely	
	Growth phase/ young age	Not Likely	
	Adult exposure (after puberty)	ALAN	
	Indirect (germline) exposure	ALAN	
	<i>Overall likelihood:</i>	<i>ALAN</i>	
Cluster:	Blood lipids		
Not applicable	Developmental (pre-natal and/or post-natal until weaning)	Not Likely	
	Developmental and adult (pre-natal and/or postnatal in pups until adulthood)	Not Likely	
	Growth phase/ young age	Not Likely	
	Adult exposure (after puberty)	ALAN	
	Indirect (germline) exposure	Not Likely	
	<i>Overall likelihood:</i>	<i>ALAN</i>	
Cluster:	Other metabolic hormones		
Not applicable	Developmental (pre-natal and/or post-natal until weaning)	Not Likely	
	Developmental and adult (pre-natal and/or postnatal in pups until adulthood)	Not Likely	
	Growth phase/ young age	Not Likely	
	Adult exposure (after puberty)	Not Likely	
	Indirect (germline) exposure	Inadequate evidence	
	<i>Overall likelihood:</i>	<i>Overall likelihood:</i>	

6166 **3.1.4.4. In vitro and mechanistic studies**

6167 Regarding scoring of likelihood of effects in the WoE for the HOC Metabolic effects, no clusters were
6168 scored Very Likely for any exposure periods, neither in animals nor humans. A few clusters and
6169 endpoints were scored Likely and several were scored ALAN. In the following, MoA studies for the Likely
6170 and ALAN clusters among the HOC Metabolic effects are described.

6171 There is some overlap in the various parts of the text on MoA, since often the included studies have
6172 examined several MoAs which may belong to more than one cluster.

6173 *Obesity*

6174 After the integration of the human and animal evidence, the overall likelihood of effects of BPA for the
6175 cluster obesity was scored ALAN.

6176 A MoA for obesity studied in many publications in experimental animals involves BPA-induced increased
6177 expression of adipogenic genes and their proteins, increasing adipogenesis, i.e. the generation of
6178 mature adipocytes. Adipogenesis is a tightly controlled differentiation process sensitive to hormones
6179 such as insulin, which is driven by key regulators such as CCAATT enhancer binding proteins (C/EBP)
6180 α , β and δ and peroxisome proliferator-activated receptor gamma (PPAR γ), which induce the expression
6181 of genes that lead to the development of the adipocyte phenotype, including formation of lipid droplets
6182 and adipokine release (Boucher et al., 2014 [RefID 667]). One such gene which is subsequently
6183 upregulated is adipocyte protein 2 (aP2), also known as fatty acid binding protein 4 (FABP4), coding
6184 for a protein involved in insulin sensitivity and glucose metabolism as well as lipid metabolism (Atlas et
6185 al., 2014 [RefID 291]).

6186 In a variety of experiments, Yang et al. (2016) [RefID 8375] investigated if BPA promoted adiposity
6187 and inflammation. In a human study of 228 subjects (mean age 62–63 years, around 39–40% men)
6188 from a Chinese population, it was found that total urinary BPA concentrations were associated with
6189 increased circulating inflammatory factors such as TNF- α , as well as leptin, in lean female subjects (BMI
6190 < 23.0 kg/m²) but not in lean males or in both sexes of overweight/obese subjects (BMI > 25.0 kg/m²).
6191 In a mouse *in vivo* experiment, 5-week-old mice of both sexes were exposed to BPA (5, 50, 500 or
6192 5000 μ g/kg bw per day) orally for 30 days and showed increased body weight and fat mass in a non-
6193 monotonic dose-dependent manner when fed a normal diet, even observed at the lowest dose. On a
6194 HFD, increased body weight was only in seen in males with 50, 500 and 5000 μ g/kg bw per day and
6195 no difference in fat mass was observed in either sex of mice, suggesting that BPA may interact with
6196 diet in affecting obesity. In this mouse *in vivo* experiment, BPA increased mRNA expression of genes
6197 involved in adipogenesis [*C/ebp-a* and *Ppar- γ* (with 5, 50, 500 and 5000 μ g/kg bw) and *Ap2* (50, 500
6198 and 5000 μ g/kg bw)] and lipogenesis [fatty acid synthase (*Fas*) (50, 500 and 5000 μ g/kg bw), *Srebp-
6199 1c* and *Scd-1* (5 and 500 μ g/kg bw)], whereas there were no effects on thermogenesis genes (uncoupling
6200 protein 1 (*Ucp1*) and PPAR γ coactivator (*Pgc-1a*), in male mice on a normal diet. Increased gene
6201 expression of *F4/80*, *Cd11c* and *Mcp-1* in WAT from male mice given BPA (only seen with 500 and/or
6202 5000 μ g/kg bw) on a normal diet, indicated macrophage WAT infiltration. mRNA of cytokines related
6203 to inflammation including *IL-6*, *IL-1 β* , *TNF- α* , *IFN- γ* and *iNos2* were also increased in WAT of male mice
6204 with BPA (500 and/or 5,000 μ g/kg bw) on a normal diet. In an *in vitro* study with differentiated
6205 adipocytes isolated from the stromal vascular fraction of adipose tissue, mRNA expression of *C/ebp-a*,
6206 *Ppar- γ* and *Ap2* was only increased with 10 and/or 50 μ M BPA, not with the lower concentrations. Based
6207 on considerations of all data derived by this study, BPA elevated adipogenesis and lipid biosynthesis,
6208 probably by a direct effect upon differentiation of adipose progenitor cells to white adipocytes, possibly
6209 via GR activation, and BPA may have a role in chronic inflammation.

6210 Overall, the available evidence from human studies was limited in the number of available studies, the
6211 study sample size, was focused on obesity, and lacked sensitivity and specificity for the remaining
6212 metabolic-related biomarkers under study.

6213 Three relevant *in vivo* studies were performed in rats. In primary adipose progenitor stem cells from
6214 newborn male rats maternally exposed to 5 mg/L of BPA in drinking water (converted oral dose 250
6215 μ g/kg bw per day) from 2 weeks before mating and during pregnancy and lactation, significantly
6216 increased protein expression of the adipogenic transcription factor PPAR γ , but not transcription factor
6217 C/EBP α or the lipogenic factor sterol regulatory element-binding protein SREBP-1, was observed at

6218 PND1 (Desai et al., 2018 [RefID 11817]). At PND21, increased protein expression of C/EBP α , but not
6219 PPAR γ , was observed, while SREBP-1 was increased in conjunction with evidence of hypertrophic
6220 adipocytes. Consistent with increased adipose tissue mass and hypertrophic adipocytes in the BPA-
6221 exposed males, protein expression of the macrophage marker cluster of differentiation 68 (CD68) and
6222 pro-inflammatory cytokine tumour necrosis factor (TNF)- α was significantly increased at 3 weeks of
6223 age.

6224 Perinatal BPA exposure on GD6–PND21 to 1 or 10 $\mu\text{g}/\text{mL}$ in drinking water (converted oral doses 50 or
6225 500 $\mu\text{g}/\text{kg}$ bw per day) had long-term adverse effects on body weight and glucose metabolism, probably
6226 associated with the downregulated expression of zinc- α 2-glycoprotein (ZAG) and adiponectin genes in
6227 adolescent female rats (Zhang et al., 2013 [RefID 8798]). In a subsequent study on offspring of female
6228 rats, changes in fat metabolism induced by the same BPA exposure were associated with downregulated
6229 ZAG gene expression caused by decreased PPAR γ mRNA expression (Zhang et al., 2014 [RefID 8797]).

6230 Compared with controls, male and female rat offspring developmentally exposed from GD3.5 to PND22
6231 to 0.5 and 50 $\mu\text{g}/\text{kg}$ bw per day of BPA had differentially expressed mRNA of genes central to lipogenesis
6232 and adipocyte adiponectin signalling in adipose tissue depending on dose, tissue and sex (Lejonklou et
6233 al., 2017 [RefID 3975]). In gonadal white adipose tissue (gWAT) from males, both BPA doses decreased
6234 the mRNA expression of adiponectin receptor 2 (AdipoR2) and acetyl-CoA carboxylase (ACC), and the
6235 50 $\mu\text{g}/\text{kg}$ bw dose decreased stearoyl-CoA desaturase (SCD1), whereas in females, the 50 $\mu\text{g}/\text{kg}$ bw
6236 dose increased AdipoR1 and the 0.5 $\mu\text{g}/\text{kg}$ bw dose decreased SREBP-1c expression. In iWAT from
6237 males, 0.5 $\mu\text{g}/\text{kg}$ bw dose decreased AdipoR1 and SCD1 expression, whereas there were no effects on
6238 these genes in females.

6239 Among the *in vitro* studies, several used human primary adipocytes or human mesenchymal stem cells
6240 (hMSC), which can differentiate into either adipocytes or osteoblasts. A study examined whether BPA
6241 (1, 10 and 100 nM) could interfere with the endocrine function that regulates metabolism in mature
6242 human adipocytes from pre-pubertal, non-obese children (7–10 years old) (Menale et al., 2015 [RefID
6243 5027]). They found that at 24 h, ER α mRNA was upregulated by 10 and 100 nM BPA, ER β mRNA levels
6244 were not affected by any dose of BPA and oestrogen-related receptor- γ (ERR γ) was downregulated by
6245 10 nM BPA. G protein-coupled receptor (GPR30) was downregulated with 10 nM BPA and leptin mRNA
6246 was upregulated by 10 and 100 nM BPA. Microarray results showed that at 24 h 10 nM BPA increased
6247 the expression of pro-inflammatory cytokines (chemokine (C-C motif) ligand 20 (CCL20), interleukin 18
6248 (IL-18) and interleukin 1 β (IL-1 β), and their increased secretion was confirmed by enzyme-linked
6249 immunosorbent assay (ELISA). BPA upregulated the expression of fatty acid binding protein 4 (FABP4)
6250 (1 and 10 nM BPA) and cluster of differentiation 36 (CD36) (10 nM BPA), genes involved in lipid binding
6251 and transport, which was confirmed by mean lipid area and triglyceride measurement in the adipocytes.

6252 The results from a study on hMSC indicated that 1 and 10 nM concentrations of BPA, which did not
6253 induce any alterations on cell viability, self-renewal and oxidative stress, could enhance adipogenic
6254 differentiation seen as changes in gene expression and increased osteogenesis (Dong et al., 2018
6255 [RefID 11840]). There was a need for pre-differentiation exposure to BPA to demonstrate marked
6256 differentiation, indicating that other mechanisms, such as epigenetic alterations, not only regulation of
6257 PPAR γ , might be involved.

6258 Cultured human adipose stromal/stem cells (ASC), the precursors to mature adipocytes, treated with 1
6259 nM, 100 nM and 1 μM BPA had an increase in adipogenesis after 21 days, with a maximal response at
6260 1 μM BPA and with cytotoxicity observed at 10 μM BPA (Ohlstein et al., 2014 [RefID 5491]). BPA for
6261 14 days increased adipogenesis with concentrations of 10 nM, 100 nM and 1 μM , with a maximal
6262 response at 100 nM. The overall proposed mechanism of BPA suggested that BPA induced adipogenesis
6263 through the upregulation of adipogenic genes in an ER-dependent manner. The expression of the
6264 adipogenesis-associated genes dual leucine zipper-bearing kinase (*DLK* (*MAP3K12*)), insulin-like growth
6265 factor 1 (*IGF1*), C/EBP α , PPAR γ and lipoprotein lipase (*LPL*) occurred earlier and was increased by 1
6266 μM BPA (the only concentration tested).

6267 In primary human subcutaneous pre-adipocytes *in vitro*, BPA concentrations (10 nM to 50 μM) did not
6268 induce significant changes in gene or protein expression of the adipogenic markers aP2 and perilipin
6269 below 100 nM (Boucher et al., 2014 [RefID 667]). However, there was a non-significant dose–response
6270 in aP2 protein throughout the dose range, with significant levels reached at 25 and 50 μM BPA. Both
6271 the ER and the GR can influence adipogenesis and lipid metabolism. BPA has been linked to modulation

6272 of both of these nuclear receptors. However, based on the results from the whole dose range used in
6273 this study, BPA induced adipocyte differentiation through a non-classical ER-mediated mechanism
6274 rather than through GR activation, and could contribute to the final maturation of cells committed to
6275 the adipocyte lineage, supporting that BPA may act as an obesogen.

6276 BPA in 1 and 100 nM concentrations impaired insulin sensitivity and 1 nM induced the release of
6277 inflammatory factors in human subcutaneous adipocytes and murine 3T3-L1 embryonic fibroblasts
6278 (Valentino et al., 2013 [RefID 7377]). A suggested mechanism was that BPA activated ERK, phospho-
6279 Thr183/Tyr185c-Jun N-terminal kinase (JNK), via TLRs or ERs, which might directly impair insulin
6280 action. Alternatively, the release of cytokines, interleukin 6 (IL-6) and interferon gamma (IFN- γ), may
6281 contribute to JNK-activation in the adipocytes and either directly or indirectly downregulate insulin-
6282 stimulated glucose uptake.

6283 The potency of BPA (0.1 nM to 0.03 μ M) to act as an ER agonist using both a sensitive oestrogen-
6284 responsive luciferase reporter assay in human T47D-KBluc breast cancer cells, and by assessing ER-
6285 mediated signals, i.e. ERK phosphorylation, mitochondrial respiration and glycolytic function, in murine
6286 3T3-L1 cells, was examined by Tsou et al. (2017) [RefID 7318]. The results indicated that BPA exposure
6287 decreased both mitochondrial respiration and glycolytic function, followed by a rapid and transient
6288 activation of ERK via ER, suggesting that BPA may affect the regulation of body fat.

6289 In murine 3T3-L1 adipocytes, dose-dependent lipid accumulation was seen with BPA, with a five-fold
6290 higher accumulation with 25 μ M versus 1.2–1.8-fold for 0.01–10 μ M BPA (Pomatto et al., 2018 [RefID
6291 12911]). No difference in lipogenesis was seen when BPA exposure occurred at early or mid-late
6292 differentiation. *In silico* molecular docking studies of BPA to PPAR γ and retinoid-X-receptor-alpha
6293 (RXR α) indicated that BPA was capable of binding with these receptors, however, the binding of BPA
6294 to PPAR γ was weak.

6295 BPA (1 nM for 3 weeks) increased both adipocyte number and lipid content by affecting murine 3T3-L1
6296 pre-adipocytic cell growth and altering timing and expression of master genes involved in adipocyte
6297 differentiation and adipose tissue development, e.g. PPAR γ , FABP4/AP2 and C/EBP α (Ariemma et al.,
6298 2016 [RefID 251]). Additionally, BPA may generate metabolic dysfunctional 3T3-L1 adipocytes with
6299 insulin resistance, indicated by downregulation of insulin signalling and reduction of glucose utilisation.

6300 Low concentrations (0.1–10 nM) of BPA dose dependently increased differentiation of murine 3T3-L1
6301 cells into adipocytes in the absence of glucocorticoids and caused the upregulation of the adipogenic
6302 marker aP2, through a potentiation of a transcriptional complex containing GR and C/EBP δ specifically
6303 on the aP2 promoter (Atlas et al., 2014 [RefID 291]). Further, BPA did not activate the nuclear GR on
6304 the mouse mammary tumour virus (MMTV) or the C/EBP α promoters, indicating that BPA was unlikely
6305 to act as a PPAR γ agonist.

6306 In porcine ovarian follicles *in vitro*, BPA (20 ng/mL (87.6 nM)) directly stimulated adiponectin secretion
6307 and expression of adiponectin and its receptors AdipoR1 and AdipoR2 (Rak et al., 2017 [RefID 6083]).
6308 It also increased adiponectin-stimulated E2 secretion, but decreased adiponectin-stimulated
6309 testosterone secretion. In addition, BPA decreased protein expression of cytochrome P450 19 (CYP19)
6310 but did not affect protein expression of 11 β -hydroxysteroid dehydrogenase type 2 (17 β -HSD) in
6311 adiponectin-stimulated ovarian follicular cells.

6312 Regulation of gene expression through epigenetic programming may lead to numerous developmental
6313 disturbances, which may contribute to or cause metabolic disorders. Epigenetic effects, especially DNA
6314 methylation, have been suggested to be involved in BPA-induced obesity.

6315 When studying the role of pre-natal BPA exposure in childhood obesity by combining epidemiological
6316 data with experimental mouse models and BPA-dependent DNA methylation changes *in vitro*, Junge et
6317 al. (2018) [RefID 12262] suggested that pre-natal BPA exposure was associated with BMI Z-scores at
6318 age 1 and 6 years, and was linked to cord blood mesoderm specific transcript (MEST) promoter
6319 methylation and MEST expression. These effects in children were confirmed in mice in which pre-natal
6320 BPA exposure (5 μ g/L in drinking water, converted oral dose 0.45 μ g/kg bw per day) from 1 week
6321 before mating to birth altered *Mest* promoter methylation and transcription with an accompanying
6322 increase in the body weight of the juvenile offspring.

6323 Other studies on epigenetic effects and obesity were also performed in mice. Perinatal BPA exposure
6324 from 2 weeks before mating and through gestation and lactation to 3, 10, 30, 100, 300, 1000 or 3000
6325 µg/kg bw per day in mice demonstrated some dose-dependent metabolic effects (van Esterik et al.,
6326 2014 [RefID 7393]). These included increased body weight and liver weights, but no effects on fat pad
6327 weights, and decreased glucagon in male offspring. Female offspring had decreased body weight, liver,
6328 muscle and fat pad weights, adipocyte size, serum lipids and serum leptin and adiponectin. These
6329 effects persisted into adulthood after termination of BPA exposure at weaning, suggesting that BPA
6330 initiated permanent functional changes when exposed during early development, affecting energy
6331 homeostasis. However, the data did not support BPA as a specific obesogen. In contrast, in a related
6332 study with the same BPA doses and exposure period as well as a high-fat diet (HFD), the observed
6333 altered metabolic phenotypes seen in their earlier work were not found to be associated with liver DNA
6334 methylation changes (van Esterik et al., 2015 [RefID 7393]).

6335 Studies on DNA methylation of hepatic genes using an epigenome-wide discovery platform were
6336 performed in adult female mice offspring after perinatal BPA exposure from 2 weeks before mating
6337 through gestation and weaning to 50 ng, 50 µg and 50 mg/kg diet of BPA (converted oral doses 0.008,
6338 7.5 and 7500 µg/kg bw) per day by Anderson et al. (2017) [RefID 187]. Genes related to energy
6339 expenditure, body weight and body fat phenotypes, Regulatory factor X-associated protein (*Rfxap*),
6340 transmembrane protein 238 (*Tmem238*), Janus kinase 2 (*Jak-2*) and Retinoid-X receptor (*Rxr*), were
6341 identified.

6342 Pre-natal exposure on GD9–GD18 to BPA (5 or 500 µg/kg bw per day) disrupted the bimodal nature of
6343 epigenetic regulation of the FGGY carbohydrate kinase domain containing (*fggy*) gene in gonadal gWAT
6344 of mouse offspring shown by genome-wide DNA methylation and messenger RNA (mRNA) expression,
6345 which may possibly contribute to adult-onset obesity (Taylor et al., 2018 [RefID 13239]).

6346 In differentiated rat primary hypothalamic neuroprogenitor cells (NPCs) from newborn offspring of dams
6347 administered BPA (5 mg/L) in drinking water (converted oral dose 250 µg/kg bw per day) from 2 weeks
6348 before mating and throughout pregnancy, BPA increased appetite peptide and reduced satiety peptide
6349 expression (Desai et al., 2018 [RefID 11816]). The mechanisms for the BPA-induced enhanced
6350 neuroprogenitor cell proliferation and differentiation may involve epigenetic modifications, particularly
6351 altered DNA methylation of the basic helix–loop–helix (bHLH) gene proliferative factor (*Hes1*). No
6352 change in the enzyme DNA methyltransferase 3a (*Dnmt3a*) was seen, however, BPA increased protein
6353 expression of lysine (K)-specific histone demethylase 1A (*LSD1*), indicating an epigenetically-mediated
6354 shift toward neurogenesis.

6355 In addition to DNA methylation, BPA could also affect other epigenetic mechanisms. BPA interacted
6356 with different families of non-coding RNAs (microRNAs, long non-coding RNAs and small nucleolar
6357 RNAs), suggesting that BPA could affect the regulation of gene transcription and various aspects of
6358 post-transcriptional mRNA processing (Verbanck et al., 2017 [RefID 7443]). Using genome-wide gene
6359 expression in differentiating human primary subcutaneous pre-adipocytes at low (10 nM) and high (10
6360 µM) BPA concentrations, microarray data indicated that chronic exposure to BPA had adverse effects
6361 on the transcriptome of human primary adipocytes during differentiation, even at the 10 nM dose.
6362 However, subsequent pathway analysis indicated that the most highlighted pathways were related to
6363 oncogenesis but not adipogenesis or inflammation.

6364 Other MoAs for an obesogenic effect of BPA have been suggested in individual studies. In mice exposed
6365 to 50 µg/kg bw per day of BPA from GD15 to PND21, it was observed that BPA induced intestinal and
6366 systemic immune imbalances at PND45, through a decrease of Th1/Th17 cell frequencies in the lamina
6367 propria concomitant to an increase of splenic Th1/Th17 immune responses (Malaisé et al., 2017 [RefID
6368 4815]). Relative to control mice, these early effects were associated with altered glucose sensitivity,
6369 defect IgA secretion into faeces and a decrease in faecal bifidobacteria. The BPA-mediated disturbed
6370 immune homeostasis and gut dysbiosis preceded infiltration of pro-inflammatory M1 macrophages in
6371 gWAT appearing with ageing, together with a decreased insulin sensitivity and an increased weight
6372 gain.

6373 Exposure of dams to BPA (10 µg/mL in drinking water, 900 µg/kg bw per day) at GD10–PND30 reduced
6374 the expression of the cannabinoid receptor 1 (*CB1*) and induced gene expression of cocaine and
6375 amphetamine regulated transcript-1 in the hypothalamus of male offspring at 78 days of age (Suglia et

6376 al., 2016 [RefID 6943]). This suggested that BPA induced activation of anorexigenic signals, causing
6377 loss of appetite, via downregulation of CB1, reducing food intake.

6378 In conclusion, the CEP Panel considered that there is substantial evidence supporting that BPA is a
6379 potential environmental obesogen based on the available studies on developmentally exposed animals
6380 as well as on cell models, although there is also evidence available against this hypothesis. Several
6381 MoAs for increased adipogenesis have been put forward in these studies, including changes in
6382 expression of adipogenic genes and their proteins (with 0.5–5000 µg/kg bw per day of BPA) leading to
6383 induced differentiation of pre-adipocytes into mature, lipid-accumulating adipocytes. BPA was
6384 considered unlikely to act as a classical oestrogen due to the high concentrations required for the
6385 transactivation of the ER by BPA, but BPA induced adipocyte differentiation through a non-classical ER-
6386 mediated mechanism. BPA does not seem to act as a GR agonist in differentiation of murine and human
6387 adipocytes. The increased gene expression may be regulated via epigenetic effects, of which DNA
6388 methylation is mostly studied (with 0.008–7500 µg/kg bw of BPA). Single studies also suggested the
6389 effect of BPA on obesity to be caused by disturbed immune homeostasis and gut dysbiosis (50 µg/kg
6390 bw) or via effects on the signalling systems of the brain regulating appetite and food intake (900 µg/kg
6391 bw). However, the underlying mechanisms resulting in adverse apical effects in humans, such as
6392 increased body weight or BMI, need further investigation.

6393 *Fat deposition in the liver*

6394 There was no human evidence available for the cluster fat deposition in the liver. Thus, the overall
6395 likelihood of effects of BPA for this cluster was scored ALAN, based on the animal evidence.

6396 Lipid metabolism and excess fat accumulation (steatosis) in the liver can be a result of abnormal
6397 synthesis, retention or breakdown of lipid. In addition to *in vivo* animal studies, *in vitro* studies on lipid
6398 metabolism often used hepatoma cell lines to examine modulation of expression of relevant genes
6399 and/or proteins.

6400 Development of hepatic steatosis in offspring of rats after exposure to BPA (40 µg/kg bw per day)
6401 during gestation and lactation (GD0–PND21) was mediated through impaired hepatic mitochondrial
6402 function, subsequent oxidative stress and upregulated hepatic lipid metabolism (Jiang et al., 2014
6403 [RefID 3190]).

6404 Hepatic steatosis in offspring of pregnant rats exposed to 100 µg/kg bw per day of BPA on GD6–PND21
6405 was associated with increased and modified hepatic triglyceride and FFA compositions in males only
6406 (Strakovsky et al., 2015 [RefID 6914]). In males, BPA increased the hepatic expression of the FFA
6407 uptake gene fatty acid translocase/cluster determinant 36 (*Fat/Cd36*) and decreased the expression of
6408 triglyceride synthesis-related and β-oxidation-related genes (diacylglycerol O-acyltransferase 1 (*Dgat1*),
6409 1-acylglycerol-3-phosphate O-acyltransferase 6 (*Agpat6*), C/EBPα (*Cebpa*), C/EBPβ (*Cebpb*),
6410 phosphoenolpyruvate carboxykinase 1 (*Pck1*), phosphoenolpyruvate carboxykinase 1 (*Acox1*), carnitine
6411 palmitoyltransferase 1a (*Cpt1a*) and cytochrome *b*-245, β polypeptide (*Cybb*)). BPA altered DNA
6412 methylation and histone marks (acetylated histone H3 or H4 (H3Ac or H4Ac), di-methylated histone H3
6413 at lysine residue 4 (H3Me2K4), tri-methylated histone H3 at lysine residue 36 (H3Me3K36)) and
6414 decreased the binding of several transcription factors (Pol II, C/EBPβ and SREBP-1) within the gene for
6415 the key β-oxidation enzyme *Cpt1a*. In females, BPA only increased the expression of genes involved in
6416 FFA uptake and triglyceride synthesis (*LPL*, fatty acid synthase (*Fasn*) and *Dgat*). The study indicated
6417 that developmental BPA exposure altered and reprogrammed hepatic β-oxidation capacity in males,
6418 potentially through the epigenetic regulation of genes.

6419 In adult male rats administered 10000 µg/kg bw per day of BPA via drinking water for 60 days, BPA
6420 increased plasma levels of triglycerides, total cholesterol, LDL-cholesterol, AST, alanine transaminase
6421 (ALT), LDH and TNF-α (Mahmoudi et al., 2018 [RefID 12656]). Immunohistochemical analysis showed
6422 increased expression of cyclooxygenase-2 (COX-2) and tumour protein p53 (p53) and decreased
6423 expression of B-cell lymphoma 2 (Bcl-2), related to liver inflammation. Thus, BPA may contribute to
6424 hepatotoxicity via oxidative stress, lipid metabolism disruption and degenerative changes in hepatic
6425 cells.

6426 Experiments were performed on adult male mice exposed to 5, 50 or 500 µg/kg bw per day of BPA for
6427 eight weeks and *in vitro* experiments in primary murine hepatocytes and the human HepG2 cell line

6428 with 0.001–10 μ M BPA (Yang SM et al., 2017 [RefID 8398]). Based on effects in mice and *in vitro* with
6429 10 μ M BPA, it was shown that BPA had dual inhibitory effects on the autophagy-lysosomal pathway,
6430 suppressing the initiation of autophagy and inhibiting autophagic degradation, thus, affecting hepatic
6431 lipid accumulation. The likely major mechanism for the inhibition of autophagy initiation was activation
6432 of the serine/threonine protein kinase mTOR, whereas to elucidate how BPA inhibits autophagic
6433 degradation will need further studies.

6434 Studies of induction of steatosis by BPA (0.2, 2, 20, 200 or 2000 nM) in the developing liver model
6435 human (female) hepatoma HepaRG cell line indicated that BPA increased lipid accumulation with a non-
6436 monotonic dose–response, with significant effects on neutral lipids and triglycerides at 2 nM only
6437 (Bucher et al., 2017 [RefID 743]). The gene expression of many enzymes involved in lipid and
6438 carbohydrate homeostasis and oxidative stress was not changed. Further, the expression of different
6439 known targets of ERR γ and pregnane X receptor (PXR), enzymes involved in BPA biotransformation,
6440 was not changed, suggesting that these nuclear receptors were not involved in BPA-induced steatosis.

6441 The overall conclusions on effects of BPA (0.3, 3, 30 or 300 ng/mL (1.3, 13.1, 131.4 or 1314.1 nM)) in
6442 rat hepatoma FaO cells were that BPA could directly induce lipid accumulation by interfering with the
6443 pathways involved in lipid oxidation and secretion, without affecting those involved in lipogenesis
6444 (Grasselli et al., 2013 [RefID 2386]). Significant increased levels of triacylglycerol (TAG) in these cells
6445 were observed dose dependently with 131.4 and 1314.1 nM BPA only. The mechanisms of action
6446 appeared to be ER-independent and involved activation of kinase-mediated pathways, in particular the
6447 phosphatidyl inositol-3 kinase (PI-3K) pathway.

6448 Several of the available studies focused on two specific MoAs for fat deposition in the liver, by measuring
6449 how BPA could modulate PPARs and SREBPs genes and proteins. PPARs are ligand-activated
6450 transcription factors and function as regulators of lipid and lipoprotein metabolism and glucose
6451 homeostasis, and also influence cellular proliferation, differentiation and apoptosis (Grygiel-Górniak,
6452 2014). PPAR α is highly expressed in tissues such as liver, muscle, kidneys and heart, where it stimulates
6453 the β -oxidative degradation of fatty acids, lowering lipid levels. PPAR γ is mostly involved in the
6454 regulation of the adipogenesis, energy balance and lipid biosynthesis. SREBPs are a family of
6455 transcription factors that regulate lipid biosynthesis and adipogenesis by controlling the expression of
6456 several enzymes required for cholesterol, fatty acid, TAG and phospholipid synthesis (Shimano and
6457 Sato, 2017).

6458 Exposure to 50 μ g/kg bw per day of BPA throughout gestation and lactation (GD0–PND21) predisposed
6459 rat offspring to fatty liver disease when fed a standard diet or a HFD after weaning (Wei et al., 2014
6460 [RefID 7890]). Perinatal exposure to BPA worsened the hepatic damage caused by the HFD. The
6461 additive effects of BPA were correlated with higher levels of hepatic oxidative stress. The expression of
6462 mRNA and protein of SREBf1 was significantly increased in the liver regardless of diet. Similarly, mRNA
6463 expression of Fasn was co-ordinately upregulated. The mRNA expressions of PPAR γ and Cd36 were
6464 elevated in BPA-exposed offspring on HFD only. The mRNA levels of PPAR α and one of its targets Cpt1a
6465 were increased on a standard diet, whereas expression of both genes was decreased on a HFD with
6466 BPA compared with HFD controls.

6467 After exposure to BPA (10 μ g/kg bw per day s.c., converted oral dose 2222 μ g/kg bw per day) during
6468 GD9–GD16 of pregnancy, hepatic triglyceride levels were increased in male mice offspring compared
6469 with controls (García-Arévalo et al., 2014 [RefID 2193]). BPA altered the expression of important genes
6470 involved in fatty acid uptake in the liver, i.e. upregulated *Ppar γ* and the 5'-adenosine monophosphate-
6471 activated protein kinase (AMPK) gene protein kinase AMP-activated catalytic subunit α 1 (*Prkaa1*). AMPK
6472 is a fuel sensor protein that inhibits anabolic pathways and activates catabolic pathways in the liver.
6473 BPA also decreased the expression of the *Cd36* gene, coding for a fatty acid transport protein.

6474 Perinatal (GD8–PND16 and PND21–PND35) BPA exposure (25 μ g/kg bw per day) of mice offspring
6475 induced persistent fat accumulation via hypomethylation of lipogenic genes and increased nuclear factor
6476 erythroid 2-related factor 2 (Nrf2) recruitment to the *Srebp-1c* promoter in the liver (Shimpi et al., 2017
6477 [RefID 6685]).

6478 BPA (0.5 μ g/kg bw per day) exposure of mice from birth to 10 months of age influenced the expression
6479 of genes involved in hepatic lipid metabolism, thereby leading to increased hepatic accumulation of
6480 cholesterol and triglycerides in middle-aged males (Ke et al., 2016 [RefID 3447]). Based on this *in vivo*

6481 study and murine hepatocyte Hepa1–6 cell line studies, downregulation of DNA methyltransferases and
6482 upregulation of the transcription factors *Srebf1* and *Srebf2*, and *Fasn* and 3-hydroxy-3-methyl glutaryl-
6483 coenzyme A reductase (*Hmgcr*) genes, key enzymes in fatty acid and cholesterol synthesis, respectively,
6484 indicated that BPA epigenetically reprogrammed DNA methylation patterns of genes involved in hepatic
6485 lipid synthesis, likely caused by disrupted expression of DNA methyltransferases.

6486 The role of KC polarisation in hepatosteatosis induced in adult male mice exposed to BPA at 5, 50 or
6487 500 µg/kg bw per day for eight weeks, or *in vitro* in primary KC cultures from the mice and the human
6488 hepatocellular carcinoma cell line HepG2 exposed to 10 µM BPA, was studied by Lv Q et al. (2017)
6489 [RefID 4697]. The effects of BPA on mRNA levels of key components involved in hepatic lipid
6490 metabolism and transport were evaluated, including SREBP-1, FAS, PPAR α , microsomal triglyceride
6491 transfer protein (MTP), adipose triglyceride lipase (ATGL), CD36 and fatty acid transport protein 1
6492 (FATP1). Overall, BPA promoted hepatic lipid synthesis without changing lipid transport at the
6493 transcription level. The results also demonstrated that pro-inflammatory M1 KC polarisation was
6494 involved in BPA-induced hepatic fat deposition, possibly associated with the ER signalling pathway.

6495 Reverse transcription quantitative polymerase chain reaction (RT-qPCR) performed on adult mouse
6496 hepatic mRNA after exposure to BPA (5, 50, 500 or 5000 µg/kg bw per day) for 8 months demonstrated
6497 an overexpression of key genes involved in cholesterol biosynthesis; *Mvd* (mevalonate
6498 diphosphodecarboxylase), *Lss* (lanosterol synthase), *Hmgcr* and *Sqle* (squalene epoxidase) (Marmugi
6499 et al., 2014 [RefID 4884]). BPA also induced the expression of *Srebp-2*, a master regulator of hepatic
6500 cholesterol biosynthesis.

6501 BPA exposure (100 µg/kg bw per day) on GD7–PND21 promoted expression of hepatic lipid synthesis
6502 and fatty acid accumulation genes in female adolescent mice offspring (Meng Z et al., 2018 [RefID
6503 12708]). *Fasn* and *Cd36* mRNA increased significantly, whereas the mRNA expression of *Scd1*, a key
6504 enzyme regulating formation of monounsaturated fatty acids (MUFA), decreased, resulting in a
6505 significant increase in two FFA (saturated fatty acid (SFA) C20:0 and MUFA C20:1n9).

6506 The effects of BPA on lipid metabolism were studied using both adult C57BL/6 mice exposed to 50
6507 µg/kg bw per day for 90 days and the human hepatocellular carcinoma cell line HepG2 (Lin Y et al.,
6508 2017 [RefID 4338]). Overall, it was concluded that BPA-induced NAFLD was caused by downregulation
6509 of the microRNA (miR)-192 both *in vivo* and *in vitro* resulting in increased SREBF1 expression in
6510 hepatocytes, thus, increasing triglyceride synthesis. However, these *in vitro* data were based on
6511 significant effects seen only above 100 nM (with 200 and 2000 nM).

6512 Human liver HepG2 cells exposed to BPA (1 fM to 1 µM) showed an increase in triglyceride storage only
6513 with 1 pM but no change in glucose uptake (Héliès-Toussaint et al., 2014 [RefID 2676]). The effects
6514 were potentially linked to increased expression of early differentiation genes SREB1c, PPAR γ and aP2,
6515 as well as ERR α and ERR γ genes.

6516 In conclusion, the CEP Panel considered that there is substantial evidence supporting that BPA may
6517 increase fat accumulation in the liver based on the available studies on animals after developmental
6518 exposure or as adults as well as in cell models. The MoAs for increased fat deposition in the liver being
6519 put forward in these studies are that BPA is causing abnormal synthesis, retention or breakdown of
6520 lipid (with 5–10000 µg/kg bw per day of BPA), often via modulation of the PPARs and SREBPs genes
6521 and proteins (with 0.5–5000 µg/kg bw per day of BPA).

6522 *Glucose regulation*

6523 There was no human evidence available for the cluster glucose regulation. Thus, the overall likelihood
6524 of effects of BPA for this cluster was scored ALAN, based on the animal evidence.

6525 The main function of β -cells in the islets of pancreas is to produce and secrete insulin, the hormone
6526 responsible for regulating levels of glucose in the blood. Several studies were concerned with BPA-
6527 induced β -cell dysfunction and disturbance of glucose homeostasis, with the underlying mechanisms
6528 possibly being loss of β -cell mass or modulation of insulin secretion from the β -cells.

6529 After exposure of mice to BPA at 10 or 100 µg/kg bw per day s.c. (converted oral doses 2222 or 22220
6530 µg/kg bw per day) during pregnancy (GD9–GD16), the offspring had decreased insulin secretion and
6531 reduced pancreatic β -cell mass (Alonso-Magdalena et al., 2015 [RefID 139]). Proliferation was

6532 decreased together with decreased expression of the cell cycle activators cyclin D2 (*Ccnd2*) and cyclin-
6533 dependent kinase-4 (*Cdk-4*). In addition, β -cell apoptosis level and expression of the cell cycle inhibitors
6534 p16 and p53 were increased. In contrast, when female non-pregnant mice were treated with BPA at
6535 the same doses no effects on glucose metabolism or insulin sensitivity were observed.

6536 BPA exposure (10 or 100 $\mu\text{g}/\text{kg}$ bw per day s.c., converted oral doses 2222 or 22220 $\mu\text{g}/\text{kg}$ bw per
6537 day) during pregnancy (GD9–GD16) affected pancreatic β -cell growth and function in mice offspring
6538 during early life (García-Arévalo et al., 2016 [RefID 2194]). BPA increased β -cell mass/area with
6539 hyperinsulinemia without insulin resistance and insulin over-secretion. Microarray analysis showed that
6540 BPA altered the expression of genes in the islets involved in β -cell growth regulation and β -cell
6541 proliferation. Excess of insulin signalling during early life may contribute to impaired glucose tolerance
6542 during adulthood and also obesity.

6543 Pancreatic islets of adult F1 and F2 mice offspring from F0 dams exposed to doses of 10 or 10000 $\mu\text{g}/\text{kg}$
6544 bw per day of BPA in the diet from two weeks before mating and during gestation and lactation showed
6545 dose-specific and sex-specific effects (Bansal et al., 2017 [RefID 9499]). The high BPA dose impaired
6546 mitochondrial function, whereas the low dose reduced β -cell mass and increased β -cell death that
6547 persisted into the F2 generation. Increased insulin-like growth factor 2 (*Igf2*) expression persisted in
6548 the islets of male F1 and F2 offspring and was associated with altered DNA methylation. Thus, BPA
6549 exposure induced impaired glucose homeostasis in the F2 offspring through the transmission of sperm,
6550 possibly via either mitochondrial dysfunction and/or epigenetic modifications.

6551 After BPA exposure at 100 $\mu\text{g}/\text{kg}$ bw per day from GD7 to PND21, adolescent female mice offspring
6552 showed changes in expression of genes related to hepatic glucose metabolism, glycolysis and glucose
6553 transport [farnesoid X receptor (*Fxr*), small heterodimer partner (*Shp*), glucose-6-phosphatase
6554 (*G6Pase*), pyruvate kinase isozymes R/L (*Pk1r*) and glycogen transporter gene (*Glut2*), and severely
6555 disturbed glucose homeostasis (Meng Z et al., 2018 [RefID 12708]).

6556 In mouse pancreatic β -cells from wild-type and oestrogen receptor beta (*Er β*)^{-/-} mice, exposure to low
6557 BPA concentrations (100 pM and 1 nM) *in vitro* decreased Ca^{2+} entry via an ER β -dependent pathway
6558 involving the transcriptional regulation of Cav2.3 ion channels, whereas high BPA concentrations (10
6559 and 100 nM) had no such effects (Villar-Pazos et al., 2017 [RefID 13316]). Higher BPA concentrations
6560 (100 nM to 1 μM) involved both ER β and *Era*, which counteracted each other. *Era* increased Ca^{2+} entry
6561 only in response to higher BPA concentrations (100 nM and 1 μM) after increasing Ca^{2+} currents in a
6562 pathway involving phosphoinositide 3-kinase (PI3K). Based on these results, BPA affected the
6563 pancreatic β -cell insulin content and secretion via oestrogen receptors ER α and ER β actions outside the
6564 nucleus. The combined opposing effect of ER α and ER β upon Ca^{2+} entry was hypothesised by the
6565 authors to potentially explain the NMDR relationship observed for BPA effects.

6566 A link between BPA exposure and the potential risk of diabetes was reported by Perreault et al. (2013)
6567 [RefID 5797], who performed two separate experiments on BPA and effects on hepatic glucokinase
6568 activity, a glucose sensor in the body. In the first experiment, adult male mice were exposed by gavage
6569 once with 50 $\mu\text{g}/\text{kg}$ bw of BPA. In the second experiment, adult male mice were exposed via drinking
6570 water to 50 $\mu\text{g}/\text{kg}$ bw of BPA once daily for two weeks. The results showed that both a single dose and
6571 exposure over a two-week period to BPA reduced hepatic glucokinase activity over a range of tested
6572 glucose concentrations (0–20 mmol/L).

6573 Whether BPA exposure at 40 $\mu\text{g}/\text{kg}$ bw per day during gestation and lactation could disrupt glucose
6574 homeostasis was studied in F2 rat offspring (Li GQ et al., 2014a [RefID 4039]). The glucokinase (*Gck*)
6575 promoter in F2 hepatic tissue was completely methylated in all CpG sites compared with five
6576 unmethylated sites in controls. In the F1 sperm, the global DNA methylation was decreased. However,
6577 there was only one CpG site (-314) that was differently methylated between BPA and controls in sperm.
6578 The study supported the hypothesis that glucose metabolism disorders can be induced trans-
6579 generationally through epigenetic alterations, especially DNA methylation changes in sperm from as
6580 early as during paternal development.

6581 There were also several studies that investigated the effect of BPA on insulin resistance and insulin
6582 signalling. Epigenetic effects from exposure of BPA (50 $\mu\text{g}/\text{kg}$ bw per day) throughout gestation and
6583 lactation (GD0–PND21) in male rat F1 offspring were investigated by Ma et al. (2013) [RefID 4748].
6584 The results showed insulin resistance caused by decreased global hepatic methylation accompanied by

6585 overexpression of DNA methyltransferase 3B mRNA, in addition to promoter hypermethylation and
6586 reduction of expression of the hepatic *Gck* gene.

6587 In four-week-old to six-week-old mice given 50 µg/kg bw per day of BPA for 12 weeks, serum insulin
6588 levels did not increase but glucose intolerance tended to increase in the growing males fed a HFD
6589 (Moon et al., 2015 [RefID 5195]). Altered serum adipocytokine level causing decreased phosphorylation
6590 of Akt, an important factor in the insulin signalling pathway, in skeletal muscle, was suggested as one
6591 mechanism by which BPA induced glucose intolerance and insulin resistance.

6592 Treatment of human liver HepG2 cells with 100 nM BPA induced significantly decreased glucose
6593 consumption, impaired insulin signalling, increased pro-inflammatory cytokines and oxidative stress,
6594 and activated signalling pathways (Geng et al., 2017 [RefID 2242]). Inhibition of JNK and p38 pathways,
6595 but not ERK nor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways,
6596 improved glucose consumption and insulin signalling, indicating a role of JNK/p38 activation in BPA-
6597 induced insulin resistance. In a later study with normal human hepatocyte LO2 cells treated with 100
6598 nM BPA, inhibition of the JNK pathway, but not the p38 nor NF-κB pathways, improved glucose
6599 consumption and insulin signalling (Geng et al., 2018 [RefID 11987]).

6600 The human pancreatic cell line (PANC-1) treated with 10 nM BPA had decreased secretion of active
6601 insulin and decreased mRNA expression of PCSK1, pro-protein convertase subtilisin/kexin type 1
6602 (PC1/3), a pro-insulin-processing enzyme that regulates insulin biosynthesis (Menale et al., 2015 [RefID
6603 5027]).

6604 Carchia et al. (2015) [RefID 852] used a toxicogenomic approach to investigate the mechanisms of
6605 effects of 1 nM of BPA in cultured *ex vivo* murine primary hepatocytes and pancreatic islets and further
6606 investigated pancreatic islet mitochondrial dysfunction, cellular pathways and response to glucose
6607 stress. *In vivo* studies on mice involved the induction of hyperglycaemia via a single dose of STZ and
6608 was used to confirm *in vitro* findings. Twenty-nine inhibited genes were identified in the islets and none
6609 in the hepatocytes. Although their expression was slightly altered, their impaired cellular level as a
6610 whole resulted in specific phenotypic changes. It was concluded that BPA affected two complementary
6611 mechanisms on mitochondria: enhancement of oxidative stress and decreased ROS scavenging
6612 systems, promoting apoptosis. The data suggested a multifactorial mechanism for BPA toxicity in
6613 pancreatic islets involving mitochondria dysfunction and NF-κB activation.

6614 Effects of BPA on metabolism and metabolomics were examined in several publications. In a study in
6615 pre-adipocytes and differentiated adipocytes obtained from non-obese children (mean age of
6616 10.5 ± 2.3 years), Menale et al. (2017) [RefID 5026] showed that BPA could alter adipocyte metabolism
6617 contributing to metabolic dysfunction, such as insulin resistance, by altering expression of adiponectin
6618 (mRNA significantly decreased with 10 and 100 nM) and resistin (induced with 1, 10 and 100 nM).

6619 BPA exposure (1 or 10 µg/mL, converted oral doses 35.7 or 357 µg/kg bw per day) in drinking water
6620 from GD6 to PND21 had long-term deleterious effects on body weight and glucose metabolism in female
6621 adolescent rats, which appeared to be associated with the downregulated expression of ZAG and
6622 adiponectin mRNA and proteins (Zhang et al., 2013 [RefID 8798]). In a further study with the same
6623 experimental design, abnormal glucose metabolism and insulin resistance were seen with both doses
6624 at PND100, but only with the high dose at PND50 (Song SZ et al., 2014 [RefID 6829]). The insulin
6625 resistance was associated with decreased adiponectin production and increased oxidative damage.

6626 After short-term (20 min or 48 h, 100–1000 nM) and long-term (four weeks, 250 nM) exposure to BPA
6627 in the oestrogen-dependent human breast cancer cell line MCF-7, BPA increased glucose uptake only
6628 with 250 and 500 nM after 20 min and had no effects after 48 h, and decreased glucose with 250 nM
6629 after long-term exposure. BPA did not affect the level of lactate, a measure of glucose oxidation
6630 efficiency, at any exposure times (Norberto et al., 2017 [RefID 5440]).

6631 Low concentrations of BPA (0.1 nM to 100 µM) reduced adipocyte-specific gene expression, but did not
6632 accelerate adipogenesis, in human adipose-derived stromal cells or murine 3T3-L1 or C3H10T1/2 cells
6633 (De Filippis et al., 2018 [RefID 11796]). The data indicated that chronic BPA exposure was unlikely to
6634 directly cause the increase in fat tissue, at least mediated through direct adipocyte differentiation. BPA
6635 exposure during differentiation led to generation of dysfunctional adipocytes with reduced insulin-
6636 stimulated glucose uptake and protein kinase B (Akt) phosphorylation associated with increased
6637 expression of pro-inflammatory cytokines such as TNF-α and IL-6, without affecting expression of

6638 adiponectin. Thus, chronic BPA exposure appeared to contribute to chronic, low grade inflammation,
6639 which subsequently may affect insulin sensitivity in adipose tissue independent of adipogenesis. This
6640 suggest that BPA may actually be a more potent diabetogen than obesogen.

6641 A metabolomics study with ¹H-nuclear magnetic resonance (NMR)-based spectroscopy examined
6642 metabolic shifts induced by perinatal (GD8–LD16) exposure to BPA (0.025, 0.25 or 25 µg/kg bw per
6643 day s.c., converted oral doses 5.6, 55.6 or 555.5 µg/kg bw per day) in male mice offspring (Cabaton et
6644 al., 2013 [RefID 776]). Variations in glucose, pyruvate, some amino acids and neurotransmitters (γ-
6645 aminobutyric acid and GLU) were found, mainly affecting energy metabolism and brain function. The
6646 shift observed for glucose would likely be involved in the disruption of pyruvate biosynthesis through
6647 glycolysis. In a later *in vitro* study, Cabaton et al. (2018) [RefID 11650] showed by bioinformatics
6648 (metabolic network modelling) that BPA could modulate major metabolic routes involved in cellular
6649 functioning and detoxification processes in human hepatoblastoma HepG2 cells. While having some
6650 commonality with E2, BPA had a distinct activity possibly reflecting a ligand-specific conformation
6651 change of the activated ER. The results also indicated that metabolism of branched-chain amino acids
6652 might be modulated by BPA with possible consequences in the promotion of protein synthesis and
6653 turnover, signalling pathways and the metabolism of glucose.

6654 Serum ¹H-NMR metabolomics studies of effects of BPA exposure at 10 µg/kg bw per day s.c. (converted
6655 oral doses 222.2 µg/kg bw per day) during gestation (GD10–GD18) on male mice offspring generally
6656 did not change expression of genes involved in glucose homeostasis compared with controls, except
6657 for an upregulation of hepatic glycogen synthase 2 (Gys2) (Wang D et al., 2018 [RefID 13341]).

6658 Using ¹H-NMR-based metabolomics, with F0 rat dams exposed to low doses of BPA (0.25, 2.5, 25 and
6659 250 µg/kg bw s.c., converted oral doses 8.9, 89.3, 892.5 and 8925 µg/kg bw per day) from GD9 to
6660 PND16, Tremblay-Franco et al. (2015) [RefID 7285] demonstrated that metabolism of glucose, lactate
6661 and fatty acids was modified over time in BPA-exposed female offspring. The data showed that BPA
6662 may modulate energy metabolism and neurotransmitter signalling.

6663 More specific MoAs by BPA, such as modulation of the homeodomain-containing transcription factor
6664 pancreatic and duodenal homeobox 1 (Pdx-1), were demonstrated. Pdx-1 is a major regulator of
6665 transcription in pancreatic cells. Glucose regulates insulin gene transcription through Pdx-1. Thus, the
6666 modulation of Pdx-1 may disturb the glucose homeostasis (Chang et al., 2016 [RefID 965]).

6667 Maternal exposure to BPA at 10 µg/kg bw per day during gestation and lactation reduced pancreatic β-
6668 cell mass at birth in rat offspring (Chang et al., 2016 [RefID 965]). This was possibly caused by
6669 reduction of Pdx-1⁺ progenitor cells during fetal development by alteration of histone modifications
6670 (histones H3 and H4 deacetylation, demethylation of histone 3 lysine 4 (H3K4) and methylation of
6671 histone 3 lysine 9 (H3K9)) in the promoter of Pdx-1, potentially increasing susceptibility to glucose
6672 intolerance in later life.

6673 Insulin-like growth factor 2 (Igf2) is a protein hormone that is structurally similar to insulin and is acting
6674 as a growth-regulating and mitogenic factor. Several studies examined changes in expression of Igf2
6675 after BPA exposure, possibly involving epigenetic mechanisms, which may lead to glucose intolerance
6676 and β-cell dysfunction.

6677 Early-life BPA exposure from two weeks before mating and through gestation and lactation to 10 or
6678 10000 µg/kg bw per day perturbed metabolic health across F1 and F2 generations in mice through
6679 stable inheritance of increased DNA methylation at the *Igf2* differentially methylated region 1 (Susiarjo
6680 et al., 2015 [RefID 7022]).

6681 Exposure of F0 mice to 10 or 10000 µg/kg bw per day of BPA from two weeks before mating and during
6682 gestation and lactation lead to induced islet inflammation in male F1 offspring that persisted into the
6683 F2 generation (Bansal et al., 2017 [RefID 9499]). The higher dose impaired mitochondrial function,
6684 whereas the lower dose reduced β-cell mass and increased β-cell death. Increased Igf2 expression
6685 persisted in the islets of male F1 and F2 offspring and was associated with altered DNA methylation.

6686 Exposure of rats to 40 µg/kg bw per day of BPA during gestation and lactation (GD0–PND21) resulted
6687 in generational transmission of glucose intolerance and β-cell dysfunction in rat offspring through the
6688 male germline from F1 to F2 generation (Mao et al., 2015 [RefID 4864]). This transfer was associated
6689 with hypermethylation of Igf2 in islets. In a subsequent study with the same experimental design, Mao

6690 et al. (2017) [RefID 4865] showed that BPA-induced pancreatic impairments in the rat offspring were
6691 associated with DNA hypermethylation and low expression of Igf2 in the islets, and that maternal dietary
6692 folate supplementation counteracted these effects, upregulating Igf2 expression.

6693 Some available studies also suggested that endoplasmic reticulum stress contributes to pancreatic β -
6694 cell loss and insulin resistance, and thus, may have a role in the pathogenesis of diabetes.

6695 In pancreatic β -cells, which produce and secrete insulin, Ca^{2+} signals contribute to insulin production
6696 and secretion. After BPA exposure (5,000 $\mu\text{g}/\text{kg}$ bw) for five days of adult male mice using the STZ-
6697 induced T1DM model, Ahn et al. (2018) [RefID 11452] found that expression of genes involved in
6698 transporting Ca^{2+} to the cytosol and endoplasmic reticulum decreased while the expression of genes
6699 affecting the removal of Ca^{2+} from the cytosol and endoplasmic reticulum increased. Depletion of
6700 calcium from the endoplasmic reticulum by BPA lead to endoplasmic reticulum stress and could induce
6701 insulin resistance in this model, confirmed by decreased expression of insulin-responsive genes, such
6702 as glucose transporter 4 (*Glut4*) and insulin response gene (*Irs2*).

6703 Long-term (35 weeks) paternal BPA exposure (50 $\mu\text{g}/\text{kg}$ per day) via the diet disrupted glucose
6704 homeostasis and pancreatic function in rat sires and offspring (Ding et al., 2014 [RefID 1620]). A HFD
6705 aggravated the adverse effects. The results indicated that autophagy was activated as a defensive
6706 response for survival during endoplasmic reticulum stress. Upregulated autophagy in the islets may
6707 deal with accumulation of misfolded and aggravated proteins and remove the abnormal unnecessary
6708 cellular components in β -cells. Increased protein expression of microtubule-associated proteins 1A/1B
6709 light chain 3 (LC3) in the pancreas indicated that autophagy was essential for maintaining the function
6710 of pancreas and that pancreatic β -cells may be protected by upregulated autophagy under insulin-
6711 resistant states caused by BPA and HFD. Thus, BPA may cause β -cell dysfunction via endoplasmic
6712 reticulum stress and enhanced autophagy.

6713 In conclusion, the CEP Panel considered that there is substantial evidence supporting that BPA may
6714 negatively affect glucose regulation based on the available studies on animals exposed during
6715 development, as adolescents or as adults, as well as in cell models. Some effects were also shown to
6716 be transferred across generations from F1 to F2 via the male germline. One MoA for disturbed glucose
6717 regulation being put forward in these studies was effects on β -cell dysfunction and disturbance of
6718 glucose homeostasis, either via loss of β -cell mass or modulation of insulin secretion (with 10–22,220
6719 $\mu\text{g}/\text{kg}$ bw of BPA). Several studies indicated effects of BPA on insulin resistance and insulin signalling,
6720 sometimes shown to include circulation of cytokines, which may lead to disruption of glucose transport,
6721 uptake and metabolism, or enhanced oxidative stress (with 50 $\mu\text{g}/\text{kg}$ bw of BPA). Some metabolomics
6722 and other studies found various effects of BPA (5.6–8,925 $\mu\text{g}/\text{kg}$ bw) on metabolism. More specific
6723 effects of BPA were modulation of the homeodomain-containing transcription factor pancreatic and
6724 duodenal homeobox 1 (*Pdx-1*) (10 $\mu\text{g}/\text{kg}$ bw), which plays a key role in pancreatic development and β -
6725 cell function, changes in expression of insulin-like growth factor 2 (*Igf2*) (10–10000 $\mu\text{g}/\text{kg}$ bw), which
6726 may lead to glucose intolerance and β -cell dysfunction, or via endoplasmic reticulum stress (with 50–
6727 5000 $\mu\text{g}/\text{kg}$ bw of BPA). Thus, apparently BPA may disturb glucose regulation via several different
6728 mechanisms, and some evidence may suggest that BPA may be a more potent diabetogen than
6729 obesogen. Unfortunately, only very limited evidence was available in the WoE for type 1 diabetes
6730 mellitus (see chapter on the cluster T1DM)) and none for type 2 diabetes mellitus, the last being the
6731 most common form of diabetes in humans.

6732 *Blood lipids*

6733 There was no human evidence available for the cluster Blood Lipids. Thus, the overall likelihood of
6734 effects of BPA for this cluster was scored ALAN, based on the animal evidence.

6735 Hypercholesterolaemia is a condition which is thought to be caused by a susceptible genotype.
6736 However, the involved genes are not yet identified. The condition is aggravated by one or more factors,
6737 including a so-called atherogenic diet, characterised by higher than needed intake of saturated fat,
6738 trans-fat and, to a lesser extent, cholesterol, obesity and sedentary lifestyle. Triglycerides in the blood
6739 (serum, plasma) are a mixture of triglycerides that are taken up from the diet, by higher than needed
6740 intake of saturated fat and trans-fat and triglycerides which are synthesised in several organs (liver,
6741 kidney, muscle and adipose tissue). Hypertriglyceridemia is often caused or worsened by the same

6742 factors as hypercholesterolaemia, e.g. sedentary lifestyle, obesity and type 2 diabetes mellitus (Mach
6743 et al., 2020).

6744 Feng et al. (2017) [RefID 1959] investigated the effect of BPA (0.1–10 nM) on the absorption of
6745 cholesterol in an *in vitro* Caco-2 intestinal cell model. BPA at 1 and 10 nM increased the absorption of
6746 cholesterol which might be related to increased Niemann–Pick C1-like 1 (NPC1L1) protein. A slight
6747 increase of sterol regulatory element-binding protein-2 (SREBP-2) which regulates the expression of
6748 NPC1L1 protein might be responsible for this finding. However, the mechanism by which BPA would
6749 influence SREBP-2 was not elucidated.

6750 In the study of Marmugi et al. (2014) [RefID 4884], a wide array of gene expression was investigated
6751 in male mice (dose range 5, 50, 500 or 5000 µg/kg bw per day of BPA for 31 weeks) (described above
6752 in the cluster fat deposition in the liver). The results of Feng et al. (2017) [RefID 1959] hint to an
6753 increased absorption of cholesterol due to BPA in µg/kg bw per day concentrations higher than 1 nM
6754 (effects found with 1 and 10 nM, but not with 0.1 nM). The study of Marmugi et al. (2014) [RefID 4884]
6755 gives some indications on elevated expression of genes involved in cholesterol synthesis. Whereas
6756 mRNA expression of Hmgcr, the classic control point in the synthesis, was increased, results of the
6757 second rate-limiting enzyme, squalene monooxygenase, were not reported (Sharpe et al., 2020). Thus,
6758 there is some indication for an influence of BPA on the mRNA expression of enzymes involved in
6759 cholesterol synthesis.

6760 Regarding triglycerides, the only mechanistic study by Bucher et al. (2017) [RefID 743] could not
6761 demonstrate an effect on gene expression of eight enzymes involved in lipid homeostasis and thus,
6762 could not contribute to provide a MoA for elevated triglycerides.

6763 In conclusion, increased absorption of cholesterol under the influence of BPA in an *in vitro* model for
6764 absorption was demonstrated, involving NPC1L1 protein and SREBP-2. However, the initiating molecular
6765 event is not identified. Regarding triglycerides, a convincing MoA was not demonstrated.

6766 *Uric acid*

6767 There was no human evidence available for the cluster uric acid. Thus, the overall likelihood of effects
6768 of BPA for this cluster was scored Likely, based on the animal evidence. The evaluation was based on
6769 three measurements reported in the same publication, scored Tier 2, in which the results of studies in
6770 two mice strains and in rats were reported. The studies were performed in males only. Uric acid
6771 concentrations were measured in liver, (in one strain) and serum (in two strains) of mice showing a
6772 monotonic dose–response (MDR) for three doses. One study was on rats (measured only in serum with
6773 results of only the highest dose reported).

6774 In the same publication (Ma et al., 2018 [RefID 12637]), the authors had examined the MoA for
6775 increased levels of uric acid. The effect of BPA on the xanthine oxidase (XO) enzyme was studied *ex*
6776 *vivo* in mouse primary hepatocytes and homogenised liver (at concentrations of 100 or 1000 nM BPA),
6777 *in vitro* using a transfected human embryonic kidney cell line HEK-293FT and *in silico* by modelling BPA
6778 binding sites. The overall conclusion was that BPA increased the formation of hepatic uric acid via
6779 increasing the activity of xanthinoxidase. Xanthinoxidase is involved in purine metabolism, catalysing
6780 the formation of hypoxanthine, which is a product in the catabolism of adenosine to xanthine. Xanthine
6781 is itself a product in the catabolism of guanosine. In a next step, xanthinoxidase catalyses the oxidation
6782 of xanthine to uric acid. By molecular modelling, it was made probable that BPA may bind to the
6783 enzyme, thus, leading to changes in enzyme conformity with resulting increased activity. Thus, this was
6784 considered a plausible MoA for how BPA may increase the levels of uric acid in the liver.

6785 *Type 1 diabetes mellitus (T1DM)*

6786 There was no human evidence available for the cluster T1DM. Thus, the overall likelihood of effects of
6787 BPA for this cluster was scored ALAN, based on the animal evidence.

6788 In the study by Cetkovic-Cvrlje et al. (2017) [RefID 916], doses of 160 and 1,600 µg/kg bw per day for
6789 11 and 50 days to adult mice, respectively, were studied for effects on splenic cells *ex vivo*. On day 11,
6790 only for the low dose, a decrease in CD3⁺, CD4⁺ and CD8⁺ cells was observed and no effects of the
6791 high dose, whereas the total cell counts and response to stimulation were not impaired in either dose
6792 groups. Inconsistent effects on cytokines (IL-6, IFN-γ, TNF-α) with increase in the low dose and

6793 decrease in the high dose were noted. On day 50, proliferation of unstimulated T cells was increased
6794 at the high dose only, and after stimulation with the mitogen concanavalin A, T-cell proliferation was
6795 decreased with the low dose only. No effects on cytokines were noted.

6796 In the study by Ahn et al. (2018) [RefID 11452], BPA at 5 mg/kg bw per day given to adult male mice
6797 lead to survival of pancreatic β -cells against the effect of STZ, showed increased insulin secretion and
6798 mitigated STZ-induced oxidative stress. Conversely, BPA induced endoplasmic reticulum stress and lead
6799 to insulin resistance especially in the STZ-induced T1DM model by disrupting calcium homeostasis.

6800 The pathogenesis of type 1 diabetes mellitus is known to be the results of T-cell-mediated autoimmune
6801 destruction of pancreatic β -cells (Atkinson et al., 2011). Given that background, the results from the
6802 two MoA studies do not shed light on the mechanism by which BPA might enhance the effect of STZ.
6803 In the study of Ahn et al. (2018) [RefID 11452], BPA led to survival of pancreatic β -cells although STZ
6804 was given, and in the study of Cetkovic-Cvrilje et al. (2017) [RefID 916], no consistent effect on T cells
6805 was shown, although a higher incidence of type 1 diabetes was already observed on day 12 in both
6806 dose group indicating the effect of STZ.

6807 Bodin et al. (2014) [RefID 623] investigated if trans-maternal BPA exposure accelerated T1DM
6808 development in NOD mice exposed to 100, 1000 or 10000 μg BPA/L in drinking water throughout
6809 mating, gestation and lactation (estimated doses approximately 30, 300 or 3000 $\mu\text{g}/\text{kg}$ bw per day
6810 during gestation, 4,500 $\mu\text{g}/\text{kg}$ bw per day for the highest dose during lactation). No effects of BPA were
6811 found on serum autoantibodies against insulin, GAD65 or HSP60, or on testosterone levels. With the
6812 highest dose, increased number of apoptotic cells, reduction in tissue resident macrophages and
6813 increase in regulatory T cells were observed in islets before insulinitis development in the trans-maternally
6814 exposed offspring. The detectable apoptotic cells were identified as mostly glucagon producing α -cells
6815 (increased with the two highest doses) but also tissue resident macrophages and β -cells (both increased
6816 with the highest dose). In the local (pancreatic) lymph node neither regulatory T cells nor natural killer
6817 T cells were affected by maternal BPA exposure. Maternal BPA exposure also induced systemic immune
6818 changes in offspring, as evidenced by alterations in lipopolysaccharide-induced (increased IL-2, IL-10
6819 and IL-17 with the highest dose) and concanavalin A-induced (reduced IL-2 with highest dose) cytokine
6820 secretion in splenocytes. Thus, trans-maternal BPA exposure, *in utero* and through lactation, increased
6821 the severity of insulinitis at 11 weeks of age and accelerated the spontaneous development of diabetes
6822 at 20 weeks of age in female NOD mice, which appeared to be related to early-life modulatory effects
6823 on the immune system.

6824 In conclusion, a convincing MoA of BPA's contribution to the development of T1DM in the STZ-induced
6825 T1DM model was not demonstrated, lowering the probability of BPA to elicit type 1 diabetes mellitus.
6826 However, in the NOD mouse model, trans-maternal BPA exposure, *in utero* and through lactation,
6827 appeared to be related to early-life modulatory effects on the immune system.

6828 *Type 2 diabetes mellitus (T2DM)*

6829 There was no animal evidence available for the cluster T2DM. Thus, the overall likelihood of effects of
6830 BPA for this cluster was scored ALAN, based on one human study. Bi et al. (2016) [RefID 571]
6831 conducted a cohort study in 2009, using a Chinese population of 2209 non-diabetic middle-aged and
6832 elderly subjects. The study sought to prospectively investigate associations of urinary BPA with incident
6833 T2DM risk and the longitudinal changes in glycaemic traits, particularly examining the interaction
6834 between genetic background and BPA exposure on the associations. Four years after the baseline study,
6835 the subjects were followed up and the population was found to include 242 diabetes cases. A genetic
6836 risk score for T2DM based on 34 common variants of single-nucleotide polymorphisms (SNPs) that was
6837 identified in genome-wide association studies and performed or validated in East Asians was created.
6838 The study found that T2DM genetic susceptibility significantly modulated the association of BPA
6839 exposure with longitudinal increase in fasting plasma glucose levels.

6840 **3.1.4.5. Conclusion on hazard identification for Metabolic effects of BPA**

6841 In EFSA 2015 opinion (EFSA CEF Panel, 2015), it was stated regarding human studies that, because of
6842 the limitations of using urinary BPA concentrations as a surrogate of exposure, the problems of
6843 interrelated dietary exposures, the mostly cross-sectional study designs and inconsistency of the results
6844 between cross-sectional and prospective studies, limited conclusions could be drawn concerning the

6845 relationship between BPA exposure and the reported findings. Notwithstanding, there were indications
6846 from cross-sectional studies that higher BPA may be associated with increased body mass in children
6847 and indication from a prospective study that pre-natal BPA exposure may be associated with reduced
6848 body mass and lower plasma adiponectin levels in girls and with higher plasma leptin levels in boys.
6849 There were no indications of note for other hormonal or metabolic endpoints.

6850 The overall conclusion on likelihood of Metabolic effects on animals exposed post-natally was that the
6851 evidence for associations between BPA exposure and these metabolic effects was inconsistent. The CEF
6852 Panel concluded further that there was reasonable evidence for effects on glucose or insulin regulation
6853 and/or effects on pancreatic morphology and/or function in shorter term studies, but no evidence that
6854 BPA was causing diabetes, insulin resistance and increases in weight (obesogenic effect) longer term.
6855 The overall conclusion on likelihood for metabolic effects and the scoring of influence on likelihood in
6856 animals exposed pre-natally were the same as for post-natal exposure.

6857 The CEF Panel assigned a likelihood level of ALAN in the WoE of metabolic effects of BPA based on the
6858 human and animal evidence, and, thus, the CEF Panel did not take any metabolic effects forward for
6859 risk characterisation (EFSA CEF Panel, 2015).

6860 In accordance with the results from 2015, in the current risk assessment of BPA, no clear causal
6861 association (scored ALAN) was found for effects of BPA on obesity based on WoE of human and animal
6862 data, including data on effects of BPA on body weight, BMI, body fat mass, visceral and non-visceral
6863 adipocyte size. However, substantial amounts of supporting evidence for plausible effects of BPA on
6864 obesity were available from MoA data, mostly from animal and *in vitro* studies. This included changes
6865 in expression of adipogenic genes and their proteins, leading to induced differentiation of pre-
6866 adipocytes into mature, lipid-accumulating adipocytes, often regulated via epigenetic effects such as
6867 DNA methylation. In addition, single studies indicated that BPA may affect obesity by disturbing immune
6868 homeostasis and gut dysbiosis or may have effects on the signalling systems of the brain regulating
6869 appetite and food intake.

6870 Increased insulin level was considered a likely effect of BPA in the growth phase of animals. For ipGTT
6871 and β -cell morphometry, the evidence was also considered likely after indirect (germline) exposure in
6872 animals. However, when considering also the effects of BPA on glucose level, glucose tolerance tests
6873 via several other administration routes (i.v., oral or unknown), ipITT, pancreas weight and α -cell and
6874 β -cell morphometry (indicating glucagon and insulin production, respectively), based on the overall
6875 WoE in animals, no clear causal association (scored ALAN) was found for effects of BPA on glucose
6876 regulation. However, also for glucose regulation, substantial amounts of supporting evidence based on
6877 MoA studies in animals and *in vitro* were available, which included BPA-induced β -cell dysfunction and
6878 disturbance of glucose homeostasis, either via loss of β -cell mass or modulation of insulin secretion,
6879 effects on insulin resistance and insulin signalling, sometimes including circulation of cytokines, which
6880 may lead to disruption of glucose transport, uptake and metabolism, or enhanced oxidative stress, and
6881 effects on metabolism and metabolomics. In addition, more specific MoAs of BPA, such as modulation
6882 of the homeodomain-containing transcription factor pancreatic and duodenal homeobox 1 (Pdx-1),
6883 changed expression of insulin-like growth factor 2 (Igf2) or endoplasmic reticulum stress, were
6884 indicated.

6885 Based on the overall WoE in animals for effects of BPA on liver cholesterol and liver triglycerides, no
6886 clear causal association (scored ALAN) was found for effects of BPA on fat deposition in the liver.
6887 Substantial supportive MoA data was available from animals and *in vitro* studies, involving abnormal
6888 synthesis, retention or breakdown of lipid, often via PPARs and SREBPs.

6889 For effects of BPA on blood lipids and T1DM, based on the WoE in animals no clear causal association
6890 (scored ALAN) was found. T2DM was also scored ALAN, based on human evidence. The MoA data were
6891 very limited and the results on T1DM depended on the animal model used.

6892 Based on the WoE of the data available, causal effects of BPA on the thyroid (human and animal data),
6893 gestational diabetes mellitus (human data), cardiometabolic effects (human data) and other metabolic
6894 hormones (animal data) were judged as Not Likely.

6895 Thus, the results in the present opinion are quite consistent with the results in the BPA opinion from
6896 2015 for the same endpoints, even when a much higher number of metabolic studies are included in
6897 the present assessment.

6898 In the 2015 EFSA opinion (EFSA CEF Panel, 2015), the endpoint uric acid was not identified in the
6899 available literature. In the current risk assessment, increased uric acid measured in liver and serum in
6900 adult animals was judged as Likely. The MoA data in the animals showed that BPA could increase the
6901 formation of hepatic uric acid by increasing the activity of the enzyme xanthinoxidase, which catalyses
6902 the conversion of purines hypoxanthine and xanthine into uric acid.

6903 Overall, the CEP Panel considers that a hazard exists for effect of BPA on increasing uric acid level.
6904 Using a WoE approach, the CEP Panel assigned a likelihood level of Likely to the effect of BPA on this
6905 parameter. Therefore, this endpoint was brought forward for BMD analysis (see Chapter 3.2.1).

6906

6907 **3.1.5. Neurotoxicity and developmental neurotoxicity**

6908 **3.1.5.1. Epidemiological studies**

6909 For the HOC Neurotoxicity and developmental neurotoxicity, a total of 18 studies was appraised by the
6910 CEP Panel. The details of the appraisals (internal validity) are reported in Annex B.

6911 *Identification of the clusters to be considered for WoE*

6912 On the basis on the approach described in Chapter 2.3.2 'Definition of Health Outcome Categories and
6913 Clusters', the following Clusters (C) and Exposure periods (Exp) were brought forward to WoE analysis:

- 6914 • C: Neurodevelopment
- 6915 – Exp: Pregnancy and Childhood.

6916 *WoE of the relevant clusters*

6917 The main information extracted from the studies included in relevant clusters in the HOC Neurotoxicity
6918 and developmental neurotoxicity are summarised in Annex C. The outcome of the weight of the
6919 evidence is described in the text below and presented in a tabulated format in Annex D.

6920 **Cluster Neurodevelopment**

6921 Exposure during pregnancy and childhood

6922 A large number of endpoints related to neurodevelopment were studied in the 18 publications pertaining
6923 to 14 longitudinal studies all assessing exposure during pregnancy (Braun et al., 2014a [RefID 707];
6924 Evans et al., 2014 [RefID 1862]; Casas et al., 2015 [RefID 878]; Roen et al., 2015 [RefID 6253]; Perera
6925 et al., 2016 [RefID 5773]; Braun et al., 2017a [RefID 704]; Braun et al., 2017c [RefID 710]¹⁸; Braun et
6926 al., 2017d [RefID 712]; Giesbrecht et al., 2017 [RefID 2272]; Lim et al., 2017 [RefID 4285]; Lin CC et
6927 al., 2017 [RefID 4292]; Minatoya et al., 2017a [RefID 5104]; Philippat et al., 2017 [RefID 5822]; Stacy
6928 et al., 2017 [RefID 6876]; Ghassabian et al., 2018 [RefID 11997]; Kim et al., 2018a [RefID 11136];
6929 Minatoya et al., 2018 [RefID 12732]; Nakiwala et al., 2018 [RefID 12789]).

6930 BPA exposure was measured via a single spot urine sample in all studies and varied between studies.
6931 The populations under study were of comparable sample size but varied in their characteristics; there
6932 were eight studies including a European-descent population. The observed heterogeneity for endpoint
6933 definitions was considerable including a large number of questionnaires evaluating cognition, behaviour,
6934 intellectual ability and psychomotor development. No statistically significant associations were observed
6935 in more than one study. Thus, the currently available longitudinal epidemiological evidence is
6936 characterised by a small number of studies, suboptimal exposure assessment and considerable
6937 heterogeneity in the assessed populations, exposure levels and endpoints. In summary, the currently

¹⁸ This study considered as a cross-sectional study in the EFSA supporting publication 'Implementation of the evidence-based risk assessment for the re-evaluation of Bisphenol A: preparatory work on cross-sectional studies' (AERU, University of Hertfordshire, 2020) was recategorized by the EFSA WG on BPA re-evaluation as a cohort study and therefore considered in the WoE assessment.

6938 available epidemiological evidence does not put forward an endpoint related to neurodevelopment as
6939 a critical one for risk assessment.

6940 In the text below, the assessed longitudinal studies are described in brief. Their detailed description
6941 and risk of bias assessment are provided in Annexes C and D.

6942 The HOME study is a birth cohort conducted in the USA (Braun et al., 2017b). A total of 389 pregnant
6943 women were enrolled between March 2003 and February 2006 in Cincinnati, Ohio, who delivered live
6944 singleton infants. Pre-natal exposure assessment was done at around 16 weeks of gestation. Post-natal
6945 exposure assessment was done at 1, 2, 3, 4 and 5 years of age. Median maternal urinary BPA
6946 concentrations during pregnancy were 2.0 mg/g creatinine (range: 0.4–49). Children were followed up
6947 at 1, 2, 3, 4 and 5 years of age for neurodevelopment, physical growth and health conditions. Three
6948 publications report on the results of various associations pertinent to the present Opinion. Braun et al.
6949 (2014a) [RefID 707] investigated the association between pre-natal exposure to BPA (n = 175) and
6950 autistic behaviours at 4 and 5 years (Social Responsiveness Scale, SRS). No statistically significant
6951 association was observed. Braun et al. (2017d) [RefID 712] assessed the association between pre-natal
6952 BPA exposure (16, 26 weeks) and neurodevelopment until 8 years old (n = 229). The included
6953 neurodevelopment endpoints pertained to behaviour (BASC-2), mental and psychomotor development
6954 (Bayley Scales of Infant Development-II, BSID-II) and child cognitive abilities (Wechsler primary and
6955 preschool scale of intelligence-III (WPPSI-III), Wechsler intelligence scale for children IV (WISC-IV)).
6956 Overall, no statistically significant associations were observed. In girls, each 10-fold increase in maternal
6957 urinary BPA concentrations was associated with a 5.9-point increase (95% CI: 1.1, 11) in BASC-2
6958 externalising scores and nearly 6-times the risk of having a score 60 (RR = 5.8; 95% CI: 1.7, 20).
6959 Finally, Braun et al. (2017a) [RefID 704] assessed children's visual-spatial abilities at 8 years of age
6960 using the Virtual Morris Water Maze (VMWM), a computerised version of the rodent Morris water maze
6961 (MWM). Pre-natal BPA exposure was not associated with VMWM performance.

6962 Braun et al. (2017c) [RefID 710] in the MIREC birth cohort assessed the association between pre-natal
6963 BPA exposure at 12 weeks of gestation and child neurobehaviour at 3 years of age (n = 812; WPPSI-
6964 III, BRIEF-P, BASC-2, Social responsiveness scale-E-2/SRS-2). Overall, while BPA exposure was not
6965 associated with WPPSI-III scores, the BASC-2 or BRIEF-P scales, a statistically significant association
6966 was observed for the SRS-2 scores (b = 0.3; 95% CI: 0, 0.7).

6967 The EDEN study (Philippat et al., 2017 [RefID 5822]) is another birth cohort conducted in France
6968 between February 2003 and January 2006 (n = 529). BPA and 19 additional phthalate metabolites and
6969 phenols (four parabens, benzophenone-3, two dichlorophenols, triclosan) were measured in spot urine
6970 samples collected during pregnancy among mothers who delivered a boy. Behaviour was investigated
6971 using the Strength and Difficulties Questionnaire (SDQ) at 3 and 5 years of age. No overall statistically
6972 significant association was observed.

6973 Nakiwala et al. (2018) [RefID 12789] assessed verbal and performance IQ at 5–6 years (WPPSI). No
6974 statistically significant association was observed.

6975 The Columbia Center for Children's Environmental Health (CCCEH, n = 727) is a birth cohort study
6976 recruiting African-American and Dominican pregnant women between 1998 and 2006 in USA. BPA was
6977 quantified in maternal urine collected during the third trimester of pregnancy and in child urine collected
6978 at ages 3 and 5 years. Neurodevelopment was assessed periodically using the Revised Children's
6979 Manifest Anxiety Scale (RCMAS) and Children's Depression Rating Scale-Revised (CDRS) at 10–12 years.
6980 Two CCCEH publications are included in the present opinion. Roen et al. (2015) [RefID 6253] and
6981 Perera et al. (2016) [RefID 5773] included 239 children in their assessment and found no statistically
6982 significant associations between pre-natal BPA and the scales' overall score for depression and anxiety.
6983 Among the numerous analyses performed, statistically significant associations were found for boys for
6984 the overall score results.

6985 Evans et al. (2014) [RefID 1862] in the SSF II study in USA evaluated pre-natal BPA exposure in relation
6986 to child behaviour at 6–10 years (Child Behaviour Checklist (CBCL), n = 153). No statistically significant
6987 association was observed.

6988 Casas et al. (2015) [RefID 878] reporting on the INMA birth cohort in Spain evaluated the association
6989 between pre-natal BPA and cognitive and psychomotor development at 1 and 4 years (Bayley Scales of
6990 Infant Development BSID, MCSA) and attention deficit hyperactivity disorder (ADHD) symptoms

- 6991 (ADHD-DSM-IV) and other behavioural problems (CPRS, SDQ) at 4 and 7 years. At 1 year of age,
6992 exposure in the highest BPA tertile was associated with a reduction of psychomotor scores (BSID, T3
6993 vs T1, $\beta = -4.28$ points, 95% CI -8.15 to -0.41). At 4 years, BPA exposure was associated with an
6994 increased risk of ADHD hyperactivity symptoms (IRR per log₁₀ BPA increase 1.72; 95% CI 1.08, 2.73)
6995 both overall and in boys. No statistically significant association was observed for the remaining
6996 endpoints.
- 6997 Lim et al. (2017) [RefID 4285] in the Environment and Development of Children study in Korea
6998 evaluated pre-natal BPA exposure and neurobehaviour at 4 years (K-SCQ, n = 304). No statistically
6999 significant association was observed for the prospective component of the study.
- 7000 Lin CC et al. (2017) [RefID 4292] in the TBP birth cohort in China assessed pre-natal BPA (cord blood,
7001 n = 208) in relation to neurodevelopment at 2 and 7 years (Comprehensive Developmental Inventory
7002 for Infants and Toddlers (CDIIT), WISC-IV). In the WISC-IV neurocognitive assessment, a significant
7003 negative association was found for the overall scale as well as for various scale domains.
- 7004 Minatoya et al. (2017a) [RefID 5104] in the Hokkaido Study on Environment and Children's Health
7005 Study in Japan investigated the association between pre-natal BPA levels (cord blood) and
7006 neurodevelopment up until 3.5 years (CBCL, K-ABC, BSID-II, n = 285). Although no overall statistically
7007 significant findings were reported among the numerous analyses performed, cord blood BPA
7008 concentration was statistically significantly positively associated only with CBCL development problems
7009 score ($\beta = 2.60$, 95% CI: 0.15, 5.06). In another publication of the same study, (Minatoya et al., 2018
7010 [RefID 12732]) examined the association between maternal (1st trimester) BPA exposure and
7011 behaviour at 5 years using the Strengths and Difficulties Questionnaire (SDQ) in 458 children. The
7012 median concentration of BPA was 0.062 ng/ml and no overall statistically significant findings were
7013 reported. Among the numerous analyses performed, BPA levels were associated with an increased risk
7014 of prosocial behaviour (OR = 1.46, 95% CI 1.04–2.06).
- 7015 Kim et al. (2018a) [RefID 11136] in the Korean CHECK birth cohort (n = 140) investigated the
7016 association between four phthalates, BPA, three heavy metals, 19 polychlorinated biphenyls (PCBs), 19
7017 organochlorine pesticides and 19 polybrominated diphenyl ethers, and early neurodevelopment (13–24
7018 months of age). For the endpoint assessment the following tools were used: BSID-II, Social maturity
7019 scale (SMS) and CBCL. No statistically significant associations were observed for BPA in the overall
7020 cohort.
- 7021 Ghassabian et al. (2018) [RefID 11997] in the Upstate KIDS birth cohort investigated the association
7022 between perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and BPA (Gathrie cards)
7023 and behaviour at age 7 (Strengths and Difficulties Questionnaire, n = 650 singletons to 138 twins). The
7024 median (interquartile range) of BPA was 7.93 ng/ml (10.79). No statistically significant association was
7025 observed for BPA and total behavioural difficulties (continuous and categorical analyses).
- 7026 Giesbrecht et al. (2017) [RefID 2272] in the Alberta Pregnancy Outcomes and Nutrition study (birth
7027 cohort, n = 132) examined the association between pre-natal maternal urinary BPA concentration and
7028 cortisol and cortisol reactivity at age 3 months. The association between maternal total BPA
7029 concentration and baseline infant cortisol ($\beta = 0.13$, 95% CI: -0.01 , 0.28) or cortisol reactivity (-18%
7030 decrease per hour for females per 10-fold increase in BPA, 95% CI: -35 , 3) was not significant when
7031 infant sex and creatinine was considered in the model.
- 7032 Four studies also assessed BPA exposure during childhood (Roen et al., 2015 [RefID 6253]; Perera et
7033 al., 2016 [RefID 5773]; Stacy et al., 2017 [RefID 6876]; Kim et al., 2018a [RefID 11136]).
- 7034 Perera et al. (2016) [RefID 5773] included 239 children in their assessment and found no statistically
7035 significant associations between post-natal BPA and the scales' overall score for depression and anxiety.
7036 Roen et al. (2015) [RefID 6253] included 250 children in their assessment and found statistically
7037 significant associations between post-natal BPA and internalising and externalising scores on the CBCL.
7038 Girls and boys were respectively showing increasing and decreasing problems. Stacy et al. (2017)
7039 [RefID 6876] in a HOME study publication attempted to extend these findings and to identify potential
7040 windows of vulnerability using repeated measures of pre-natal BPA exposures. Among all children, there
7041 was not strong evidence that the associations between BPA and neurobehaviour varied by the timing
7042 of exposure (Visit \times BPA p-values ≥ 0.16). Finally, Kim et al. (2018a) [RefID 11136] in the Korean
7043 CHECK birth cohort investigated the association between post-natal BPA exposure via breast milk

7044 sampling at 30 days after delivery (n = 73) and early neurodevelopment (13–24 months of age; BSID-II, SMS, CBCL). No statistically significant associations were observed for BPA in the overall cohort.

7046 Overall conclusions

7047 On the basis of the above, the CEP Panel concluded that the evidence for an association between BPA
7048 exposure and impaired neurodevelopment is Not Likely.

7049 *Cross-sectional studies*

7050 Ten cross-sectional studies investigated BPA exposure and childhood behaviour, learning disabilities
7051 and autism (Findlay and Kohen, 2015 [RefID 2029]; Stein et al., 2015 [RefID 6899]; Arbuckle et al.,
7052 2016 [RefID 237]; Kardas et al., 2016 [RefID 3383]; Kondolot et al., 2016 [RefID 3646]; Perez-Lobato
7053 et al., 2016 [RefID 5786]; Tewar et al., 2016 [RefID 7178]; Rahbar et al., 2017 [RefID 6058]; Li Y et
7054 al., 2018c [RefID 11160]; Metwally et al., 2018 [RefID 11177]). All but one (Rahbar et al., 2017 [RefID
7055 6058]) yielded statistically significant results pertaining to various clinical endpoints (autism, n = 3
7056 (Stein et al., 2015 [RefID 6899]; Kardas et al., 2016 [RefID 3383]; Metwally et al., 2018 [RefID 11177]);
7057 ADHD = 3 (Arbuckle et al., 2016 [RefID 237]; Tewar et al., 2016 [RefID 7178]; Li Y et al., 2018c [RefID
7058 11160]); Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS), n = 1 (Kondolot et
7059 al., 2016 [RefID 3646])) and scales ((SDQ), n = 2 (Findlay and Kohen, 2015 [RefID 2029]; Arbuckle et
7060 al., 2016 [RefID 237]); CBCL, n = 1 (Perez-Lobato et al., 2016 [RefID 5786])) and across different
7061 populations and studies of varying power. The available cross-sectional evidence is not aligned with the
7062 available longitudinal evidence where most associations neither reached statistical significance, nor
7063 were replicated by subsequent research.

7064 **3.1.5.2. Animal studies**

7065 For the HOC Neurotoxicity and developmental neurotoxicity a total of 94 studies was appraised by the
7066 CEP Panel. The details of the appraisals (internal and external validity) are reported in Annex E.

7067 The endpoints for each study identified as relevant in this opinion are reported in Annex F. Effects on
7068 behaviour were already key endpoints in the uncertainty analysis in the 2015 EFSA opinion (EFSA CEF
7069 Panel, 2015, Section 4.3.2). For more details see Annex A, Section 2.5.

7070 *Identification of clusters of relevant endpoints*

7071 Endpoints for which statistically significant changes were reported were extracted from the available
7072 literature and grouped into several clusters related to brain morphology and function. These clusters
7073 have been evaluated in a WoE approach and the results are described here.

7074 Rather than identifying all the multiple pathways these endpoints can interact with, the CEP Panel
7075 decided to group them into three empirical clusters that represent different aspects of brain function:
7076 neuromorphology, nervous system functionality and behaviour. However, it is important to note that
7077 they are more or less interrelated and are not independent of each other; for example, dendritic spine
7078 plasticity as a driver or consequence of behaviour (Gipson and Olive, 2017). They all contribute to
7079 perception, cognition and integrated responses that enable an organism to cope with its changing
7080 environment. Potential connections between these clusters will be explored in the section on hazard
7081 identification:

- 7082 1) Neuromorphology (including neuronal dendrite morphology, dendritic spine density
7083 (hippocampus, neocortex), brain nucleus size/volume, number of neurons/glia in various brain
7084 regions).
- 7085 2) Nervous system functionality (neurochemistry, electrophysiology, brain regional
7086 neurotransmitter/receptor/hormone content, neuronal responsiveness).
- 7087 3) Behaviour (anxiety-related behaviour, learning and memory, social behaviour, taste preference,
7088 locomotor activity, sensory/motor coordination) as the most apical outcome of brain function.

7089 **Table 11:** Clusters of relevant endpoints.

Clusters	Relevant endpoints
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Neuromorphology	<p>Dendrite branching Dendrite intersections Dendrite length Hypothalamic histology (kisspeptin-ir cells in anteroventral periventricular nucleus (AVPV), rostral periventricular area (rPen), caudal periventricular nucleus (cPen)) Number of dopamine (DA) neurons in neocortex Number of DA neurons in ventral mesencephalon (VM) Number of glia within medial prefrontal cortex (mPFC) Pro-opiomelanocortin (POMC) projection into the paraventricular nucleus (PVN) POMC projections to hypothalamus Dendritic spine density in CA1 pyramidal cells Dendritic spine density in hippocampus Dendritic spine density in CA1 region of hippocampus Dendritic spine synapses in CA1 region Dendritic spine density in hippocampal dentate gyrus Dendritic spine head size of dentate gyrus neurons Dendrite length (hippocampus) Number of hippocampal CA1 neurons Number of hippocampal CA3 neurons Dendritic spine density on CA1 pyramidal cells Dendritic spine density in CA1 regions Dendritic spine density on layer II/III on mPFC pyramidal cells Dendritic spine density in layers 2/3 of dorsolateral prefrontal cortex (DLPFC) Hypothalamic histology (number of kisspeptin-ir cells in AVPV) Spine synapses in CA1 regions Spine synapses in layers 2/3 of DLPFC</p>
Nervous system functionality	<p>Gamma amino butyric acid (GABA) (hippocampus) GABA (cortex) Aspartate (hippocampus) Aspartate (cortex) Glutamine (GLN) (hippocampus) GLN (cortex) Noradrenaline (NA) (hippocampus) Dopamine and metabolite (hippocampus) Serotonin (hippocampus) Oxytocin receptor (OTR) density (in posterior bed nucleus of stria terminalis (BNST_p), ventromedial hypothalamus (VMH), PVN and dorsolateral bed nucleus of the stria terminalis (BNST_d)) Excitatory and inhibitory responsiveness of recorded medial amygdala neurons to neurochemical signals Leptin blood concentration Leptin sensitivity Serum corticosterone Corticosterone production Acetylcholinesterase (AChE) activity (in prefrontal cortex, hypothalamus, cerebellum and hippocampus) GLU (cortex) GLU (hippocampus) Glycine (GLY) (cortex) GLY (hippocampus) Monoamino-oxidase (MAO) activity Serum corticosterone Taurine (cortex) Taurine (hippocampus)</p>
Behaviour	<p>Anxiety/emotionality (avoidance of predator odour, elevated plus maze (EPM), elevated zero maze (EZM), forced swimming test (FST), open field test (OFT), dark light test (DLT)) Learning and memory (radial arm maze (RAM), reference memory, Barnes maze, Morris water maze (MWM), object recognition, fixed interval reinforcement, object placement, Y maze; working memory, spatial memory)</p>

	Locomotor activity (exploratory behaviour, open field test (OFT), running activity, spontaneous activity, hole poke test, tremor activity (electronic balance test), EPM, EZM, mirrored maze) Preference behaviour (sodium salt intake; sweet preference; water intake) Social behaviour (maternal behaviour; female sexual behaviour; male sexual behaviour; interaction) Sensory-motor coordination (rotarod experiment; string test)
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7090

7091 **Neuromorphology**

7092 The first cluster comprises endpoints related to brain development, including effects on neurogenesis
 7093 and on the morphology of the various brain regions. It includes the number of cells, the dendritic spine
 7094 density and the degree of connectivity between cells, as well as brain volume and growth. Such relevant
 7095 endpoints can be considered at the level of the whole brain or a specific brain area.

7096 **Nervous system functionality**

7097 The second cluster considers endpoints related to brain function, including several systems of
 7098 neurotransmission (GLU, GABA, serotonin, noradrenaline, dopamine) within the brain and hormone
 7099 communication (noradrenaline, steroid and peptide hormones) with the whole body.
 7100 Electrophysiological neuronal responsiveness is also considered within this cluster as neurons propagate
 7101 chemical signals by generating electric potentials.

7102 **Behaviour**

7103 The third cluster comprises behavioural endpoints including anxiety-related behaviour, learning and
 7104 memory, social behaviour and sensory-motor function, which were considered by EFSA in 2015 (EFSA
 7105 CEF Panel, 2015) to be ALAN related to BPA exposure. Behaviour represents the highest level of
 7106 integrated function between the brain, the organism and the environment. Behavioural disturbances
 7107 may cause problems for human health and be indicative of neurodevelopmental syndromes such as
 7108 attention deficits, autism, schizophrenia and neurodegenerative processes later in life such as dementia,
 7109 Alzheimer's or Parkinson's disease. However, even subtle effects can potentially impact all stages of
 7110 human life from birth to old age, starting with mother-child bonding and parenting, the education
 7111 process and the interaction with peers.

7112 For adversity to human, endpoints of the clusters Neuromorphology and Nervous system functionality
 7113 are highly interrelated, representing anatomical signalling pathways, and signal generation and
 7114 transduction, respectively.

7115 Depending on the study, endpoints of the clusters of Neuromorphology and Nervous system
 7116 functionality were studied at the level of the whole brain or a specific brain region. A neurofunctional
 7117 or neuroanatomical effects with the same causal mechanism but occurring in different parts of the brain
 7118 may have different outcomes depending on the brain region. As a consequence, measurement done at
 7119 the level of the whole brain is less indicative of specific BPA-related changes. In addition, effects
 7120 observed in any of the three clusters may not be identical for males and females due to the known sex-
 7121 specific development of the brain. These gender-related aspects of brain function may necessitate
 7122 separate evaluations in males and females to determine if sex could influence the neurotoxicity of BPA.

7123 Finally, brain weight, which is a general endpoint used in neurotoxicity, does not seem to be specifically
 7124 relevant due to a lack of significant effects of BPA in any of the reported studies. The lack of significant
 7125 effects could be explained by the fact that the brain is a well protected organ against most insults and
 7126 the brain weight is an endpoint that remains very difficult to disrupt.

7127 *WoE of the clusters of relevant endpoints*

7128 The main information extracted from the studies addressing relevant endpoints in the HOC
 7129 Neurotoxicity and developmental neurotoxicity are summarised in Annex G. The outcome of the WoE
 7130 is described in the text below and presented in a tabulated format in Annex H.

7131 The clusters of the effects of BPA on Neurotoxicity and developmental neurotoxicity considered for
 7132 this assessment were the following:

- 7133 • Neuromorphology
- 7134 • Nervous system functionality
- 7135 • Behaviour

7136 **Neuromorphology**

7137 The cluster Neuromorphology includes various endpoints which relate to different types of actions or
7138 responses that can be observed in animals or humans. For the sake of clarity, the results of the WoE
7139 exercise will be presented by endpoint to demonstrate which neuromorphological measurements were
7140 found to be affected by BPA exposure of a certain age group and which were not. The specific
7141 measurements that were included for the effects of BPA on neuromorphology concern the cell number
7142 and/or volume (mostly concerning dopaminergic neurons), the dendritic morphology and/or density,
7143 and the number of spine synapses. It has to be noticed that most studies report such measurements
7144 in various parts of the brain, cover more than one endpoint and assess such endpoints relatively to
7145 specific behavioural performances.

7146 The complete cluster Neuromorphology includes 15 studies, eight being conducted in rats, six in mice,
7147 one in rhesus monkeys and one in vervet monkeys. Animals were exposed during development in seven
7148 studies (two in rats, four in mice and one in rhesus monkeys) or during the growth phase/young age
7149 in six studies (four in rats, one in mice and one in rhesus monkeys). Two studies were conducted at
7150 the adult stage, one in mice and one in vervet monkeys.

7151 Developmental exposure (pre-natal and/or post-natal until weaning)

7152 Seven studies were identified concerning this period of exposure. Two studies were performed in rats,
7153 four in mice, and one in rhesus monkeys (*Macaca mulatta*). One of the rat studies was allocated to Tier
7154 2 (Liu ZH et al., 2014 [RefID 10411]) and one to Tier 3 (Liu ZH et al., 2015 [RefID 4535]). In mice,
7155 two studies were allocated to Tier 1 (Komada et al., 2014 [RefID 3641]; MacKay et al., 2017 [RefID
7156 4767]) and two to Tier 2 (Kimura et al., 2016 [RefID 3566]; Naule et al., 2014 [RefID 5335]). The
7157 study performed in rhesus monkeys was allocated to Tier 1 (Elsworth et al., 2013 [RefID 1810]).

7158 Four studies reported effects of BPA on dendritic spine density measured in two highly plastic parts of
7159 the brain, namely hippocampus and PFC. The Tier 1 study from Elsworth et al. (2013) [RefID 1810]
7160 reported that a gestational exposure to BPA for 50 days using subcutaneous implants in rhesus monkeys
7161 (*Macaca mulatta*), delivering an estimated level of exposure of 550 µg/kg bw per day, was associated
7162 with a significant loss of spine synapses in CA1 hippocampus, but not in the dorsolateral part of PFC.
7163 Two Tier 2 studies revealed similar results in the same brain region, one in mice (Kimura et al., 2016
7164 [RefID 3566]) and one in rats (Liu ZH et al., 2014 [RefID 10411]). In mice (Kimura et al., 2016 [RefID
7165 3566]) BPA exposure from GD8.5 to GD18.5 at the highest dose of 400 µg/kg bw per day induced a
7166 significant reduction in the three different endpoints measured at PND21, all related to the synaptic
7167 connectivity in the hippocampal CA1 area. These endpoints are the number of 5th order branching, the
7168 number of intersections >40 µm from cell body and the dendrite length. No changes were observed at
7169 the lowest dose level (40 µg/kg bw per day). Both reductions were observed only in basal dendrites at
7170 the dose of 400 µg/kg bw per day, whereas the apical part of dendrites remained unaffected at any
7171 dose. Only males were studied. In rats (Liu ZH et al., 2014 [RefID 10411]) a 7-day post-natal exposure
7172 from PND7 to PND14 induced a significant BPA dose-related decrease in the dendritic spine density of
7173 dentate gyrus neurons (–5% at 50 µg/kg bw per day, –12% at 250 µg/kg bw per day and –15% at
7174 the dose of 500 µg/kg bw per day). A concomitant 25% reduction in the dendritic spine head size of
7175 the same neurons was observed in the same region at all three dose levels.

7176 The Tier 3 study from Liu ZH et al. (2015) [RefID 4535] revealed a dose-related reduction in the
7177 dendritic spine density of CA1 hippocampal neurons (0%, –4% and –13%) in 12-week-old rats post-
7178 natally exposed to BPA (50, 250 or 500 µg/kg bw per day from PND7 to PND14, i.p. administration). A
7179 more important reduction has been measured in the same study in 12-week-old rats after chronic
7180 exposure to BPA via oral maternal dosing (GD0–PND21) and post-natal dosing (PND21–12 weeks of
7181 age) through drinking water. Two levels of exposure were applied (0.15 and 7.5 µg/kg bw per day)
7182 leading to 14% and 21% decreases in the dendritic spine density, respectively. Only males were tested
7183 in both experiments. This study is reported here despite its allocation to Tier 3 because it revealed
7184 comparable results related to the same endpoints as reported in other Tier 1 or Tier 2 studies (Elsworth

7185 et al., 2013 [RefID 1810]; Kimura et al., 2016 [RefID 3566]; Liu ZH et al., 2014 [RefID 10411]) and
7186 then highly contributes to the final likelihood per endpoint (Likely) and per cluster (Likely). The effect
7187 of BPA on the dendritic spine density in the same brain region in three different species was judged as
7188 Likely.

7189 A Tier 3 study conducted in rats (Sadowski et al., 2014b [RefID 6362]) reported a significant increase
7190 in the number of neurons (+15%) and glial cells (+19%) in layers 5–6 of the medial PFC at PND140
7191 only in males early exposed to the highest dose of BPA (GD0–PND9, 400 µg/kg bw per day). No effects
7192 were observed in males at the low and mid doses studied (4 and 40 µg/kg bw per day) or in females
7193 at any dose. No effect was observed in layers 2–3 of the same part of the brain in males and females.
7194 This Tier 3 study is the only one available exploring the number of glial cells in medial PFC. The evidence
7195 was considered Inadequate to conclude on the likelihood of the effect.

7196 Two studies investigated the plasticity of the dopaminergic system in various parts of the brain, one in
7197 mice (Komada et al., 2014 [RefID 3641]) and one in monkeys (Elsworth et al., 2013 [RefID 1810]).

7198 The Tier 1 study (Komada et al., 2014 [RefID 3641]) was performed in mice and showed at PND3 a
7199 significant reduction in the projection of dopaminergic neurons in the neocortex (from layer 1 to 6) of
7200 pre-natally BPA-exposed newborns at the two doses tested. BPA was administered orally from GD6 to
7201 GD18 at a dose of 20 or 200 µg/kg bw per day. Effects of BPA on this endpoint was judged as ALAN
7202 for concluding on the likelihood of the BPA effects.

7203 The other Tier 1 study related to the dopaminergic system (Elsworth et al., 2013 [RefID 1810])
7204 investigated the effects of a single-dose of BPA on the same endpoint considered by Komada et al.
7205 (2014) [RefID 3641] but in rhesus monkeys (*Macaca mulatta*). Results showed a significant reduction
7206 in the number of dopaminergic TH-immunoreactive neurons in VM, a part of the brain including
7207 dopaminergic regions like substantia nigra and ventral tegmental area, in newborn monkeys
7208 gestationally exposed to BPA (400 µg/kg bw per day, GD100–GD165, oral administration by food). The
7209 evidence was considered Inadequate to conclude on the likelihood of the effect.

7210 The Tier 2 study from Naule et al. (2014) [RefID 5335] investigated the number of kisspeptin-
7211 immunoreactive cells in three brain areas, the AVPV and the rostral and caudal parts of the
7212 periventricular nucleus. Results showed a significant increase in the number of kisspeptin-
7213 immunoreactive cells at both doses of BPA 50 and 5000 µg/kg bw per day (+25% and +35%,
7214 respectively) limited to the rostral part of the periventricular nucleus. This endpoint was judged as ALAN
7215 for concluding on the likelihood of the BPA effects.

7216 A Tier 1 study (MacKay et al., 2017 [RefID 4767]) assessed the effects of a perinatal exposure to BPA
7217 (3 µg/kg bw per day through the diet) from GD0 to PND21 on hypothalamic feeding circuitry in mice
7218 pups. At PND21, BPA induced a significant reduction in density of POMC immunolabeled fibres in the
7219 periventricular nucleus in males and females whereas the counting of POMC neurons in the arcuate
7220 nucleus was unchanged in both sexes, suggesting that BPA did not alter the embryonic neurogenesis
7221 of POMC neurons. The evidence was considered to be Inadequate to conclude on the likelihood of the
7222 effect.

7223 The CEP Panel assigned a likelihood level of Likely to the Neuromorphology effect of BPA in the
7224 developmental exposure period, based on Likely effects for dendritic spine density (Kimura et al., 2016
7225 [RefID 3566]; Liu ZH et al., 2014 [RefID 10411]). Therefore, these studies were taken forward for BMD
7226 analysis (see Chapter 3.2.1). Moreover, the Likely and ALAN endpoints were also considered in the
7227 uncertainty analysis (see Appendix D)

7228 Developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood)

7229 No studies were available for this exposure period.

7230

7231 Growth phase/young age exposure

7232 Six studies were identified in this exposure period, all of them allocated to Tier 1. Four studies were
7233 conducted in rats (Chen Z et al., 2018 [RefID 11734]; Wise et al., 2016 [RefID 7970]; Bowman et al.,
7234 2014 [RefID 687]; Bowman et al., 2015 [RefID 688]), one in mice (Zhou YX et al., 2017 [RefID 9083])
7235 and one in vervet monkeys (*African green monkeys, Chlorocebus aethiops sabaeus*) (Elsworth et al.,
7236 2013 [RefID 1810]).

7237 These studies explored the neuro-morphological effects of BPA in three different brain regions, namely
7238 hippocampus, PFC and mesencephalon.

7239 In hippocampus, the Tier 1 study from Zhou YX et al. (2017) [RefID 9083] revealed a reduced number
7240 of neurons in hippocampal CA1 and CA3 areas) of juvenile male rats (PND56) exposed orally to BPA for
7241 8 weeks from birth to post-natal week (PNW) 8 at three doses, 0.5, 50 and 5000 µg/kg bw per day. In
7242 the CA1 area, the number of neurons was significantly lower (about 10%) compared with controls at
7243 the highest dose of BPA (5000 µg/kg bw per day). In the CA3 area, the number of neurons was
7244 significantly lower than in controls (also about 10%) at 0.5 and 5000 µg/kg bw per day. Only males
7245 were tested. The evidence for these endpoints was judged as Likely.

7246 A Tier 1 study in rats (Chen Z et al., 2018 [RefID 11734]) reported a decrease in the hippocampal CA1
7247 dendritic spine density in animals exposed to 40, 400 and 4000 µg/kg bw per day of BPA after weaning
7248 (PND21–PND49) but it was significant only at the highest dose compared with controls. No effect of
7249 BPA on the dendritic length was observed in the same region. Only males were tested.

7250 Two Tier 1 studies (Bowman et al., 2014 [RefID 687]; Bowman et al., 2015 [RefID 688]) investigated
7251 the dendritic spine density of hippocampal CA1 pyramidal neurons in both basal and apical parts of the
7252 cell at two post-natal ages (PND49 or PND91) in rats exposed subcutaneously to 40 µg/kg bw per day
7253 of BPA, from PND42 to PND49. At PND49, spine density was lower in the BPA group in both basal
7254 (–24%) and apical (–15%) parts of the CA1 pyramidal cells (Bowman et al., 2014 [RefID 687]). Males,
7255 both controls and BPA-treated animals, had fewer spines in CA1 basal dendrites (–9%) than control
7256 and BPA-exposed females, with no significant interaction between treatment and sex. At adulthood
7257 (PND91), a reduction in basal and apical CA1 dendritic spine densities was observed in both males and
7258 females compared with control animals (–19% and –21%, respectively). No significant interaction
7259 between treatment and sex was noted. Considering the above evidence, the endpoint dendritic spine
7260 density of pyramidal cells in hippocampal CA1 area was judged as Likely.

7261 The Tier 1 study of Elsworth et al. (2013) [RefID 1810], reported no difference in the hippocampal CA1
7262 dendritic spine density regions measured at the end of dosing in vervet monkeys (*African green monkeys*,
7263 *Chlorocebus aethiops sabaues*, 14–18 months of age) continuously exposed to BPA (550 µg/kg bw per
7264 day) through subcutaneous implants for 30 days during the pre-pubertal period. The evidence was
7265 considered to be Inadequate to conclude on the likelihood of the effect

7266 Regarding the PFC, the Tier 1 study from Wise et al. (2016) [RefID 7970] explored the total number of
7267 glial cells in the medial part of the PFC in both adult male and female rats (PND150) exposed during
7268 the brain growth phase (PND27–PND46) to BPA at a dose of 4, 40 or 400 µg/kg bw per day. Results
7269 showed similar increases in the number of glial cells in females, rather microglial cells than astrocytes,
7270 and similar decreases in males. However, such differences were statistically significant referred to
7271 controls only at the dose of 40 µg/kg bw per day in females and 4 µg/kg bw per day in males. No
7272 effects on the number of neurons were observed at any dose in males and females. This endpoint was
7273 judged ALAN for the number of glial cells.

7274 The two Tier 1 studies from Bowman et al. (2014) [RefID 687] and Bowman et al. (2015) [RefID 688]
7275 investigated at two post-natal ages (PND77 or PND91) the dendritic spine density of pyramidal neurons
7276 (basal and apical parts of the cell) in the medial part of PFC of rats exposed subcutaneously to 40 µg/kg
7277 bw per day of BPA from PND42 to PND49. At PND77, the BPA group had lower spine density in both
7278 basal (–21%) and apical (–10%) parts of the prefrontal pyramidal cells (Bowman et al., 2014 [RefID
7279 687]). Both sexes showed the same effect on this endpoint in this part of the brain. At adulthood
7280 (PND91), no effect on basal and apical dendritic spine densities was observed in either males or females.
7281 The endpoint dendritic spine density of pyramidal cells in PFC was judged as Likely.

7282 The Tier 1 single-dose study of Elsworth et al. (2013) [RefID 1810] reported no difference in the
7283 prefrontal dendritic spine synapses measured in the cortical layers 2 and 3 at the end of the period of
7284 exposure in vervet monkeys (*African green monkeys*, *Chlorocebus aethiops sabaues*, 14–18 months of
7285 age) continuously exposed to BPA through subcutaneous implants for 30 days during the pre-pubertal
7286 period (plasma levels 13.1 ± 1.4 ng/mL at termination after 30 days exposure; equivalent to 4500
7287 µg/kg bw per day oral). The same study reported also the same absence of effect of BPA on the number
7288 of dopaminergic neurons measured in the VM of the same animals.

7289 The evidence for dendritic spine density of pyramidal cells in PFC and the number of dopaminergic
7290 neurons in mesencephalon was judged Inadequate to conclude on the likelihood of the effect.

7291 Overall, the CEP Panel assigned a likelihood level of Likely to the Neuromorphology effects of BPA in
7292 the growth phase/young age exposure period. Since the likelihood for Neuromorphology is Likely for
7293 the endpoints Number of hippocampal CA1 neurons (Zhou YX et al., 2017 [RefID 9083]), Number of
7294 hippocampal CA3 neurons (Zhou YX et al., 2017 [RefID 9083]) and dendritic spine density on CA1
7295 pyramidal cells (Chen Z et al., 2018 [RefID 11734]), these studies were taken forward for BMD analysis
7296 (see Chapter 3.2.1). The endpoint dendritic spine density on layer II/III pyramidal cells in the PFC, also
7297 Likely, was not taken forward for BMD analysis because only single-dose studies were identified for this
7298 endpoint. However, the Likely and ALAN endpoints were considered in the uncertainty analysis (see
7299 Appendix D).

7300 Adult exposure (after puberty)

7301 Only two studies from two different species were identified in this exposure period, one in mice and
7302 one in vervet monkeys.

7303 The study performed in mice (Wang XL et al., 2014 [RefID 7784]) was allocated to Tier 2 and reported
7304 a lack of changes in the number of kisspeptin-immunoreactive cells in the anteroventral part of the
7305 periventricular nucleus of females 6 hours after a unique oral administration of 20 µg/kg bw per day of
7306 BPA. The second study (Elsworth et al., 2015 [RefID 1809]) is a Tier 1 study performed in adult male
7307 vervet monkeys (*Chlorocebus sabaeus*) that consisted in administering BPA subcutaneously using an
7308 osmotic minipump to achieve a dose of 50 µg/kg bw per day for 30 days (this level of dose was
7309 calculated to correspond to an equivalent oral dose of 5556 µg/kg bw per day). The results showed a
7310 significant reduction in the number of spine synapses measured at the end of exposure in hippocampus
7311 (CA1 stratum radiatum) and PFC (layers 2/3 of the dorsolateral part of PFC) compared with controls. It
7312 is noted that measurements done 4 weeks after the removal of the minipump and the end of BPA
7313 exposure showed a partial recovery of the number of synapses into the same regions.

7314 These studies were considered to be of Inadequate evidence for the endpoint related to the
7315 hypothalamic histology (Wang XL et al., 2014 [RefID 7784]) and for the endpoints number of spine
7316 synapses in hippocampus and PFC (Elsworth et al., 2015 [RefID 1809]).

7317 The CEP Panel considered the evidence Inadequate to assign a likelihood level to the Neuromorphology
7318 effects of BPA in the adult exposure period. Therefore, none of the endpoints was taken forward for
7319 BMD analysis.

7320 Indirect (germline) exposure

7321 No studies were available for this exposure period.

7322 Overall cluster selection of the endpoints/studies for BMD analysis for Neuromorphology

7323 Overall, the CEP Panel assigned a likelihood level of 'Likely' to the effects of BPA on neuromorphology
7324 in the exposure periods developmental (pre-natal and/or post-natal until weaning) and growth
7325 phase/young age, and Inadequate evidence in the period adult exposure (after puberty).

7326 The CEP Panel considered that the evidence from the studies available showed a Likely effect of BPA
7327 in the exposure period developmental (pre-natal and/or post-natal until weaning) for the endpoint
7328 'Dendritic spine density of pyramidal cells in hippocampus (CA1 and dentate gyrus areas)' (Kimura et
7329 al., 2016 [RefID 3566]; Liu ZH et al., 2015 [RefID 4535]; Elsworth et al., 2013 [RefID 1810]; Liu ZH et
7330 al., 2014 [RefID 10411]). The endpoint 'Dendritic spine density of pyramidal cells in hippocampus (CA1
7331 and dentate gyrus areas)' (Kimura et al., 2016 [RefID 3566]; Liu ZH et al., 2014c [RefID 10411]) was
7332 taken forward for BMD analysis (see Chapter 3.2.1). The same endpoint assessed in the studies by Liu
7333 ZH et al. (2015) [RefID 4535] and Elsworth et al. (2013) [RefID 1810] was not taken forward because
7334 the first is a Tier 3 study and the second is a single-dose Tier 1 study.

7335 The CEP Panel considered that the evidence from the studies available showed a Likely effect of BPA
7336 in the exposure period Growth phase/young age for the endpoints 'Number of neurons in hippocampus
7337 (CA1 and CA3 areas)' (Zhou YX et al., 2017 [RefID 9083]), 'Dendritic spine density in CA1 pyramidal
7338 cells' (Bowman et al., 2014 [RefID 687]; Bowman et al., 2015 [RefID 688]; Chen Z et al., 2018 [RefID
7339 11734]), and 'Dendritic spine density in pyramidal cells in medial part of PFC' (Bowman et al., 2014

7340 [RefID 687]; Bowman et al., 2015 [RefID 688]). The endpoints 'Number of neurons in hippocampal
7341 CA1 area' (Zhou YX et al., 2017 [RefID 9083]), 'Number of neurons in hippocampal CA3 area' (Zhou
7342 YX et al., 2017b [RefID 9083]) and 'Dendritic spine density in CA1 pyramidal cells' (Chen Z et al., 2018
7343 [RefID 11734]) were taken forward for BMD analysis (see Chapter 3.2.1). Both endpoints 'Dendritic
7344 spine density in CA1 pyramidal cells' and 'Dendritic spine density in pyramidal cells in medial part of
7345 PFC (Bowman et al., 2014 [RefID 687]; Bowman et al., 2015 [RefID 688]) were not taken forward for
7346 BMD analysis because these two Tier 1 studies are both single-dose studies.

7347 The CEP Panel assigned a likelihood level of Inadequate evidence to the effects of BPA on
7348 neuromorphology in the exposure period 'adult exposure (after puberty)'. Therefore, none of the
7349 endpoints was taken forward for BMD analysis.

7350 The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster
7351 Neuromorphology, was Likely.

7352

7353 **Nervous system functionality**

7354 As for the cluster Neuromorphology, this cluster includes various endpoints that relate to different types
7355 of actions or responses that can be observed in animals or humans. For the sake of clarity, the results
7356 of the WoE exercise will be presented by endpoint to demonstrate which brain functioning
7357 measurements were found to be affected by BPA exposure of a certain age group and which were not.
7358 The specific measurements that were included for the effects of BPA on brain functionality concern
7359 various neurotransmitter systems (GABA, GLU, aspartate (ASP), TAU, GLY, monoaminergic systems),
7360 acetylcholine esterase (AChE) activity, leptin secretion and corticosterone secretion and regulation.
7361 Most of these studies report such measurements in various parts of the brain, cover more than one
7362 endpoint and assess such endpoints relative to specific behavioural performances.

7363 The complete cluster Nervous system functionality includes 10 studies, six in rats and four in mice.
7364 Animals were exposed during development in four studies (two in rats, two in mice) or during the
7365 growth phase/young age in two studies (one in rats and one in mice). Four studies were performed in
7366 adult animals (one in mice and three in rats). Two studies in rats exposed the tested animals by indirect
7367 germline exposure.

7368 Developmental exposure (pre-natal and/or post-natal until weaning)

7369 Four studies in two animal species were identified for this part, two in mice (Xin et al., 2018 [RefID
7370 13482]; MacKay et al., 2017 [RefID 4767]) and two in rats (Witchey et al., 2019 [RefID 13782];
7371 Fujimoto and Aou, 2018 [RefID 11960]).

7372 The Tier 2 study from Xin et al. (2018) [RefID 13482] investigated the brain levels of various
7373 neurotransmitters in the hippocampus of adult mice (21 weeks after birth) born from dams exposed to
7374 BPA from pre-conception (2 weeks before mating) until weaning (PND21) through the diet at two doses
7375 (10 and 10000 µg/kg bw per day). Results showed a significant dose-dependent decrease in serotonin
7376 (5HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in high-dose BPA-exposed adult male,
7377 while females were unaffected. Levels of noradrenaline (NA) were sex-specifically affected by BPA with
7378 a significant reduction in noradrenaline in low-dose BPA-exposed females. Dopamine (DA) levels were
7379 not affected in either sex at the low dose (10 µg/kg bw per day). A reduced level of the dopamine
7380 metabolite homovanillic acid (HVA) was also observed in low-dose BPA-exposed females. GABA,
7381 noradrenaline, dopamine and its metabolite and serotonin are endpoints which were judged as ALAN
7382 whereas the others (ASP, GLU and GLN) were judged as Not Likely.

7383 The Tier 1 electrophysiological study from Fujimoto and Aou (2018) [RefID 11960] investigated the
7384 extracellular responses of neurons in the medial amygdala to the presentation of three plant odours
7385 and three predator odourants in adult male rats exposed to BPA during early development (GD0–
7386 PND14, 15 µg/kg bw per day through drinking water). Results showed a greater activity of amygdala
7387 odour-responsive neurons to two odourants (fox odour and whisky lactone) in BPA-exposed rats
7388 compared with the control animals. Females were not tested.

7389 This study was considered to be Inadequate evidence for the BPA brain risk assessment for the endpoint
7390 of excitatory and inhibitory responsiveness of recorded medial amygdala neurons to neurochemical
7391 signals.

7392 A Tier 1 study (Witchey et al., 2019 [RefID 13782]) revealed significant sexually dimorphic differences
7393 in the density of oxytocin receptors (OTR) between control male and female rats aged 28 days after
7394 birth in three hypothalamic regions [Bed Nucleus of Striatum Terminalis (BNST), Paraventricular Nucleus
7395 (PVN) and Ventromedial Hypothalamus (VMH)]. In rats of the same age (PND28) exposed to BPA from
7396 GD6 to PND21 at 2.5, 25 or 2500 µg/kg bw per day, OTR binding was significantly increased compared
7397 with controls at 2.5 and 25 µg/kg bw per day in males only in the BNST_{dl}, which eliminated the sex-
7398 specific difference observed in the control group. No significant main effects of BPA exposure were
7399 measured in any other hypothalamic areas (BNST_p, PVN and VMH). No effects of BPA were found in
7400 females. This endpoint was judged as ALAN for concluding on the likelihood of the BPA effects.

7401 The Tier 1 study from MacKay et al. (2017) [RefID 4767] revealed a delayed serum leptin surge from
7402 PND8 in control pups to PND10–12 in BPA-exposed rats. Exposure to BPA was performed in dams from
7403 GD0 to GD12 and in the offspring up to PND21 at a dose of 3 µg/kg bw per day. In addition, results
7404 showed a decrease in leptin sensitivity reflected by a lack of reduction in body weight in adult (PND130)
7405 BPA early exposed females and males after two days of leptin administration. Females were more
7406 strongly affected compared with the males.

7407 This evidence was considered to be Inadequate for concluding on the likelihood of the BPA effect on
7408 the endpoint leptin (sensitivity and blood concentration).

7409 The CEP Panel assigned a likelihood level of ALAN to the neurofunctional effects of BPA in the
7410 developmental exposure period, therefore none of the endpoints was taken forward for BMD analysis.
7411 However, the ALAN endpoints were considered in the uncertainty analysis (see Appendix D).

7412 Developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood)

7413 No studies were available for this exposure period.

7414

7415 Growth phase/young age

7416 Two studies were identified during this exposure period. One was performed in mice (Luo et al., 2013
7417 [RefID 11330]), while the other one was done in rats (Bowman et al., 2015 [RefID 688]).

7418 The Tier 1 study from Luo et al. (2013) [RefID 11330] reported a significant decrease in hippocampal
7419 AChE activity in young adult male rats immediately after the end of the period of exposure to BPA
7420 (PND30 to PND70; 10000 µg/kg bw per day) whereas the enzyme activity remained unchanged in the
7421 other brain regions studied (PFC, hypothalamus, cerebellum). Females were not tested. The evidence
7422 was considered to be Inadequate to conclude on the likelihood of the BPA effect on the endpoint 'AChE
7423 activity'.

7424 The second study is also a Tier 1 study (Bowman et al., 2015 [RefID 688]) which reported a slight non-
7425 significant increase in corticosterone blood levels in male (+24%) and female (+16%) adult rats
7426 (PND91) exposed to BPA from PND42 to PND49 (40 µg/kg bw per day, s.c. equivalent to an oral dose
7427 of 1428 µg/kg bw per day) in a model of restraint-induced stress (1 hour, 21°C). The evidence was
7428 considered to be Inadequate to conclude on the likelihood of the effect.

7429 The CEP Panel assigned a likelihood level of Inadequate evidence to the neurofunctional effects of BPA
7430 in the growth phase/young age exposure period, so, none of the endpoints was taken forward for BMD
7431 analysis.

7432 Adult exposure (after puberty)

7433 Four studies in two animal species were identified for this exposure period, one in mice (Khan et al.,
7434 2018 [RefID 12311]) and three in rats (Fan et al., 2013 [RefID 1907]; Khadrawy et al., 2016 [RefID
7435 3462]; Fan et al., 2018 [RefID 11915]).

7436 The study from Khan et al. (2018) [RefID 12311], which was allocated to Tier 2, reported a concomitant
7437 30% decrease in AChE activity and significant increase (+40%) in monoamine oxidase (MAO) activity
7438 in the brain homogenate of adult male mice exposed to BPA at a dose of 10000 µg/kg per day (PND63–

7439 PND91). Only males were tested. The evidence was considered Inadequate for the endpoint MAO and
7440 as Likely for the other endpoint, AChE activity.

7441 A Tier 1 study (Fan et al., 2013 [RefID 1907]) reported a significant reduction of AChE activity measured
7442 in hippocampus of adult male rats at 144 days after birth and exposed to BPA 50 µg/kg per day for 10
7443 weeks. Females were not considered for this study. The evidence for this endpoint was judged as Likely.

7444 The Tier 2 study from Khadrawy et al. (2016) [RefID 3462] intend to assess the effects of BPA on AChE
7445 activity and excitatory (GLN, GLU and aspartate (ASP)) and inhibitory (GABA, GLY and TAU) amino
7446 acid neurotransmitter levels in the cortex and hippocampus of adult male rats. Females were not tested.
7447 Two protocols of exposure to BPA were used: (1) BPA administered at two levels of doses, 10000 and
7448 25000 µg/kg bw per day, for 6 weeks, and (2) BPA administered at only one level of dose, 10000 µg/kg
7449 bw per day, with two exposure times, 6 or 10 weeks. The results related to the AChE activity measured
7450 in the cortex and hippocampus revealed a significant dose-dependent and duration-dependent increase
7451 in the enzyme activity in both regions. Significant increases were observed in the cortex whatever the
7452 treatment regimen used. In the hippocampus, AChE activity was significantly increased only at the two
7453 dose levels administered for 6 weeks.

7454 There were concomitant increases in hippocampal levels of both excitatory (GLN, GLU and ASP) and
7455 inhibitory (GABA, GLY and TAU) amino acid neurotransmitters with the two levels of doses given during
7456 6 weeks. In the cortex, the level of the GLU and ASP metabolic precursor GLN was reduced regardless
7457 of the dose or duration used for exposure. Cortical GLU and ASP levels were increased in a significant
7458 way only in animals exposed to 10000 µg/kg bw per day for 10 weeks, or to 25000 µg/kg bw per day
7459 for 6 weeks. Cortical GABA and GLY were significantly decreased in the same exposure groups. Finally,
7460 TAU was decreased in a significant way only in rats exposed to 25000 µg/kg bw per day of BPA during
7461 6 weeks. The endpoint AChE activity was judged as Likely while the other ones (excitatory and inhibitory
7462 amino acid neurotransmitters) were judged as ALAN.

7463 A Tier 1 study performed in adult male rats (Fan et al., 2018 [RefID 11915]) dosed 0 or 50 µg/kg bw
7464 per day for 21 weeks through the diet) reported a significant increase in corticosterone blood levels 30
7465 minutes after being tested in the open field compared with the basal level measured before the open
7466 field. The highest variation was observed in BPA-exposed rats compared with controls. Females were
7467 not included in this study. The evidence on serum corticosterone was judged Inadequate to conclude
7468 on the likelihood of the effect.

7469 The CEP Panel assigned a likelihood level of Likely to the Neurofunctional adverse effect of BPA in the
7470 adult exposure period. The likelihood level for this cluster is Likely for the endpoint 'AChE activity' based
7471 on Khadrawy et al. (2016) [RefID 3462], therefore this endpoint was taken forward for BMD analysis.

7472 Other endpoints related to this period of exposure, namely 'excitatory or inhibitory neurotransmitters
7473 (GABA, ASP, GLU, GLN, GLY and TAU)' (Khadrawy et al., 2016 [RefID 3462]), 'MAO activity' (Khan et
7474 al., 2018 [RefID 12311]) and 'blood corticosterone production' (Fan et al., 2018 [RefID 11915]) were
7475 not considered for BMD analysis due to a likelihood level of ALAN based on a Tier 2 study (Khadrawy et
7476 al., 2016 [RefID 3462]) or considered of Inadequate Evidence since only two single-dose Tier 1 or Tier
7477 2 studies (Fan et al., 2018 [RefID 11915] and Khan et al., 2018 [RefID 12311], respectively) were
7478 available.

7479 The Likely and ALAN endpoints were considered in the uncertainty analysis (see Appendix D).

7480 Indirect (germline) exposure

7481 Two Tier 1 studies both performed in rats were identified (Fan et al., 2018 [RefID 11915]; Fan et al.,
7482 2013 [RefID 1907]) regarding this period of exposure.

7483 The Tier 1 study from Fan et al. (2018) [RefID 11915] reported a significant increase (+25%) in
7484 corticosterone blood levels measured in adult female rats (PND56) derived from male parents exposed
7485 to BPA 50 µg/kg bw per day for 21 weeks before mating. Blood was examined 30 minutes after the
7486 FST which was the last one of a series of three consecutive behavioural tests including the Open Field,
7487 the EPM and the Forced Swim Test. No effect was observed in males.

7488 This evidence was considered to be Inadequate to conclude on the likelihood of the effect The second
7489 Tier 1 study (Fan et al., 2013 [RefID 1907]) reported a lack of significant changes in the hippocampal

7490 AChE activity measured in adult F1 male and female rats (PND56) issued from non-exposed F0 females
7491 being mated with F0 males exposed to BPA (50 µg/kg bw per day) for 10 weeks before mating.

7492 This evidence was considered to be Inadequate to conclude on the likelihood of the effect. The CEP
7493 Panel assigned a likelihood level of Inadequate Evidence to the neurofunctional effects of BPA in the
7494 indirect (germline) exposure period, so, none of the endpoints was taken forward for BMD analysis.

7495 Overall cluster selection of the endpoints/studies for BMD analysis for Nervous system functionality

7496 The CEP Panel considered that the evidence from the studies available showed a Likely effect of BPA
7497 in the adult exposure (after puberty) period for the endpoint 'AChE activity' (Fan et al., 2013 [RefID
7498 1907]; Khadrawy et al., 2016 [RefID 3462]; Khan et al., 2018 [RefID 12311]). However, only the study
7499 Khadrawy et al., 2016 [RefID 3462] assessing this endpoint was taken forward for BMD analysis and
7500 not the other two because these were all single-dose studies/experiments. Other endpoints related to
7501 this period of exposure, namely 'excitatory or inhibitory neurotransmitters (GABA, ASP, GLU, GLN, GLY
7502 and TAU)' (Khadrawy et al., 2016 [RefID 3462]), 'MAO activity' (Khan et al., 2018 [RefID 12311]) and
7503 'blood corticosterone production' (Fan et al., 2018 [RefID 11915]) were not considered for BMD analysis
7504 due to a likelihood level of ALAN based on a Tier 2 study (Khadrawy et al., 2016 [RefID 3462]) or
7505 considered of Inadequate evidence since only two single-dose Tier 1 or Tier 2 studies (Fan et al., 2018
7506 [RefID 11915]; Khan et al., 2018 and [RefID 12311], respectively) were available.

7507 The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster Nervous
7508 system functionality, was Likely.

7509

7510 Behaviour

7511 The cluster Behaviour comprises a number of different endpoints that relate to different types of actions
7512 or responses that can be observed in animals or humans. For the sake of clarity, the results of the WoE
7513 exercise will be presented by endpoint to demonstrate which behavioural measurements were
7514 susceptible to BPA at different exposure periods. The specific measurements that were included for the
7515 effects of BPA on behaviour belong to the endpoints of anxiety-related and emotional behaviour,
7516 learning and memory, locomotor activity and exploration, social behaviour and preference behaviour.
7517 Note that most studies cover more than one endpoint and some cover different stage of the animal life.

7518 Overall, the cluster Behaviour comprises 17 studies performed in rats (of which two studies have indirect
7519 germline exposure through the male parent before conception, 10 studies relate to exposed animals
7520 during development until weaning, two studies relate to growth phase/young age and five studies relate
7521 to exposure in adulthood) and 13 studies performed in mice. Among these, two studies relate to indirect
7522 exposure of the male or the female parent before conception, seven studies relate to exposure during
7523 development until weaning, two to exposure in growth phase/young age and five to exposure in
7524 adulthood.

7525 In addition, there were three studies performed in monkeys and one study in prairie voles.

7526 Developmental exposure (pre-natal and/or post-natal until weaning)

7527 For effects of BPA in the developmental exposure period, 20 studies in total (in rats, mice, monkeys or
7528 prairie voles) were identified. From the rat studies, five were allocated to Tier 1 (Ferguson SA et al.,
7529 2014 [RefID 1998]; Fujimoto et al. 2013 [RefID 2102]; Fujimoto et al., 2015 [RefID 2104]; Hicks et al.,
7530 2016 [RefID 2704]; Johnson et al., 2016 [RefID 3241]), three to Tier 2 (Hass et al., 2016 [RefID 2610];
7531 Wang C et al., 2016 [RefID 7576]; Wang C et al., 2014 [RefID 7579]), and two to Tier 3 (Rebuli et al.,
7532 2015 [RefID 6127]; Sadowski et al., 2014a [RefID 6361]). One mouse study was allocated to Tier 1
7533 (Luo et al., 2014 [RefID 4660]), six to Tier 2 (Kumar and Thakur, 2017 [RefID 3737]; Nagao et al.,
7534 2014 [RefID 5295]; Naule et al., 2014 [RefID 5335]; Picot et al., 2014 [RefID 5830]; Sobolewski et al.,
7535 2014 [RefID 6802]; Xin et al., 2018 [RefID 13482]) and one to Tier 3 (Kundakovic et al., 2013 [RefID
7536 3755]). The study in cynomolgus monkeys (*Macaca fascicularis*) (Negishi et al., 2014 [RefID 5351])
7537 was in Tier 2 and the study in prairie voles (*Microtus ochrogaster*) (Sullivan et al., 2014 [RefID 6954])
7538 was allocated to Tier 3.

7539 • Anxiety/emotionality

7540 For the endpoint anxiety/emotionality, five studies in rats, four studies in mice and one study in prairie
7541 voles were included. In most cases, the exposure started at implantation of the conceptus (2 studies
7542 in rats, 1 study in mice) or before or directly after mating of the parent animals (3 studies in mice, 1
7543 study in rats). One study in rats examined animals exposed during the fetal period, whereas another
7544 study in rats and the study in prairie voles restricted exposure to the post-natal period.

7545 No changes of anxiety or emotionality parameters were found in adult male or female rats in a Tier 2
7546 study by Hass et al. (2016) [RefID 2610] with pre-natal and post-natal exposure covering a large dose
7547 range (25, 250, 5000, 50000 µg/kg bw per day). The test was performed in an EPM. The finding is
7548 supported by a Tier 3 study by Rebuli et al. (2015) [RefID 6127]. These authors examined the behaviour
7549 of juvenile and adult rat offspring after pre- and post-natal exposure to 2.5, 25 and 250 µg/kg bw per
7550 day in the EPM and the Open Field and tested adult offspring also in the Zero Maze. None of the tests
7551 gave evidence for changes in anxiety-related behaviour following developmental BPA exposure. In
7552 contrast, Hicks et al. (2016) [RefID 2704] found evidence for increased anxiety in the Open Field in a
7553 single-dose study with exposure through the drinking water throughout pregnancy and lactation (80–
7554 195 µg/kg bw per day). Young adult male and female offspring from the BPA group spent less time in
7555 the centre area, away from the walls. Fujimoto et al. (2013) [RefID 2102] did not find effects on anxiety
7556 either in the EPM in young adult rats of both sexes in a single-dose study (24 µg/kg per day) with
7557 exposure during the first pre-natal week. However, they observed an influence on depression-like
7558 behaviour in the Forced Swim Test in which males and females of the BPA group displayed a decrease
7559 in latency to immobility and males showed a prolonged duration of immobility. Another indication for
7560 effects of developmental BPA exposure on anxiety-related parameters comes from a single-dose study
7561 (15 µg/kg bw per day) by Fujimoto et al. (2015) [RefID 2104]. The authors reported an increase in the
7562 avoidance response to fox odour by adult males and females after exposure to BPA during the second
7563 half of the gestation period. Overall, the data in rats indicate effects of BPA only in the more stressful
7564 tests that examine the endpoint of anxiety/emotionality.

7565 A single-dose Tier 2 study with mice detected increased anxiety in pre-natally and post-natally exposed
7566 males in the Open Field and the EPM at a dose of 50 µg/kg bw per day (Kumar and Thakur, 2017
7567 [RefID 3737]). The males were tested at the age of 8 weeks and the BPA group showed a lower
7568 inclination to visit the unprotected areas in both tests. Females were not examined in this study, but
7569 data from the single-dose study of Luo et al. (2014) [RefID 4660] conducted with a much higher dose
7570 (10000 µg/kg bw per day) indicate a comparable effect in female mice shortly after the end of exposure
7571 at weaning in the Open Field and in the EZM. The finding in females is supported by a Tier 3 study with
7572 pre-natal exposure. Females tested at the age of 8 weeks spent less time in the central area of the
7573 Open Field at the dose levels of 20 and 200 µg/kg bw per day (Kundakovic et al., 2013 [RefID 3755]).
7574 However, male mice in this study exhibited a change in the opposite direction at all doses tested (2, 20
7575 and 200 µg/kg bw per day). They spent more time in the unprotected area, a behaviour that resembles
7576 the normal pattern of control females.

7577 Xin et al. (2018) [RefID 13482] did not observe increased anxiety in adult male mouse offspring from
7578 dams treated with 10 or 10000 µg/kg bw per day from before pregnancy until weaning when testing
7579 them in the EZM. However, they discovered an increase in depression-like behaviour (time spent
7580 immobile) in the Forced Swim Test which was similar in extent at both dose levels. Females did not
7581 show an increase in depression-like behaviour in this test.

7582 Effects of BPA exposure appear Likely based on effects in Open Field, Elevated Maze and Forced Swim
7583 Test in Tier 1 and Tier 2 mouse studies and on findings in the Forced Swim Test and a predator odour
7584 avoidance test in rats.

7585 • Learning and memory

7586 The endpoint learning and memory was examined in a single-dose study in mice (Tier 2) and five
7587 studies in rats with three or more dose groups and a control. Sobolewski et al. (2014) [RefID 6802]
7588 exposed pregnant/lactating mice from implantation until weaning to a dose of 50 µg/kg bw per day.
7589 Male and female offspring were tested as young adults in a novel object exploration and recognition
7590 test that examined response to novelty and short-term memory of familiar objects. In the BPA group
7591 both sexes showed a significant decrease in their initial exploration time spent with a novel object and
7592 the males also exhibited a decrease in overall exploration time. Females compensated the decreased
7593 duration of the exploration bouts with an increase in the number of approaches to the object. No effects

7594 were seen on short-term memory in this test. During fixed interval (60 s) reinforcement sessions, BPA-
7595 group males, but not females, exhibited significant reductions in response rates which could indicate
7596 an attention deficit or a lack of motivation to respond for food rewards.

7597 In rats, Hass et al. (2016) [RefID 2610] did not identify learning deficits in a MWM in 4–6-month-old
7598 male or female offspring after developmental exposure (GD7 to PND22) to doses between 25 and
7599 50000 µg/kg bw per day in their Tier 2 study.

7600 In a Tier 3 study, Sadowski et al. (2014a) [RefID 6361] did not find changes in working or reference
7601 memory in BPA-exposed offspring tested as adults in a 17-arm radial maze. The rats had been exposed
7602 from GD0 until PND9 to doses between 4, 40 or 400 µg/kg bw per day. However, with male rats tested
7603 after weaning and exposed only during pre-natal development (GD9 to GD20), Wang C et al. (2014)
7604 [RefID 7579] found an increase in 8-arm radial maze working memory errors compared with the control
7605 group. The effect was seen at all doses (50, 500, 5000 and 50000 µg/kg bw per day), especially during
7606 the first half of the 14-day trial period. A similar, albeit smaller, effect was seen for reference memory
7607 errors in this Tier 2 study. An increased error rate (incidence of sniffing incorrect holes) was also
7608 observed in a Tier 1 study by Johnson et al. (2016) [RefID 3241] in male and female rats exposed from
7609 GD6 to PND21 to a dose of 2500 µg/kg per day and tested as adults in a Barnes maze. In addition, the
7610 female rats exhibited an increased latency to locate the escape box in this test. No effects on Barnes
7611 maze performance were seen at lower doses (2.5 and 25 µg/kg per day).

7612 In another Tier 2 study, a test for object recognition in weanling male rats exposed from GD9 to GD20
7613 resulted in a decrease of the object recognition index as a measure of short-term memory (1.5 hours)
7614 at 500, 5000 and 50000 µg/kg bw per day. Long-term memory (24 hours) was also impaired but only
7615 at 5000 and 50000 µg/kg bw per day. No effect on either measure was seen at the dose of 50 µg/kg
7616 bw per day (Wang C et al., 2016 [RefID 7576]). This is compatible with the negative result in mice
7617 after developmental exposure to this low dose only (Sobolewski et al., 2014 [RefID 6802]).

7618 Based on findings of memory impairment, impaired novel object exploration/recognition and increased
7619 error rate an effect of BPA on these endpoints was judged to be Likely.

7620 • Locomotor activity/exploration

7621 Regarding the endpoint locomotor activity/exploration the motor activity was studied either in the home
7622 cage of the animals or in an apparatus like the open field that allowed spontaneous movement and
7623 exploration in most cases. Five studies in mice with either pre-natal (Nagao et al., 2014 [RefID 5295];
7624 Kundakovic et al., 2013 [RefID 3755]) or pre-natal and post-natal exposure (Luo et al., 2014 [RefID
7625 4660]; Sobolewski et al., 2014 [RefID 6802]; Xin et al., 2018 [RefID 13482]) were available. Four of
7626 these studies did not identify an effect of BPA on this endpoint at doses between 2 and 10000 µg/kg
7627 bw per day. Only the Tier 3 study of Kundakovic et al. (2013) [RefID 3755] described a sexually
7628 dimorphic effect on distance travelled in the Open Field for males at all doses tested (2, 20 and 200
7629 µg/kg bw per day) and for females at the two highest doses. Similarly, out of eight studies in rats, only
7630 the study of Wang C et al. (2014) [RefID 7579], found a slight reduction between 10 and 30% in
7631 locomotor activity over a wide dose range (50, 500, 5000 or 50000 µg/kg bw per day) in males after
7632 pre-natal exposure. No clear dose dependency was observed. Females were not included in this Tier 2
7633 study. In a different test setting, the same group (Wang C et al., 2016 [RefID 7576]) detected no
7634 change in the time period the males spent exploring. Other investigators did not report changes in
7635 motor activity of rats after either pre-natal (Fujimoto et al., 2015 [RefID 2104]), pre-natal and post-
7636 natal (Rebuli et al., 2015 [RefID 6127]; Hicks et al., 2016 [RefID 2704]; Ferguson SA et al., 2014 [RefID
7637 1998]; Hass et al., 2016 [RefID 2610]) or post-natal exposure (Fujimoto et al., 2013 [RefID 2102]),
7638 although the study by Hass et al. covered a similar dose range (25, 250, 5000, 50000 µg/kg bw per
7639 day) as the study by Wang C et al. (2014) [RefID 7579]. The only other study in which an effect was
7640 observed was a Tier 3 study in prairie voles (Sullivan et al., 2014 [RefID 6954]) that identified a
7641 reduction of activity after exposure during the second PNW to the dose of 50000 µg/kg bw per day in
7642 the female sex only. No effects occurred at lower doses or in males.

7643 Overall, the available data suggest that an effect of BPA on this endpoint is Not Likely.

7644 • Social interaction

7645 The effects of developmental exposure of BPA on various aspects of social behaviour were examined
7646 in rats (pre-natal and post-natal), mice (pre-natal and post-natal), prairie voles (post-natal) and
7647 cynomolgus monkeys (pre-natal). No effects on sexual behaviour were seen in intact female rats
7648 exposed to doses of 2.5 or 25 µg/kg per day from implantation to weaning in a Tier 1 study (Ferguson
7649 SA et al., 2014 [RefID 1998]) and in male mice exposed to doses of 50 or 5000 µg/kg bw per day from
7650 the fetal period until weaning in a Tier 2 study (Picot et al., 2014 [RefID 5830]). BPA also did not
7651 change non-sexual social interactions in male and female rat offspring in a single-dose Tier 1 study
7652 with exposure through the drinking water amounting to 80–195 µg/kg bw per day (Hicks et al., 2016
7653 [RefID 2704]) or in a Tier 2 study with male mice exposed to 10 or 10000 µg/kg bw per day (Xin et al.,
7654 2018 [RefID 13482]). Only the Tier 3 study by Kundakovic et al. (2013) [RefID 3755] reported a
7655 decrease in chasing behaviour directed to cage mates in male mice at the highest dose tested (200
7656 µg/kg bw per day).

7657 A Tier 2 study in cynomolgus monkeys reported a decrease in social interaction in males after pre-natal
7658 exposure to BPA after subcutaneous exposure equivalent to an oral dose of about 1000 µg/kg bw per
7659 day (Negishi et al., 2014 [RefID 5351]). In addition, the exposure to BPA abolished the known difference
7660 in discriminant scores that is present between control males and females.

7661 In a Tier 3 study with a monogamous rodent species, the prairie vole, Sullivan et al. (2014) [RefID
7662 6954] observed BPA effects on social interaction after exposure on PND8 to PND14. Females at a dose
7663 of 50000 µg/kg bw per day spent more time investigating a same sex stimulus animal, whereas males
7664 at 50 and 50000 µg/kg bw per day spent less time. Consequently, the normal sex difference of the
7665 response was lost or inverted. In the same study, female prairie voles seemed to show a decrease in
7666 formation of opposite sex partner preference bonding at all doses tested (5, 50 or 50000 µg/kg bw per
7667 day) based on a low number of females that spent time with a strange male in addition to their partner.
7668 This parameter was not affected in males.

7669 There were no effects on social behaviour in Tier 1 and Tier 2 studies in rats and mice; weak effects
7670 were observed in a small single-dose level Tier 2 study in monkeys and in a Tier 3 study in female
7671 prairie voles. Overall, an effect of BPA on this endpoint was judged as Not Likely.

7672 • Preference behaviour

7673 A Tier 1 (Ferguson SA et al., 2014 [RefID 1998]) and a Tier 2 study (Hass et al., 2016 [RefID 2610])
7674 examined preference for sweet solution in male and female rats exposed during *in utero* development
7675 until weaning. No effect of BPA was detected in either study at doses equal to or lower than 5000 µg/kg
7676 bw per day. The dose of 50000 µg/kg bw per day used in the study of Hass et al. decreased the intake
7677 of saccharin solution by approximately 25% in females only, which may indicate masculinisation or de-
7678 feminisation of this behaviour at high doses.

7679 BPA exposure to doses of 2.5 or 25 µg/kg bw per day did not change the preference for sodium chloride
7680 solution in males and females (Ferguson SA et al., 2014 [RefID 1998]).

7681 The available data indicate that changes in preference behaviour are Not Likely.

7682 Overall, the CEP Panel assigned a likelihood level of Likely to the behavioural effects of BPA in the
7683 developmental exposure period (pre-natal and/or post-natal until weaning). Since the likelihood level
7684 for this cluster is Likely for the endpoints anxiety/emotionality (Xin et al., 2018 [RefID 13482]) and
7685 learning and memory (Johnson et al., 2016 [RefID 3241]; Wang C et al., 2016 [RefID 7576]; Wang C
7686 et al., 2014 [RefID 7579]), these were taken forward for BMD analysis (see Chapter 3.2.1) and
7687 uncertainty analysis (see Appendix D). The endpoints anxiety/emotionality investigated in the studies
7688 by Kumar and Thakur (2017) [RefID 3737] and Luo et al. (2014) [RefID 4660] were not taken forward
7689 for BMD analysis because both are single-dose studies.

7690 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

7691 No studies were available for this exposure period.

7692 Growth phase/young age exposure

7693 For this exposure period, two studies in rats (Bowman et al., 2015 [RefID 688]; Chen Z et al., 2018
7694 [RefID 11734]), two studies in mice (Zhou YX et al., 2017 [RefID 9083]; Lou et al., 2013 [RefID 11330])

7695 and one study in vervet monkeys (*Chlorocebus sabaeus*) (Elsworth et al., 2013 [RefID 1810]) were
7696 identified. All studies were allocated to Tier 1.

7697 • Anxiety/emotionality

7698 The endpoint anxiety/emotionality was studied in rats and mice. Chen Z et al. (2018) [RefID 11734]
7699 tested young adult rats that had been dosed orally with 40, 400 and 4000 µg/kg bw per day from
7700 weaning until PND49. They observed a statistically significant increase of latency to first entry into the
7701 centre area of an open field in males at 4000 µg/kg bw per day, as well as slight, non-significant
7702 increases in both lower dose groups. No effects were seen in females. Lou et al. (2013) [RefID 11330]
7703 examined adult male mice that had been given 10000 µg/kg bw per day from PND30 until PND70 in a
7704 dark/light test and the EPM and found evidence for increased anxiety at this dose with both tests.

7705 Based on these findings, effects of BPA on anxiety/emotionality were judged as Likely for males.

7706 • Learning and memory

7707 For the endpoint of learning and memory, studies in rats, mice and monkeys were available. In the
7708 study of Chen Z et al. (2018) [RefID 11734] male rats displayed reduced memory for platform location
7709 in the MWM in the mid and the high-dose group (400 and 4000 µg/kg bw per day). Memory was
7710 unaffected in females. In male mice treated with doses of 0.5, 50 and 5000 µg/kg bw per day from 4–
7711 12 weeks of age, Zhou YX et al. (2017) [RefID 9083] found a decrease in learning performance in the
7712 high-dose group. These animals required an increased number of trials to qualify to the learning
7713 standard (90% correct response) in a Y maze test.

7714 The other studies for this endpoint were conducted with subcutaneous exposure. In a study with rats
7715 employing only a single dose (40 µg/kg bw per day s.c., equivalent to 1428 µg/kg bw per day orally)
7716 Bowman et al. (2015) [RefID 688] observed a decrease in overall exploration in males and in females,
7717 but no effect on spatial memory performance. In addition, males but not females spent less time with
7718 novel objects than the control group. No effect was seen on the working memory of pre-pubertal male
7719 and female monkeys exposed to plasma levels of about 15 ng/ml (equivalent to 4500 µg/kg bw per day
7720 orally) for 30 days in the study by Elsworth et al. (2013) [RefID 1810].

7721 The available data indicate a Likely effect of BPA on learning/memory for males but not for females.

7722 • Locomotor activity/exploration

7723 No or no consistent effects on the other two endpoints examined for this exposure period, preference
7724 behaviour and locomotor behaviour, were found by Bowman et al. (2015) [RefID 688] in their single-
7725 dose study with rats. Since this single-dose study was the only study available for these endpoints,
7726 there was Inadequate evidence to judge the likelihood of these effects.

7727 Overall, the CEP Panel assigned a likelihood level of Likely to the behavioural effects of BPA in the
7728 exposure period Growth phase/young age. Since the likelihood level for this cluster is Likely for the
7729 endpoints anxiety/emotionality and learning and memory (in Chen Z et al., 2018 [RefID 11734] and
7730 Zhou YX et al., 2017 [RefID 9083], respectively) in males, these were taken forward for BMD analysis
7731 (see Chapter 3.2.1) and uncertainty analysis (see Appendix D).

7732 Adult exposure (after puberty)

7733 In this exposure group, 10 studies from three species were identified, four in rats, five in mice and one
7734 in vervet monkeys (*Chlorocebus sabaeus*). Three of the rat studies were allocated to Tier 1 (Fan et al.,
7735 2018 [RefID 11915]; Fan et al., 2013 [RefID 1907]; Nuñez et al., 2018 [RefID 11199]), one to Tier 2
7736 (Nojima et al., 2013 [RefID 5435]). The database for mice consisted of one Tier 1 study (Xu XH et al.,
7737 2015 [RefID 8232]), three Tier 2 studies (Picot et al., 2014 [RefID 5830]; Khan et al., 2018 [RefID
7738 12311]; Xin et al., 2018 [RefID 13482]) and one Tier 3 study with subcutaneous exposure (Liang et al.,
7739 2018 [RefID 12508]) which was considered supportive for the endpoints anxiety/emotionality and
7740 locomotor activity/exploration identified in Tier 1 studies. The single study in monkeys (Elsworth et al.,
7741 2015 [RefID 1809]) was allocated to Tier 1.

7742 • Anxiety/emotionality

7743 For the endpoint anxiety/emotionality, one study in rats and two studies in mice identified an increase
7744 of the level of anxiety in male animals. Fan et al. (2018) [RefID 11915] observed that male rats treated

7745 with the single dose of 50 µg/kg bw per day for 23 weeks spent about 40% less time in the centre area
7746 of an open field, whereas Xu XH et al. (2015) [RefID 8232] and Liang et al. (2018) [RefID 12508] found
7747 that male mice were not responsive in this type of test in their studies. However, they displayed dose-
7748 dependent increases in anxiety or emotionality in two other tests, EPM and the FST. In the study by Xu
7749 XH et al. (2015) [RefID 8232], the effect was noted from the lowest dose tested (40 µg/kg bw per day)
7750 in the FST and for all doses in the EPM except the lowest one. Female mice showed either no or opposite
7751 effects (decreased anxiety/emotionality), consistent with possible sexual dimorphic effects of BPA in
7752 the brain. An effect of BPA on the endpoint anxiety/emotionality was judged as Likely.

7753 • Learning and memory

7754 With respect to learning and memory, the data available from single-dose studies in three species (rats,
7755 mice and vervet monkeys) indicate impaired cognition in males. Fan et al. (2013) [RefID 1907] observed
7756 that male rats given 50 µg/kg bw per day for 10 weeks in a small amount of food (5 g) exhibited
7757 increased swimming distance and escape latency during the learning phase for the location of the
7758 platform in the MWM test . In the probe trial that tested how well platform location had been
7759 memorised, they spent less time in the target quadrant and thus displayed a reduced capacity to
7760 remember. In the study of Khan et al. (2018) [RefID 12311], male mice showed a reduced attraction
7761 to novel objects after treatment with 10000 µg/kg bw per day for 5 weeks. This can be interpreted as
7762 a lack of discrimination between objects of different familiarity or, alternatively, as a fear of novelty. A
7763 study conducted with young adult male vervet monkeys (Elsworth et al., 2015 [RefID 1809]), pretrained
7764 in a two-choice spatial delayed response test, found a decrease in median per cent correct responses
7765 from 90% to about 83% 1 week after the start of exposure to 5555 µg/kg bw per day. The effect was
7766 reversible and full recovery was achieved 2 weeks after the end of the exposure. Considering the above
7767 evidence, the effects of BPA on the endpoint learning/memory are judged as Likely.

7768 • Locomotor activity/exploration

7769 No effects of BPA on locomotor activity in the open field test (OFT) was seen in a Tier 1 study in male
7770 rats with a single-dose level of 50 µg/kg bw per day (Fan et al., 2018 [RefID 11915]), a Tier 3 study in
7771 male mice with oral equivalent dose levels of 8888, 88880, 888800 µg/kg bw per day (Liang et al.,
7772 2018 [RefID 12508]), and a Tier 1 study in male and female mice with dose levels of 40, 400, 4000
7773 and 40000 µg/kg bw per day (Xu XH et al., 2015 [RefID 8232]). In the latter study, the animals were
7774 also tested in the EPM and a mirrored maze that examine entry into specific parts of the maze as an
7775 indicator for anxiety and may give some information on locomotor activity when total activity is
7776 considered. In both sexes, no effects were seen in the mirrored maze which is more similar to the OFT.
7777 BPA treatment also did not affect overall locomotion of female mice in the EPM, whereas males showed
7778 reduced overall activity at the two highest doses. However, it is considered that the lower activity of
7779 males resulted from the dose-related increase in anxiety and not from a direct impact on locomotor
7780 behaviour.

7781 Nojima et al. (2013) [RefID 5435] conducted a single-dose study (Tier 2) with intraperitoneal exposure
7782 by minipump that measured spontaneous motor activity of male rats in their home cage. At a dose
7783 equivalent to 918 µg/kg bw per day orally they noted a difference in activity to the control group that
7784 was most pronounced shortly before and after the transition from dark to light phase. The deviation
7785 became statistically significant for the light phase only on days 11–12 after implantation of the
7786 minipump. However, the circadian activity pattern was not changed, and the BPA group showed slightly
7787 higher overall activity throughout the test period. As no pre-treatment data are reported in the study it
7788 is not entirely clear if this is a substance effect or if the rats chosen for the BPA group already showed
7789 a higher home cage activity before the experiment started.

7790 Overall, there is sufficient evidence to conclude that effects of BPA on the endpoint locomotor activity
7791 are Not Likely.

7792 • Sensory-motor coordination

7793 Khan et al. (2018) [RefID 12311] found sensory-motor coordination deficits in male mice exposed to
7794 10000 µg/kg bw per day. The animals showed a decreased performance on the rotarod and in a grip
7795 strength test. Since only one Tier 2 single-dose study was available the evidence was considered
7796 Inadequate.

7797 • Social interaction

7798 Two Tier 2 studies in mice examined the effects of BPA on adult social behaviour. Xin et al. (2018)
7799 [RefID 13482] studied maternal behaviour in females on lactation day 1 after exposure from before
7800 mating and throughout pregnancy. They found no effects on nest building, pup retrieval or time spent
7801 in the nest at doses of 10 or 10000 µg/kg bw per day. In the study of Picot et al. (2014) [RefID 5830],
7802 sexual behaviour was affected in male mice exposed from week 8 to week 12 of age at a dose of 50
7803 µg/kg bw per day but not at 5000 µg/kg bw per day. The males in the low dose required longer latencies
7804 to accomplish their first mount, intromission, thrust and ejaculation; in addition, the number of mounts
7805 with intromission and thrusts was also reduced. Therefore, it is judged as Likely that BPA affects male
7806 sexual behaviour.

7807 • Preference behaviour

7808 Nuñez et al. (2018) [RefID 11199] examined the preference for sodium salt intake in male and
7809 ovariectomised (OVX) female rats during seven days of subcutaneous exposure to BPA in oral equivalent
7810 doses of 357, 1785, 3570 and 17850 µg/kg bw per day. The males decreased both their spontaneous
7811 water intake and the consumption of NaCl solution at all doses except in the lowest dose group and
7812 showed a decreased preference for saline after fluid deprivation at 357, 3570 and 17850 µg/kg bw per
7813 day. No differences in preference were noted between the groups. For OVX females, decreased water
7814 intake was only observed at the highest dose, but they showed reductions in spontaneous salt intake
7815 and reduced preference for salt after fluid deprivation at the same doses as the males. The treated
7816 females showed a reduced preference for 2.7% NaCl at all doses when compared with the control
7817 group, but the effect was only statistically significant at 3570 µg/kg bw per day. The findings may
7818 indicate an effect of BPA on body fluid regulation that leads to differences in intake behaviour. The BPA
7819 effects on this endpoint is judged as Likely.

7820 Overall, the CEP Panel assigned a likelihood level of Likely to the behavioural effects of BPA exposure
7821 in adult males and females based on increased anxiety/depression-like behaviour in male mice (Xu XH
7822 et al., 2015 [RefID 8232] and Liang et al., 2018 [RefID 12508]) and decreased anxiety in female mice
7823 (Xu XH et al., 2015 [RefID 8232]); impaired male sexual behaviour in mice (Picot et al., 2014 [RefID
7824 5830]) and changes in salt preference in rats (Nuñez et al., 2018 [RefID 11199]). Therefore, these
7825 endpoints were taken forward for BMD analysis (see Chapter 3.2.1) and for uncertainty analysis (see
7826 Appendix D). The endpoints anxiety/depression-like behaviour in male rats (Fan et al., 2018 [RefID
7827 11915]) and impaired learning and/or memory in male rats (Fan et al., 2013 [RefID 1907]), mice (Khan
7828 et al., 2018 [RefID 12311]) and monkeys (Elsworth et al., 2015 [RefID 1809]) were not taken forward
7829 for BMD analysis because the studies are single-dose studies.

7830 Indirect (germline) exposure

7831 Behavioural endpoints after exposure through the germline were examined in the offspring of rats and
7832 mice that had been treated with BPA before mating of the animals began. For effects through the
7833 female germline one study in mice (Xin et al., 2018 [RefID 13482]) allocated to Tier 2 with a low and
7834 a high-dose group (10 and 10000 µg/kg bw per day) was available, in which the female parents had
7835 been exposed throughout their own development until weaning. This study did not identify any changes
7836 in the two endpoints that were examined in their male offspring (anxiety/emotionality, locomotor
7837 activity) at any dose. Female offspring was not tested.

7838 Two Tier 1 studies in rats (Fan et al., 2018 [RefID 11915]; Fan et al., 2013 [RefID 1907]) and one Tier
7839 1 study in mice (Luo et al., 2017 [RefID 4661]) dealt with possible consequences of the exposure of
7840 the male parent and compared only a single-dose group of BPA of either 50 µg/kg bw per day (in rats)
7841 or 10000 µg/kg bw per day (in mice) to a control group.

7842 • Anxiety/emotionality

7843 The endpoint of anxiety/emotionality was examined by three different procedures in young adult rats
7844 offspring (Fan et al., 2018 [RefID 11915]) and identified increased anxiety in females with all three
7845 tests (Forced Swim Test, Open Field and Elevated Maze). In the males, this finding was only obvious
7846 in the Forced Swim Test, which can be considered to impose a higher level of stress on the animals
7847 compared with the other testing procedures, open field and elevated maze. In the mouse study (Luo
7848 et al., 2017 [RefID 4661]), these latter tests indicated increased anxiety in juvenile and young adult

7849 male offspring, respectively. Female mice were not tested. Effects on this endpoint were judged as
7850 Likely.

7851 • Learning and memory

7852 Spatial learning of male and female offspring was affected in the MWM in a rat study (Fan et al., 2013
7853 [RefID 1907]). The animals needed more time and longer swimming distances to locate the escape
7854 platform during the acquisition process. However, memory of the learned positions appeared affected
7855 only in females, not in males. As only a single-dose study was available, this evidence was considered
7856 Inadequate.

7857 • Locomotor activity/exploration

7858 For the endpoint locomotor activity/exploration one study in rats (Fan et al., 2018 [RefID 11915]) and
7859 one study in mice (Luo et al., 2017 [RefID 4661]) were available. In young adult rats, no effects were
7860 observed on the distance travelled in the open field in either sex. Juvenile male mice showed reduced
7861 overall locomotor activity in the same test. No data were obtained for female mice. In addition, a study
7862 with male mice (Xin et al., 2018 [RefID 13482]) did not find any changes of exploratory/locomotor
7863 activity in a hole board test. An effect of BPA on locomotor behaviour was considered Not Likely.

7864 • Social interaction

7865 Social interaction was studied in male mice only (Luo et al., 2017 [RefID 4661]) and revealed a decrease
7866 in exploration time of same sex strangers and a reduced preference for social contact. As only a single-
7867 dose study was available, this evidence was considered Inadequate.

7868 Overall, The CEP Panel assigned a likelihood level of Likely to the behavioural effects of BPA exposure
7869 through the male germline based on consistent findings in two species (rats and mice) for the endpoint
7870 anxiety/emotionality (in Fan et al., 2018 [RefID 11915] and Luo et al., 2017 [RefID 4661]). However,
7871 both of these studies were conducted with only a single dose of BPA and none of them was taken
7872 forward for BMD analysis.

7873 Overall cluster selection of the endpoints/studies for BMD analysis for Behaviour

7874 Overall, the CEP Panel assigned a likelihood level of Likely to effects of BPA on behaviour in the exposure
7875 periods developmental until weaning, growth phase/young age, adult age and indirect (germline)
7876 exposure periods.

7877 The CEP Panel considered that the evidence from the studies available showed a Likely effect of BPA
7878 in the exposure period developmental (pre-natal and/or post-natal development until weaning) for the
7879 endpoints anxiety/emotionality (Xin et al., 2018 [RefID 13482]) and learning and memory (Johnson et
7880 al., 2016 [RefID 3241]; Wang C et al., 2016 [RefID 7576]; Wang C et al., 2014 [RefID 7579]).
7881 Therefore, these endpoints were taken forward for BMD analysis (see Chapter 3.2.1).

7882 The CEP Panel considered that the evidence from the studies available showed a Likely effect of BPA
7883 in the exposure period growth phase/young age for the endpoints anxiety/emotionality (Chen Z et al.,
7884 2018 [RefID 11734]; Luo et al., 2013 [RefID 11330]) and learning and memory (Chen Z et al., 2018
7885 [RefID 11734]; Zhou YX et al., 2017 [RefID 9083]) in males.

7886 The CEP Panel considered that the evidence from the studies available showed a Likely effect of BPA
7887 in the exposure period adult age for the endpoints anxiety/emotionality (Xu XH et al., 2015 [RefID
7888 8232]), sensory-motor coordination (Khan et al., 2018 [RefID 12311]), and salt preference (Nuñez et
7889 al., 2018 [RefID 11199]). Therefore, these endpoints were taken forward for BMD analysis (see Chapter
7890 3.2.1).

7891 The CEP Panel considered that the evidence from the studies available showed a Likely effect of BPA
7892 in the exposure period of the male germline for the endpoint anxiety/emotionality (Fan et al., 2018
7893 [RefID 11915]; Luo et al., 2017 [RefID 4661]). These endpoints were not taken forward for BMD
7894 analysis because single-dose studies.

7895 The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster effects
7896 on behaviour, was Likely.

7897 **3.1.5.3. Integration of likelihoods from human and animal studies**

7898 Table 12 presents the overall likelihood per cluster for the human and animal stream separately, as
 7899 well as the integration of the likelihoods from the human and animal studies for Neurotoxicity and
 7900 developmental neurotoxicity.

7901 **Table 12:** Integration of the human and animal studies for Neurotoxicity and developmental
 7902 neurotoxicity.

Human stream		Animal stream	Integrated likelihood
Cluster: Neurodevelopment (behaviour after developmental exposure)		Cluster: Behaviour	
Exposure during Pregnancy	Not Likely	Developmental (pre-natal and/or post-natal until weaning)	Likely
Exposure during Childhood	Not Likely	Growth phase/young age	Likely
		Adult exposure (after puberty)	Likely
		Indirect (germline) exposure	Likely
<i>Overall likelihood:</i>	<i>Not Likely</i>	<i>Overall likelihood:</i>	<i>Likely</i>
Cluster: Neuromorphology		Cluster: Neuromorphology	
Not applicable		Developmental (pre-natal and/or post-natal until weaning)	Likely
		Growth phase/young age	Likely
		Adult exposure (after puberty)	Inadequate evidence
		<i>Overall likelihood:</i>	<i>Likely</i>
Cluster: Nervous system functionality		Cluster: Nervous system functionality	
Not applicable		Developmental (pre-natal and/or post-natal until weaning)	ALAN
		Growth phase/young age	Inadequate evidence
		Adult exposure (after puberty)	Likely
		Indirect (germline) exposure	Inadequate evidence
		<i>Overall likelihood:</i>	<i>Likely</i>

7903

7904 **3.1.5.4. In vitro and Mechanistic studies**

7905 BPA effects are reported over a huge range of effective concentrations/doses (low nM and µg/kg per
 7906 day to high µM and >1 mg/kg). This always needs to be considered; there may be qualitative as well
 7907 as quantitative differences in mechanisms, depending on dose. In addition, apparent inconsistencies
 7908 between studies may result from heterogeneity in study design (experimental models, route and
 7909 window of exposure, dose of BPA, age at time of assessment, testing procedure, etc.).

7910 It is clear that many of the reported effects of BPA on Neurotoxicity and developmental neurotoxicity
 7911 endpoints are downstream pleiotropic effects (e.g. altered expression of many genes and proteins;
 7912 ERK1/2 signalling, Wnt/β-catenin, Tmprss2, Foxa1, NGF, SOX2, Pax6, Grn2b, JNK, CREB and p53-
 7913 mitochondrial apoptosis pathways, Gnrh1, Calbindin-D28, Kisspeptin (Kiss1), GNRH, DNA methylation
 7914 (Dnmt, MECP2), hypotaurine, NMDA, GABA, oxytocin, serotonin and dopamine signalling,
 7915 hypothalamic–pituitary axes (HPA, HPT and HPG), BDNF-NTRK2 neurotrophin system, etc.)

7916 Details of upstream mechanisms, which are more likely to be BPA specific, are unclear, but receptor
7917 interactions are an obvious candidate mechanism. In particular, ER-dependent pathways have been
7918 implicated in a number of studies (Mhaouty-Kodja et al., 2018).

7919 Mhaouty-Kodja et al. (2018) concluded that effects of BPA on learning and memory appear to be
7920 associated with the disruption of oestrogen-dependent pathways and of cerebral glutamate-NMDAR
7921 pathway (downstream targets leading to gene transcription of ERK, CREB, Brain-derived neurotrophic
7922 factor BDNF and synaptic proteins involved in synaptic plasticity).

7923 It is unfortunately difficult to generalise data from studies reporting receptor-dependency of BPA
7924 effects, because steroid receptor properties and interactions are dynamic; substance effects can vary
7925 by tissue, life-stage and/or dose. In fact, much of the complexity of steroid receptor mechanisms has
7926 been revealed by research with BPA.

7927 Oxidative stress generation is an additional candidate mechanism for BPA effects on AChE activity and
7928 other endpoints. There is some evidence that BPA-induced oxidative stress is involved in brain functional
7929 effects at both low and high doses, but the data are not consistent. In humans, Condolot et al. (2016)
7930 [RefID 3646] sought to identify correlations between BPA levels and oxidant-antioxidant parameters in
7931 autistic and non-autistic children. No significant correlations were observed.

7932 **Neuromorphology**

7933 There was no human evidence available for the cluster Neuromorphology. Thus, the overall likelihood
7934 of effects of BPA for this cluster was scored Likely, based on the animal evidence.

7935 Low-dose BPA-related reduction of dendritic spine density and/or cell number during development
7936 and/or growth phase (hippocampal CA1 and CA3) was judged as Likely.

7937 BPA-induced mitochondria-related oxidative stress is one possible MoA.

7938 Agarwal et al. (2016) [RefID 52] concluded that pre-natal and post-pubertal low dose (40 µg/kg bw
7939 per day) of BPA impaired rat hippocampus mitochondrial fusion/fission dynamics and autophagy-
7940 mediated mitochondrial turnover, leading to increased oxidative stress, mitochondrial fragmentation,
7941 and apoptosis in hippocampal neural stem cells (NSCs), and inhibited hippocampal derived NSC
7942 proliferation and differentiation.

7943 A possible mechanism for BPA effects on mitochondria is interaction with calcium channels.

7944 Michaela et al. (2014) [RefID 5081] reported that nanomolar concentrations of BPA inhibited calcium
7945 current through T-type calcium channels (TCCs) in HEK cells. They suggested that this high-affinity
7946 low-efficacy inhibition may be caused by direct binding of BPA to TCCs in their resting state.

7947 Chen et al. (2020) reported that TCC blockade (in C2C12 myoblasts) induced mitochondria-related
7948 apoptosis and reduced mitochondrial transmembrane potential (MMP), induced mito-ROS generation,
7949 and enhanced expression of mitochondrial apoptosis proteins.

7950 Jiang et al. (2015) [RefID 3189] reported BPA-related decreased activities of mitochondrial respiratory
7951 complexes and abnormalities in mitochondrial morphology in rat cardiac myofibrils, including decreased
7952 mitochondrial volume density, reduced cristae density and increased vacuoles, after low-dose BPA (50
7953 µg/kg bw per day) for 48 weeks. They considered it possible that the changes in mitochondrial function
7954 were a primary event to observed BPA-induced myocardial hypertrophy.

7955 Mitochondria are the major source of cellular ROS, and there is evidence that neural stem cell activity
7956 is modulated by ROS (Hou et al., 2012; Adusumilli et al., 2021). BPA-induced ROS could be a possible
7957 MoA for the reported alterations in hippocampal cell numbers.

7958 Overall, there is some evidence for a connection between BPA-induced mitochondria-related oxidative
7959 stress, possibly related to calcium channel blockade, and effects on neuromorphology.

7960 **Nervous system functionality**

7961 There was very limited human evidence available for the cluster Nervous system functionality, not fitting
7962 for the WoE. Thus, the overall likelihood of effects of BPA for this cluster was scored Likely, based on
7963 the animal evidence.

7964 More specifically, Alavian-Ghavanini et al. (2018) [RefID 11471] investigated initially if low-dose
7965 developmental BPA exposure affects DNA methylation and expression of Grin2b (glutamate ionotropic
7966 receptor NMDA type subunit 2B) in brains of adult rats. They reported that developmental exposure to
7967 BPA changes the DNA methylation level in the Grin2b promoter, results in altered gene expression
7968 levels in female, but not in male, rats 1 year after the exposure had ceased. Extending their investigation
7969 to humans, the authors report that pre-natal BPA exposure was associated with increased methylation
7970 levels in girls. Attempting an indirect link they also report that low APGAR scores, a predictor for
7971 increased risk for neurodevelopmental diseases, were associated with higher Grin2b methylation levels
7972 in girls than boys.

7973 Kundakovic et al. (2015) [RefID 3756] showed that pre-natal exposure to BPA induces lasting DNA
7974 methylation changes in the transcriptionally relevant region of the BDNF gene in the hippocampus and
7975 blood of BALB/c mice. Extending their work in humans, they examined BDNF IV DNA methylation in
7976 cord blood samples from the CCCEH cohort where high maternal BPA exposure had been associated
7977 with adverse behavioural effects in different sex groups. High pregnancy BPA levels (based on maternal
7978 spot urine) were associated with altered DNA methylation of two CpG sites in the human cord blood
7979 with an observed trend for a sex-specific effect of high BPA on CpG1A methylation and a significant
7980 sex-specific effect of high BPA exposure on CpG1B methylation levels.

7981 Yang CW et al. (2014) [RefID 8324] identified candidate genes of neuronal development by
7982 implementing a gene ontology analysis and formed a reconstructed neuronal sub-network.
7983 Subsequently, their gene expressions were determined in 20 umbilical cord blood samples dichotomised
7984 into high and low BPA level tiers. Two neuronal genes, sex determining region Y-box 2 (Sox2) and
7985 paired box 6 (Pax6), had preferentially downregulated expression in response to BPA exposure. Fetal
7986 cord blood samples had the obviously attenuated gene expression of Sox2 and Pax6 in high BPA group
7987 referred to low BPA group. Visualised gene network of Cytoscape analysis showed that Sox2 and Pax6
7988 which were contributed to neural precursor cell proliferation and neuronal differentiation might be
7989 downregulated through sonic hedgehog (Shh), vascular endothelial growth factor A (VEGFA) and Notch
7990 signalling. These results indicated that trans-placental BPA exposure downregulated gene expression
7991 of Sox2 and Pax6 potentially underlying the adverse effect on childhood neuronal development.

7992 In animals, changes in neurotransmitters (GABA, noradrenaline NA, dopamine DA, 5-hydroxytryptamine
7993 5HT serotonin) and OR were judged ALAN during developmental (pre-natal and/or post-natal until
7994 weaning) exposure.

7995 At high doses (≥ 10000 $\mu\text{g}/\text{kg}$ bw per day), BPA increased hippocampal neurotransmitters and also
7996 increased markers of oxidative stress and lipid peroxidation (Khadrawy et al., 2016 [RefID 3462]). It is
7997 therefore possible that these neurotransmitter changes were downstream to (i.e. a result of) BPA-
7998 induced oxidative stress.

7999 At low dose (10 $\mu\text{g}/\text{kg}$ bw per day), mouse hippocampal DA was increased in males only, and NA was
8000 decreased in females only, without changes in GABA and GLN (Xin et al., 2018 [RefID 13482]).

8001 Low-dose BPA (≤ 2500 $\mu\text{g}/\text{kg}$ bw per day) increased male OR density, and thus eliminated sex
8002 differences, in various sexually dimorphic brain nuclei (BNST_{dl}, VMH, paraventricular hypothalamic
8003 nucleus PVN (Witchey et al., 2019 [RefID 13782])).

8004 Given that these low-dose effects are gender-specific, it is possible that the MoA involves interactions
8005 with oestrogen (ER) and androgen (AR) receptors.

8006 Altered AChE activity after adult exposure to BPA was judged Likely.

8007 Low dose BPA (50 $\mu\text{g}/\text{kg}$ bw per day) decreased AChE activity (-36%) in the rat hippocampus and
8008 impaired spatial memory (Fan et al., 2013 [RefID 1907]). Given that BPA is reported by others to induce
8009 oxidative stress at low doses, this reduced AChE activity could be caused by oxidative stress, cf.
8010 Schallreuter et al. (2004).

8011 At higher doses (20000–50000 $\mu\text{g}/\text{kg}$ bw per day), BPA increased cortical AChE activity (by about 20–
8012 50%) (Khadrawy et al., 2016 [RefID 3462]). Hippocampal AChE activity was similarly significantly
8013 increased after 10000 $\mu\text{g}/\text{kg}$ for 6 weeks and 25000 $\mu\text{g}/\text{kg}$ for 6 weeks, but not after 25000 $\mu\text{g}/\text{kg}$ bw
8014 per day for 6 weeks, i.e. this was not a consistent effect.

8015 A possible mechanism for the increased brain AChE activity at high doses is altered membrane fluidity
8016 leading to increased extracellular enzyme exposure, as proposed in the study by Macczak et al. (2017)
8017 [RefID 4760] for erythrocyte AChE activity ('AChE is located in erythrocyte membrane on
8018 phosphatidylinositol, and thus changes in membrane fluidity may lead to stronger exposure of this
8019 enzyme outside of the cell, which results in an increase of its activity.'). Such a mechanism implies cell
8020 damage, but brain histopathology, reported by others, was not measured in Macczak et al. (2017)
8021 [RefID 4760].

8022 By contrast, Khan et al. (2018) [RefID 12311] reported reduced AChE activity (about –35%) in whole
8023 brain of adult mice dosed orally with BPA 10000 µg/kg bw per day for 30 days. Oxidative stress
8024 biomarkers in brain homogenates were also significantly increased (and neurobehavioural and cognitive
8025 performance was reduced). These study data support the hypothesis that oxidative stress is a causative
8026 upstream event leading to the observed decrease in AChE activity.

8027 The reported relationship between both high-dose and low-dose BPA exposure and brain AChE activity
8028 is inconsistent, thus, it is not possible to propose a single mechanism, although there is some evidence
8029 suggesting that BPA-induced oxidative stress may be involved.

8030 **Behaviour**

8031 After the integration of the human and animal evidence, the overall likelihood of effects of BPA for the
8032 cluster Effects on behaviour was scored Likely.

8033 Changes in behavioural endpoints are expressed downstream of functional and/or structural changes
8034 in the brain and are considered the apical indication that a living system has exhausted its innate ability
8035 to compensate for changes induced in the underlying processes.

8036 Effects of BPA on learning and memory performances were judged as Likely in both sexes for two
8037 periods of exposure, i.e. the developmental (pre-natal and/or post-natal until weaning) and the adult
8038 period (after puberty), whereas it was limited to the males for BPA exposure in the growth phase and/or
8039 the young age. Neurofunctional clusters interrelated with learning and memory performances were
8040 judged as Likely for the hippocampal and cortical AChE activity for an exposure occurring at the adult
8041 stage (after puberty), and ALAN for neurotransmitter systems in various parts of the brain following a
8042 developmental exposure (pre-natal and/or post-natal until weaning). Concomitant neuromorphological
8043 changes including dendritic morphology and spine density in hippocampus and cortex were also judged
8044 as Likely for the two earliest periods of exposure (development until weaning and growth phase and/or
8045 young age) and as ALAN for exposure limited to the adult stage (after weaning).

8046 Effects of BPA on anxiety and depression-like behaviour were judged as Likely in all exposure periods:
8047 during pre-natal and/or post-natal exposure until weaning (in mice); during the growth phase and
8048 young age (in male rats and mice); in adult animals; and also, after indirect exposure through the
8049 germline of the male parent. Possible mechanisms include changes in corticosteroid regulation and in
8050 Wnt/β-catenin signalling.

8051 *Corticosterone regulation*

8052 Sex-specific functional alterations of the hypothalamic–pituitary–adrenal (HPA) axis and concomitant
8053 changes in anxiety-like behaviour have been described after developmental exposure to BPA. Chen F
8054 et al. (2014, 2015) [RefID 1012, RefID 1013] reported a reduction in the HPA axis response to stress
8055 for females ('anti-anxiety-like' behaviour), whereas in males they found a hyperactivation. BPA-exposed
8056 males, but not females, had higher basal levels of serum corticosterone and adrenocorticotrophic
8057 hormone (ACTH), as well as an increase of corticotropin releasing hormone (CRH) mRNA in the
8058 hypothalamic PVN.

8059 Pubertal female rats exposed to BPA exposure at 40 µg/kg bw per day during pregnancy and lactation
8060 showed increased basal corticosterone and reduced hypothalamic GR levels. A stress challenge elicited
8061 more anxiety-like behavioural coping and a weaker corticosterone response compared with control
8062 females (Panagiotidou et al., 2014 [RefID 5627]). In female rat offspring exposed to BPA at 40 µg/kg
8063 bw per day during pregnancy and lactation, Zhou et al. (2015) [RefID 9062] found a significant increase
8064 in both basal (morning) and peak (afternoon) corticosterone release compared with controls and
8065 increased basal and peak plasma ACTH at the same time points. In the hippocampus, mRNA expression

8066 for GR, mGlu2 and mGlu3 receptors as well as proteins levels of mGlu2/3 receptors were decreased,
8067 suggesting that both increased corticosterone levels and decreased signalling through hippocampal
8068 mGlu2/3 contribute to the anxiety and depression-like behaviour observed after BPA exposure.

8069 The increased corticosterone production observed in BPA-exposed animals seems to involve impaired
8070 feedback from the hippocampus to the HPA axis which leads to increased levels in CRH and ACTH. The
8071 CRH signalling pathway was identified among the top scoring pathways in the transcriptome of the
8072 amygdala in male and female neonatal rats after pre-natal BPA exposure at 25 µg/kg bw per day
8073 (Arambula et al., 2018 [RefID 11050]). An activation of Cyp11A1 (P450scc) in the adrenal was shown
8074 by Medwid et al. (2016) [RefID 4996] in mice at 5000 µg/kg bw per day and by Lan et al. (2015) [RefID
8075 3825] in mouse Y1 adrenal cortex cells in the BPA concentration range 50–1000 nM, and in rats injected
8076 subcutaneously with BPA (0.5 µg/kg bw per day for 3 days, equivalent to oral 17.85 µg/kg bw per day).
8077 In addition to this increase of adrenal steroid synthesis, an upregulation of corticosterone production
8078 could also occur in the neurons themselves; hippocampal neurons contain the complete pathway of
8079 corticosteroid synthesis and normally produce low levels of corticosterone (approximately 7 nM) that
8080 are sufficient to modulate synaptic plasticity (Hojo et al., 2011). Thus, a cell-autonomous
8081 overproduction could augment the adverse effects of circulating corticosteroids on synaptic plasticity
8082 and affective behaviours.

8083 A possible mechanism for the effects of BPA on both corticosterone and anxiety is via ER-mediated
8084 altered hippocampal expression of FKBP5 (FKBP51), a GR binding protein that negatively regulates GR,
8085 which is upregulated by stress, and is connected to neuronal synaptic plasticity (Qiu et al., 2019).
8086 Increased Fkbp5 promoter methylation (decreased protein expression) has been reported to be
8087 associated with an anxiety phenotype and increased corticosterone levels in mice after pre-natal trauma
8088 (Plank et al., 2021). Kitraki et al. (2015) [RefID 3589] found an increase in DNA methylation of this
8089 gene at a BPA dose of 40 µg/kg bw per day in male rats, which probably resulted from a reduction of
8090 ERβ binding to a site in intron 5 of the gene.

8091 Similar to BPA, increased corticosterone levels have been associated with a reduction in dendritic spine
8092 density in hippocampus and PFC and an increase in anxiety/depression-like behaviour (Wang G et al.,
8093 2013a).

8094 In conclusion, it appears that the effects on the HPA axis at the molecular level, the morphological
8095 decrease of dendritic spine density in hippocampus and PFC, and the apical change in affective
8096 behaviour (anxiety/depression-like behaviour) are causally connected and delineate a MoA for BPA
8097 neurotoxicity. In the dose range between 2 and 40 µg/kg bw per day, BPA exposure increased serum
8098 corticosterone by 10–60%. It decreased dendritic spine density or dendritic spine synapses in layer
8099 II/III mPFC pyramidal cells and hippocampus for all exposure periods (lowest effective dose reported
8100 was 40 µg/kg bw per day) and increased anxiety/depression-like behaviour (lowest effective dose
8101 reported at 10–20 µg/kg bw per day). A MoA involving increased corticosterone production could be
8102 envisaged as follows:

- 8103 • Binding of BPA to ERβ changes specificity for target genes (including Fkbp5 in hippocampus)
8104 imply a decreased Fkbp5 expression, which would lead to an increase in CRH and ACTH. This
8105 activates Cyp11A1 (P450scc) in mitochondria of adrenal cells (and possibly in hippocampal
8106 neurons), causing an increase of corticosterone production through the c-Jun JNK) signalling
8107 pathway, an increase of serum corticosterone and a corticosterone-dependent decrease in
8108 dendritic spine density associated with increased anxiety/depression.

8109 Altered Wnt/β-catenin pathway activity is a further possible MoA. Arambula et al. (2018) [RefID 11050]
8110 identified Wnt/β-catenin signalling (four genes) as one of the top pathways affected by BPA in neonate
8111 amygdala at 25 µg/kg bw per day in males. BPA was shown to downregulate this pathway and to
8112 increase β-catenin degradation in rat brain and in cultured hippocampal neurons in studies by Liu ZH
8113 et al. (2014, 2015) [RefID 10411, RefID 4535] and by Tiwari et al. (2015, 2016) [RefID 7220, RefID
8114 7221]. BPA exposure upregulated GSK3β which phosphorylates β-catenin and marks it for degradation
8115 in the absence of ligand. As a consequence, nuclear translocation of β-catenin was decreased in the
8116 hippocampus and the subventricular zone (SVZ). Proliferation and neuronal differentiation of
8117 hippocampus-derived neural stem cell as well as hippocampal and subventricular zone neurogenesis
8118 were impaired (Tiwari et al., 2015 [RefID 7220]). BPA-induced decreases in dendritic spine density
8119 were noted in the dentate gyrus and the CA1 area of the hippocampus by Liu ZH et al. (2015) [RefID

8120 4535]. This finding was replicated in cultured CA1 neurons with BPA concentrations of 10 nM or greater.
8121 Importantly, this *in vitro* effect was abolished by addition of a Wnt ligand (Wnt7a) to the culture,
8122 suggesting that Wnt is causally involved in the effect.

8123 Wnt signalling together with BDNF cooperatively regulates dendritic spine formation; Wnt signalling
8124 inhibition in cultured cortical neurons disrupts dendritic spine development (Hiester et al., 2013). BDNF
8125 is a direct target of Wnt signalling, being induced through Wnt-dependent TCF/LEF transcription factors
8126 (Yi et al., 2012) and then can exert a positive feedback on Wnt signalling through induction of Wnt2
8127 which is sufficient to promote cortical dendrite growth and dendritic spine formation *in vitro*. BPA
8128 diminished the expression of LEF-1 and TCF mRNAs and proteins in the hippocampus of rats (Tiwari et
8129 al., 2015 [RefID 7220]).

8130 Activation of Wnt-dependent β -catenin signalling decreased the expression of steroidogenic genes
8131 (including Cyp11a1) in an adrenocortical cell line and caused a reduction in the release of corticosterone
8132 (Walczak et al., 2014). The inhibitory effect of BPA on the Wnt pathway may contribute to the
8133 upregulation of corticosterone production.

8134 In addition to the canonical (β -catenin dependent) pathway, non-canonical Wnt pathways appear to be
8135 involved in BPA effects on neurons. Liu ZH et al. (2014) [RefID 10411] reported a dose-dependent
8136 decrease of the canonical ligand Wnt7a while the non-canonical ligand Wnt5a increased in hippocampal
8137 dentate gyrus homogenates. Wnt5a has been shown to modulate mitochondrial dynamics in cultured
8138 hippocampal neurons and to promote fission of mitochondria through recruitment of the fission
8139 regulator Drp1 from the cytosol to the mitochondria. The change in mitochondrial morphology was
8140 associated with significant increases in cytosolic and mitochondrial Ca^{2+} levels (Godoy et al., 2014).
8141 Agarwal et al. (2016) [RefID 52] showed that BPA increased mitochondrial fragmentation in the
8142 hippocampus (including dentate gyrus and CA regions) of the rat brain at a dose level of 40 μ g/kg bw
8143 per day and in hippocampal NSC-derived neuron cultures at a concentration of 100 μ M.

8144 In conclusion, these studies support the involvement of canonical and non-canonical Wnt pathways in
8145 the effects of BPA on functional and structural parameters in the brain and their downstream
8146 consequences for behavioural endpoints.

8147 **3.1.5.5. Conclusion on hazard identification for Neurotoxicity and developmental** 8148 **neurotoxicity of BPA**

8149 In the EFSA opinion 2015 (EFSA CEF Panel, 2015), a likelihood level of ALAN was assigned to
8150 neurological, neurodevelopmental and neuroendocrine effects of BPA in a WoE approach. This was
8151 based on the results from prospective epidemiological studies examining children exposed to BPA during
8152 the pre-natal period. The studies provided evidence for an association with sex-dependent behavioural
8153 problems but not sufficient proof for a causal link, due to inconsistent findings across studies. Animal
8154 studies, while indicating a possible impairment of brain functions and behavioural parameters such as
8155 anxiety-like behaviour, learning and memory, social behaviour and sensory-motor function, presented
8156 methodological shortcomings as well as inconsistent results from different studies. Therefore, the CEP
8157 Panel decided not to take these effects forward to derive the toxicological RP but used them in the
8158 analysis of uncertainty for hazard characterisation and risk characterisation.

8159 In the current assessment, epidemiological evidence derived from newly available longitudinal studies
8160 examining children with exposure during pregnancy or post-natally did not suggest any endpoints
8161 related to neurodevelopment as critical for risk assessment. The children were followed for various time
8162 periods, up to the age of 10 years. Although some statistically significant associations were observed,
8163 none of them occurred in more than one study and subsequent research failed to replicate the results.

8164 The available cross-sectional studies produced some evidence for associations of BPA exposure and
8165 various neurodevelopmental endpoints in children but are not considered robust enough on their own
8166 to support an adverse association.

8167 With respect to the animal studies, the results of the present evaluation extend the previous database
8168 and indicate possible effects of BPA during development and in adults mainly on anxiety and depression-
8169 related behaviours, learning and memory, as well as on dendritic spine density and AChE activity in the
8170 hippocampus and the PFC. Loss of spines in these brain regions has also been observed in association
8171 with impaired cognitive ability and mood disturbances in a number of other animal models. Thus, it can

8172 be assumed that the reduction of dendritic spine density forms the structural basis for the behavioural
8173 changes induced by BPA. The mechanisms that underly the effect on spine density are less clear. Spine
8174 formation may be affected by various signals, including neurosteroids, neurotransmitters and their
8175 receptors, synaptic plasticity-promoting proteins, signalling pathways and oxidative stress, most of
8176 which have also been identified in studies that examined possible MoAs for BPA. This includes for
8177 example reductions in the expression of ERs, overproduction of corticosterone, downregulation of
8178 NMDA receptors, changes in PSD-95 expression and interference with Wnt/ β -catenin signalling.

8179 Overall, the CEP Panel considers that the different effects observed on brain structure, neurochemistry
8180 and functional outcome, e.g. behaviour, can be integrated into a convincing picture that indicates the
8181 existence of a neurotoxic hazard of BPA in developing, growing and adult animals.

8182 Using a WoE approach, the CEP Panel assigned a likelihood level of Likely to the effect of BPA on
8183 anxiety/emotionally, learning and memory, salt preference, dendritic spine density and AChE activity.
8184 Therefore, these endpoints were brought forward for BMD analysis (see Chapter 3.2.1).

8185

8186 **3.1.6. Reproductive and developmental toxicity**

8187 **3.1.6.1. Epidemiological studies**

8188 For the HOC Reproductive and developmental toxicity, a total of 47 studies was appraised by the CEP
8189 Panel. The details of the appraisals (internal validity) are reported in Annex B.

8190 *Identification of the clusters to be considered for WoE*

8191 On the basis on the approach described in Chapter 2.3.2 'Definition of Health Outcome Categories and
8192 Clusters', the following Clusters (C) and Exposure periods (Exp) were brought forward to WoE analysis:

- 8193 • C: Fetal and post-natal growth
 - 8194 – Exp: Adulthood
- 8195 • C: Prematurity
 - 8196 – Exp: Pregnancy
- 8197 • C: Pre-eclampsia
 - 8198 – Exp: Adulthood
- 8199 • C: Male fertility
 - 8200 – Exp: Adulthood
- 8201 • C: Female fertility
 - 8202 – Exp: Adulthood

8203 *WoE of the relevant clusters*

8204 The main information extracted from the studies included in relevant clusters in the HOC Reproductive
8205 and developmental toxicity are summarised in Annex C. The outcome of the weight of the evidence is
8206 described in the text below and presented in a tabulated format in Annex D.

8207

8208 **Cluster Fetal and post-natal growth**

8209 Exposure during pregnancy

8210 A total of 13 studies reported results on indices of fetal growth (Burstyn et al., 2013 [RefID 762];
8211 Philippat et al., 2014 [RefID 5821]; Smarr et al., 2015 [RefID 6784]; Veiga-Lopez et al., 2015 [RefID
8212 7422]; Birks et al., 2016 [RefID 591]; Casas et al., 2016 [RefID 879]; Ferguson et al., 2016b [RefID
8213 1995]; Huang YF et al., 2017a [RefID 2925]; Pinney et al., 2017 [RefID 5838]; Woods et al., 2017
8214 [RefID 8003]; Lee YM et al., 2018 [RefID 11150]; Lester et al., 2018 [RefID 12446]; Mustieles et al.,

8215 2018b [RefID 12784]). Of these two studies examined associations with measures of growth *in utero*
8216 based on ultrasound measures (Philippat et al., 2014 [RefID 5821]; Lee YM et al., 2018 [RefID 11150])
8217 and two studies examined associations between maternal pregnancy BPA concentrations with measures
8218 of post-natal growth up to 3 (Philippat et al., 2014 [RefID 5821]) and 6 years of age (Lee YM et al.,
8219 2018 [RefID 11150]). Overall, no consistent associations with BW, birth length, head circumference or
8220 other indices measured *in utero* or at birth were observed. In most cases the effect estimates were
8221 centred around the NULL with wide confidence intervals but significant associations were observed in
8222 a few studies. As an example, a study by Lee YM et al. (2018) [RefID 11150] reported a significant
8223 increase in BW with higher maternal exposure while a significant decrease in femoral length *in utero*
8224 was observed. Another study by Mustieles et al. (2018b) [RefID 12784] examined associations with BW
8225 among 346 subjects who were having their fertility status examined. In that study a significant inverse
8226 association between maternal pre-pregnancy BPA concentration and BW was observed [~ 79 g decrease
8227 in BW (95%CI: $-153, -5$) for ~ 3 -fold increase in BPA exposure]. For maternal samples collected during
8228 pregnancy this association was in the same direction but non-significant [-38 g (95%CI: $-101, 25$)].
8229 Although interesting, this inverse association for maternal pre-pregnancy concentration would need to
8230 be replicated in another study before any robust conclusions can be drawn.

8231 In terms of possible high exposures, Birks et al. (2016) [RefID 591] used individual data from 13
8232 European birth cohorts to identify pregnant women who had been occupationally exposed (based on
8233 self-report) to BPA during pregnancy. Of 133,957 individuals a total of 59 women with occupational
8234 exposure were identified. Mean birth weight among these exposed women was not significantly different
8235 from those unexposed.

8236 The few studies that examined more clinically relevant birth outcomes such as small for gestational age
8237 (i.e. babies with LBW (below the 10th percentile), when controlling for gestational age) and LBW did
8238 not find any association with maternal BPA exposure (Burstyn et al., 2013 [RefID 762]; Lester et al.,
8239 2018 [RefID 12446]). However, only the case-control study by Burstyn et al. (2013) [RefID 762] had
8240 sufficient statistical power to evaluate this outcome with some accuracy.

8241 Concerning post-natal growth, the study by Lee YM et al. (2018) [RefID 11150] also reported some
8242 significant associations between maternal concentrations of BPA in pregnancy and weight and weight
8243 for length from age 3 to 6 years of age. In contrast, no association with weight or length up to 3 years
8244 of age was observed in the study by Philippat et al. (2014) [RefID 5821].

8245 Overall conclusions

8246 On the basis of the above, the CEP Panel concluded that an association between maternal BPA exposure
8247 and impaired pre-natal and post-natal growth is Not Likely.

8248

8249 **Cluster Prematurity**

8250 Exposure during pregnancy

8251 A total of seven studies examined the association between maternal BPA concentrations and length of
8252 gestation or preterm delivery (Weinberger et al., 2014 [RefID 7909]; Cantonwine et al., 2015 [RefID
8253 823]; Smarr et al., 2015 [RefID 6784]; Veiga-Lopez et al., 2015 [RefID 7422]; Birks et al., 2016 [RefID
8254 591]; Casas et al., 2016 [RefID 879]; Pinney et al., 2017 [RefID 5838]).

8255 In a cohort of 72 pregnant women Weinberger et al. (2014) [RefID 7909] reported a significant inverse
8256 association between total BPA concentration in urine and length of gestation (~ 1 day shorter gestation
8257 for each interquartile increase in exposure). In contrast, a relatively large case-control study sampling
8258 130 preterm cases and 352 random controls sampled from a larger birth cohort ($n = 2246$) did not find
8259 any association between maternal urinary BPA exposure (samples collected minimum three times during
8260 pregnancy) and preterm delivery (Cantonwine et al., 2015 [RefID 823]). No association was consistently
8261 observed in other studies looking at length of gestation.

8262 In studies examining associations with length of gestation no associations were observed (Smarr et al.,
8263 2015 [RefID 6784]; Veiga-Lopez et al., 2015 [RefID 7422]; Casas et al., 2016 [RefID 879]; Pinney et
8264 al., 2017 [RefID 5838]).

8265 In terms of possible high exposures Birks et al. (2016) [RefID 591] reported a slightly longer gestation
8266 (~4 days) among 59 that were likely to have been occupationally exposed to BPA (based on self-report)
8267 compared with unexposed women (n = 116,358).

8268 Overall conclusions

8269 On the basis of these studies, the CEP Panel concluded an association between BPA exposure and
8270 shorter duration of gestation or increased risk of preterm delivery is Not Likely.

8271

8272 **Cluster Pre-eclampsia**

8273 Exposure during adulthood

8274 Two case-control studies, Ye et al. (2017) [RefID 8483] n = 173 and Cantonwine et al. (2016) [RefID
8275 824], n = 481 for a total number of cases of 123, assessed the association between BPA exposure
8276 measured in spot urine (n = 1) or serum (n = 1) samples in pregnancy and endpoints related to pre-
8277 eclampsia (onset and/or severity). Their detailed description and risk of bias assessment related to
8278 these studies are provided in Annexes C and D. One study was conducted in USA (Cantonwine et al.,
8279 2016 [RefID 824]) and one study was conducted in China (Ye et al., 2017 [RefID 8483]) with similar
8280 endpoint definitions. In the latter, BPA exposure (continuous and per tertile) was statistically
8281 significantly associated with pre-eclampsia, pre-eclampsia onset and pre-eclampsia severity.

8282 Overall conclusions

8283 On the basis of the above, the CEP Panel concluded that the evidence for a positive association between
8284 BPA exposure and pre-eclampsia is ALAN.

8285

8286 **Cluster Male fertility**

8287 Exposure during adulthood

8288 A total of five studies (Buck Louis et al., 2014 [RefID 4599]; Bae et al., 2015 [RefID 347]; Dodge et
8289 al., 2015 [RefID 1648]; Goldstone et al., 2015 [RefID 2304]; Buck Louis et al., 2018 [RefID 12602]) in
8290 three different cohorts examined the relationship between urinary concentrations of BPA and fertility in
8291 males and females.

8292 Among couples (n = 218) seeking fertility treatment Dodge et al. (2015) [RefID 1648] found no
8293 association between urinary BPA concentrations in men and fertilisation or live birth following *in vitro*
8294 fertilisation or insemination. Similarly, Buck Louis et al. (2014) [RefID 4599] examined associations
8295 between urinary concentrations in both male and female couples (n = 501) with fecundability. No
8296 association was observed for urinary BPA concentrations in males and females.

8297 In a later study of 339 males from the same cohort (Buck Louis et al., 2018 [RefID 12602]) no
8298 association between BPA concentrations in seminal plasma and fecundity was also observed.

8299 These findings are in line with a study by Goldstone et al. (2015) [RefID 2304] where no association
8300 was observed between urinary BPA concentrations and semen quality (total count, concentration or
8301 morphology) in 418 males.

8302 Finally, Bae et al. (2015) [RefID 347] reported association between paternal BPA exposure and fewer
8303 male births (lower male to female sex ratio). As no other studies have reported on this outcome and in
8304 the light of the fact that none of the other studies found consistent associations with live birth rate,
8305 fecundability or other fertility outcomes a chance finding seems plausible.

8306 Overall conclusions

8307 On the basis of the above, the CEP Panel concluded that an association between exposure to BPA
8308 measured in spot urine and reduced fertility is considered Not Likely.

8309

8310 **Cluster Female fertility**

8311 Exposure during adulthood

8312 A total of 11 studies examined the association between exposure to BPA during adult life with fertility
8313 in females (Souter et al., 2013 [RefID 6856]; Buck Louis et al., 2014 [RefID 4599]; Lathi et al., 2014
8314 [RefID 3864]; Bae et al., 2015 [RefID 347]; Minguez-Alarcon et al., 2015 [RefID 5118]; Chavarro et
8315 al., 2016 [RefID 988]; Jukic et al., 2016 [RefID 3276]; Minguez-Alarcon et al., 2016 [RefID 5117]; Chin
8316 et al., 2018 [RefID 11745]; Pollack et al., 2018 [RefID 12909]; Wang B et al., 2018 [RefID 13333]).

8317 These included associations between BPA exposures during pregnancy with fecundability and
8318 miscarriage and offspring sex ratio in the more general population (Buck Louis et al., 2014 [RefID
8319 4599]; Lathi et al., 2014 [RefID 3864]; Bae et al., 2015 [RefID 347]; Jukic et al., 2016 [RefID 3276];
8320 Chin et al., 2018 [RefID 11745]; Wang B et al., 2018 [RefID 13333]) or associations with fertility
8321 outcomes in a more selective group of women seeking fertility treatment (Souter et al., 2013 [RefID
8322 6856]; Minguez-Alarcon et al., 2015 [RefID 5118]; Minguez-Alarcon et al., 2016 [RefID 5117]).

8323 For the studies recruiting the more general population associations with fecundability were inconsistent
8324 with three studies reporting no association (Buck Louis et al., 2014 [RefID 4599]; Jukic et al., 2016
8325 [RefID 3276]; Chin et al., 2018 [RefID 11745]). One study reported associations between maternal
8326 BPA exposure and decreased fecundability (Wang B et al., 2018 [RefID 13333]). A study by Lathi et al.
8327 (2014) [RefID 3864] also reported a significant positive association between BPA and aneuploid
8328 pregnancy and risk of miscarriage.

8329 In a group of selected women seeking fertility treatment, Chavarro et al. (2016) [RefID 988] reported
8330 a significant decrease in live births among strata of women who did not consume soy (n = 100), while
8331 no such association were observed among majority of study participants that did consume soy
8332 (n = 247). As women who seek treatment may change their dietary habits before treatment it is
8333 possible that women who did not consume soy in a group of selected women may have underlying
8334 different fertility compared with those who reported to consume soy. Similarly, in a group of selected
8335 women (Minguez-Alarcon et al., 2016 [RefID 5117]) reported decreased probability of implantation and
8336 clinical pregnancies with higher BPA exposures.

8337 Overall conclusions

8338 Overall, the results from these studies conducted in both selected and more unselected group of women
8339 are inconsistent and an association between BPA exposure in adult life and reduced fertility was judged
8340 as ALAN.

8341

8342 **Cluster Pubertal/endocrine**8343 Exposure during pregnancy

8344 Watkins et al. (2017b) [RefID 7876] examined association between pregnancy exposure to BPA and
8345 pubertal development in 109 male offspring aged 8 to 14 years. In a separate publication based on the
8346 same cohort associations with pubertal development were also examined in 120–129 female offspring
8347 aged 8 to 13 years (Watkins et al., 2014 [RefID 7877]; Watkins et al., 2017a [RefID 7875]). No
8348 associations with markers of pubertal development were observed in either studies.

8349 Similarly, no association between pregnancy exposure to BPA and markers of puberty was observed in
8350 112 boys in a study by Ferguson KK et al. (2014) [RefID 1996]. However, a study by Berger et al.
8351 (2018) [RefID 11600] examining the relation between maternal BPA exposure and pubertal
8352 development in 179 females and 159 male offspring at age 9 to 13 years found associations with later
8353 and earlier pubertal development in female and male offspring, respectively.

8354 Finally, three studies reported associations with anogenital distance (Barrett et al., 2017 [RefID 434];
8355 Arbuckle et al., 2018 [RefID 11520]; Sun et al., 2018 [RefID 13199]), an outcome that is often used
8356 as predictor for later reproductive disorders. Overall, despite a few significant findings observed the
8357 overall findings from these three studies were inconsistent.

8358 On the basis of these studies, the CEP Panel concluded that an association between BPA exposure
8359 during pregnancy and pubertal development is considered as ALAN.

8360 Exposure during childhood

8361 A total of five studies examined prospectively associations between exposure to BPA measured in urine
8362 during childhood and pubertal development (Lee et al., 2013 [RefID 3911]; Wolff et al., 2015 [RefID
8363 7982]; Kasper-Sonnenberg et al., 2017 [RefID 3401]; Wolff et al., 2017 [RefID 9215]; Binder et al.,
8364 2018 [RefID 11612]). However, two of these studies were conducted in the same study population but
8365 with different length of follow-up (Wolff et al., 2015 [RefID 7982]; Wolff et al., 2017 [RefID 9215]).

8366 Overall, no association between childhood exposures to BPA and pubertal development were observed
8367 in these studies.

8368 On the basis of these studies, the CEP Panel concluded that an association between BPA exposure
8369 during childhood and pubertal development is considered Not Likely.

8370 Overall conclusions

8371 On the basis of these studies, the CEP Panel concluded that an association between BPA exposure and
8372 pubertal development is considered ALAN.

8373 *Cross-sectional studies*

8374 **Fetal and post-natal growth**

8375 Several cross-sectional studies using maternal blood or urine drawn at end of pregnancy or cord blood
8376 examined the associations with length of gestation and measures of fetal growth. Several studies found
8377 no significant associations with these outcome measures (Tang et al., 2013 [RefID 7111]; Ding et al.,
8378 2017 [RefID 1610]; Huang YF et al., 2018 [RefID 12183]; Mammadov et al., 2018 [RefID 11173]; Wan
8379 et al., 2018 [RefID 13328]). One reported a positive association with length at birth (Xu XJ et al., 2015
8380 [RefID 8238]) and one study reported an inverse association (Youssef et al., 2016 [RefID 9542]). One
8381 study reported higher placental BPA concentration in LBW infants (<2,500g) compared with controls
8382 (Huo et al., 2015 [RefID 2951]). Since BPA has very short elimination half-life it is difficult to explain
8383 how placental concentration at time of birth might reflect concentrations during previous months when
8384 the fetus was growing making it likely that the observed association is coincidental rather than causal.
8385 Overall, the results from these cross-sectional studies were in line with those from prospective studies
8386 showing relatively consistent NULL associations.

8387 **Prematurity**

8388 One cross-sectional study found no association between maternal urine (n = 567) concentration at time
8389 of delivery and length of gestation (Tang et al., 2013 [RefID 7111]), while another small (n = 80) study
8390 reported an inverse association (Youssef et al., 2016 [RefID 9542]). Overall, no meaningful conclusions
8391 on possible association with length of gestation can be drawn from these two studies.

8392 **Pre-eclampsia**

8393 One cross-sectional study investigated the relationship between BPA exposure (maternal and fetal
8394 serum, placenta) and pre-eclampsia (Leclerc et al., 2014 [RefID 3888]) and found no statistical
8395 significance. Only in placental tissue, concentrations of BPA were higher in preeclamptic women
8396 compared with normotensive pregnant women (p-value = 0.04).

8397 **Male fertility**

8398 A total of 15 studies (see external report) (Knez et al., 2014 [RefID 3608]; Lassen et al., 2014 [RefID
8399 3859]; Komarowska et al., 2015 [RefID 3642]; La Rocca et al., 2015 [RefID 3791]; Liu XQ et al., 2015
8400 [RefID 4492]; Vitku et al., 2015 [RefID 7499]; Zhuang et al., 2015 [RefID 9129]; Vitku et al., 2016
8401 [RefID 7498]; Liang H et al., 2017 [RefID 4236]; Wang ZL et al., 2017 [RefID 7850]; Adoamnei et al.,
8402 2018 [RefID 11046]; Joensen et al., 2018 [RefID 12254]; Mustieles et al., 2018a [RefID 11188]; Omrán
8403 et al., 2018 [RefID 12844]; Radwan et al., 2018 [RefID 12945]) examined cross-sectionally the
8404 association between BPA and male reproduction. Of those four studies focused on reproductive
8405 hormones only (Liu XQ et al., 2015 [RefID 4492]; Zhuang et al., 2015 [RefID 9129]; Liang H et al.,
8406 2017 [RefID 4236]; Mustieles et al., 2018a [RefID 11188]), while others also focused on more direct
8407 measures of male fertility (e.g. semen quality, n = 9) (Knez et al., 2014 [RefID 3608]; Lassen et al.,
8408 2014 [RefID 3859]; La Rocca et al., 2015 [RefID 3791]; Vitku et al., 2015 [RefID 7499]; Vitku et al.,
8409 2016 [RefID 7498]; Adoamnei et al., 2018 [RefID 11046]; Joensen et al., 2018 [RefID 12254]; Omrán
8410 et al., 2018 [RefID 12844]; Radwan et al., 2018 [RefID 12945]) or pubertal development (n = 2) (Wang

8411 ZL et al., 2017 [RefID 7850]; Mustieles et al., 2018a [RefID 11188]) or birth malformation
8412 (cryptorchidism, n = 1) (Komarowska et al., 2015 [RefID 3642]). Among the studies examining
8413 associations between BPA exposure (in blood or urine) and semen quality, four studies reported lower
8414 sperm concentrations with higher serum or urinary BPA exposure (Knez et al., 2014 [RefID 3608]; Vitku
8415 et al., 2015 [RefID 7499]; Adoamnei et al., 2018 [RefID 11046]; Omran et al., 2018 [RefID 12844]);
8416 while other studies reported inverse association with other parameters (e.g. sperm motility but not
8417 sperm concentrations) of semen quality (Lassen et al., 2014 [RefID 3859]; La Rocca et al., 2015 [RefID
8418 3791]; Joensen et al., 2018 [RefID 12254]; Radwan et al., 2018 [RefID 12945]). One study also
8419 quantified BPA in seminal fluid where an inverse association with sperm count was also observed (Vitku
8420 et al., 2016 [RefID 7498]). Significant associations between serum or urinary BPA concentrations and
8421 reproductive hormones were also reported in several studies. Although, the associations were not
8422 entirely consistent in terms of directionality, findings from some individual studies could be interpreted
8423 as being adverse for male reproductive function.

8424 The results from these cross-sectional analyses are in stark contrast to the few prospective cohorts on
8425 male fertility described above where no association for semen quality, fecundability and live birth rate
8426 were observed. The cross-sectional inverse association observed for reduced semen quality is also
8427 difficult to interpret in terms of the temporal separation between the exposure and outcome. A single
8428 measure of urinary or blood BPA reflects only few hours of past exposures while the process of
8429 spermatogenesis takes around 3 months. With only a single measure of BPA cross-sectionally it seems
8430 biologically implausible that such short time exposure quantified at that point in time could affect sperm
8431 quality. An alternative explanation for these cross-sectional findings is that those with reduced fertility
8432 may be differently exposed to BPA through difference in lifestyle or behaviour. Alternatively, these
8433 associations may also reflect biological differences in rate of excretion of BPA. Studies relying on
8434 repeated measures of BPA would to some extent be able to address these limitations. Similarly, the
8435 cross-sectional association between urinary BPA concentration in 1–4-year-old boys and cryptorchidism
8436 (Komarowska et al., 2015 [RefID 3642]) is hard to explain biologically as this malformation has origin
8437 during pre-natal but not post-natal development.

8438 In summary, in the absence of support from other lines of evidence, including prospective studies or
8439 results from animal experiments, the associations reported in these studies are most likely to be
8440 coincidental rather than causal.

8441 **Female fertility**

8442 Few cross-sectional associations were reported on reproductive hormones in adult women. No
8443 consistent findings were reported in these studies. Seven studies examined cross-sectionally using
8444 case–control design association between BPA concentration and polycystic ovary syndrome (PCOS).
8445 Higher BPA concentrations were observed in four of these studies (Akin et al., 2015 [RefID 93]; Vahedi
8446 et al., 2016 [RefID 7370]; Rashidi et al., 2017 [RefID 9297]; Konieczna et al., 2018 [RefID 12363]),
8447 while no association was observed in three studies (Vagi et al., 2014 [RefID 7369]; Yang QY et al.,
8448 2015 [RefID 8385]; Gu et al., 2018 [RefID 12041]). Two studies reported higher BPA controls among
8449 women diagnosed with endometriosis compared with controls (Upson et al., 2014 [RefID 7361];
8450 Simonelli et al., 2017 [RefID 6742]). Similarly higher BPA concentrations were reported in women with
8451 diminished ovarian reserves (Cao et al., 2018 [RefID 11666]) and women diagnosed as infertile (La
8452 Rocca et al., 2014 [RefID 3792]) compared with controls. In addition, three cross-sectional studies
8453 found higher BPA concentration among women with uterine leiomyoma compared with controls (Jeong
8454 et al., 2013 [RefID 11125]; Shen et al., 2013 [RefID 6644]; Shen et al., 2016 [RefID 6640]). The
8455 directionality of these findings showing higher BPA concentrations among women diagnosed with PCOS,
8456 endometriosis, women with no or reduced fertility and uterine leiomyoma is difficult to evaluate. The
8457 measured BPA concentrations reflect exposure during the previous few hours and as such, in the
8458 absence of further evidence, are unlikely to reflect past exposure that may have led to development of
8459 the underlying disease condition. Differences in lifestyle factors that may affect exposure to BPA or rate
8460 of uptake or excretion among cases may equally explain the observed findings, particularly in the
8461 absence of similar findings from prospective studies.

8462 **Pubertal/endocrine**

8463 A study of 665 girls 9–18 years of age reported positive association between BPA concentrations in
8464 urine and age at menarche (Miao et al., 2017 [RefID 5074]), while another study recruiting 987

8465 adolescent girls from the US National Health and Nutrition Examination Survey (NHANES) found no
8466 such association (McGuinn et al., 2015 [RefID 4984]). One small (n = 88) study reported higher BPA
8467 concentration in girls with more advanced puberty (Supornsilchai et al., 2016 [RefID 7015]). Another
8468 study also found higher BPA concentrations in girls with isolated breast development before the age of
8469 8 compared with controls (Durmaz et al., 2018 [RefID 11867]). Limited conclusions can be drawn from
8470 these studies. Also, a few cross-sectional associations were reported on reproductive hormones in
8471 adolescent girls. No consistent findings were reported in these studies.

8472 3.1.6.2. Animal studies

8473 For the HOC Reproductive and developmental toxicity a total of 153 studies were appraised by the CEP
8474 Panel. The details of the appraisals (internal and external validity) are reported in Annex E.

8475 The endpoints for each study identified as relevant in this opinion are reported in Annex F.

8476 *Identification of clusters of relevant endpoints*

8477 Endpoints for which statistically significant changes were reported were extracted from the available
8478 literature in accordance with the protocol and grouped into three clusters:

- 8479 • Developmental toxicity
- 8480 • Female reproductive toxicity
- 8481 • Male reproductive toxicity.

8482 These clusters thus include endpoints identified as relevant in the 2015 opinion and considered in the
8483 uncertainty analysis (EFSA CEF Panel, 2015, Section 4.3.2), i.e. endometrial hyperplasia, ovarian cysts
8484 and anogenital distance (AGD), the first two of which were also identified as relevant in the newly
8485 compiled studies. Because it was previously identified as relevant, AGD was included and considered
8486 relevant in the new assessment, together with the others. For more details see Annex A, Section 2.5.

8487 MoAs of developmental toxicants are generally distinctly different from those of male and female
8488 reproductive toxicants. Developmental toxicants can have multiple pleiotropic effects.

8489 *WoE of the clusters of relevant endpoints*

8490 The main information extracted from the studies addressing relevant endpoints in the HOC
8491 Reproductive and developmental Toxicity are summarised in Annex G. The outcome of the weight of
8492 the evidence is described in the text below and presented in a tabulated format in Annex H.

8493 **Developmental toxicity**

8494 Within the cluster developmental toxicity, nine studies were on mice, of which seven studies had
8495 exposure during development until weaning, two had exposure during development until adulthood,
8496 one had exposure during the growth phase. Of the 13 studies on rats, 10 studies had exposure during
8497 the development until weaning, four had exposure during development until adulthood and one was
8498 exposed as adults. Some studies assessed multiple exposure periods.

8499 The specific endpoints that were included for effects of BPA on the developmental toxicity cluster were
8500 blastocyte outgrowth incidence, embryo development, age/day of first oestrus, AGD, mammary gland
8501 histology, mammary gland weight, bone development and body weight of F1/F2/F3 generation, as well
8502 as body weight of F0 dams.

8503 The assessment of body weight for each exposure period is described in detail in the Metabolic effects
8504 category (Chapter 3.1.4.2).

8505 Developmental exposure (pre-natal and/or post-natal until weaning)

8506 **AGD:** One Tier 2 study in rats (Spörndly-Nees et al., 2018 [RefID 13164]) was available. There was a
8507 not statistically significant increase of AGD at nominal 0.5 (achieved 0.4) µg/kg bw per day after 12
8508 months only but no changes on PND35, and at nominal 50 (achieved 40) µg/kg bw per day at both
8509 time points. The study assessed males only. As only one Tier 2 study showed an unexpected increase
8510 without a clear dose–response, the endpoint is rated as Not Likely.

8511 **Age at first oestrus:** One Tier 1 study in mice (Tucker et al., 2018 [RefID 13275]) was available. No
8512 effect was seen at 500, 5000 and 50000 µg/kg bw per day, twice daily, from GD10.5–17.5. A change
8513 in the age at first oestrus was judged Not Likely after developmental exposure.

8514 **Bone development:** Two Tier 1 studies in rats (Lejonklou et al., 2016 [RefID 3974]; Lind et al., 2017
8515 [RefID 4350]) and one Tier 1 study in mice were available (van Esterik, 2014 [RefID 7393]). In
8516 Lejonklou et al. (2016) [RefID 3974] in Wistar rats, femur cortical thickness was increased in males,
8517 while in females femur length was increased. In Lind et al. (2017) [RefID 4350] in F344 rats; femur
8518 length was decreased in males; in females no effects were observed. In van Esterik (2014) [RefID
8519 7393] no effect on femur length was observed in males or females in C57BL/6J mice. Femoral
8520 diaphyseal cortex thickness (F1 males, 25 µg/kg bw per day) and the femoral length (F1 females, 25
8521 and 5,000 µg/kg bw per day) were increased in Wistar rats (Lejonklou et al., 2016 [RefID 3974])
8522 whereas the femur length (F1 males) was decreased dose dependently, at 0.5 and 50 µg/kg bw per
8523 day in F344 rats (Lind et al., 2017 [RefID 4350]). No effect on femur length was described after
8524 exposure of mice to 3, 10, 30, 100, 300, 1000 or 3000 µg/kg bw per day) 2 weeks before mating until
8525 PND21 (van Esterik, 2014 [RefID 7393]). Both Tier 1 studies in rats show inconsistent effects according
8526 to doses, directions, sex, and strains and mice are without effect. The CEP Panel considered effects on
8527 bone development ALAN.

8528 **Mammary gland weight:** For effects on mammary gland weight in this exposure period only one Tier
8529 1 study in rats was identified (Montévil et al., 2020 [RefID 13788]).

8530 The mammary gland weight in female rats was determined at PND90 (Montévil et al., 2020 [RefID
8531 13788]). Based on the authors' description it appears that a data-driven approach was used for
8532 identifying a NMDR by defining a step function around the doses of 25 and 250 µg/kg bw per day.
8533 However, when using more conventional statistical methods, e.g. modelling the data in PROAST (Hill
8534 and Exponential models) or using spline and polynomial fit (without overfitting the data) no dose–
8535 response was identified by the CEP Panel. The CEP Panel considered that alternative interpretations of
8536 the data may be plausible, e.g. a significant difference between the dose group with 25 µg/kg bw per
8537 day and the controls – in the absence of dose–response – may be likely explained by random
8538 fluctuations and variability in the data. The CEP Panel assigned a likelihood level of Not Likely to the
8539 mammary gland weight effects of BPA in the developmental exposure period.

8540 **Mammary gland histology:**

8541 Males: Out of six rat and one mouse studies, three rat studies (all Tier 1) (Kass et al., 2015 [RefID
8542 3402]; Mandrup et al., 2016 [RefID 4831]; NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
8543 11370]) assessed the mammary gland histology of male pups. There were no neoplastic changes but
8544 changes in mammary gland growth observed in Kass et al. (2015) [RefID 3402] and Mandrup et al.
8545 (2016) [RefID 4831]. In Kass et al. (2015) [RefID 3402] male pups from dams exposed to BPA by s.c.
8546 injection of 25 and 250 µg/kg bw per day (converted to the equivalent oral doses 892.5 and 8925 µg/kg
8547 bw per day) from GD9 until GD23, showed an increased ductal growth at PND5 (transient effect) and
8548 a delay in ductal growth when assessed on PND30, both at the higher dose. A parallel experiment
8549 applying 64 µg/kg bw per day (oral dose from drinking water) (GD9–PND21) showed a reduced number
8550 of terminal end buds (TEBs) at PND15 and 30. The other oral study (Mandrups et al., 2016 [RefID 4831])
8551 showed a decrease in male and increase in female-like mammary gland structures in male rat pups
8552 assessed PND100 when doses of 5,000 and 50000 µg/kg bw per day were applied from GD7 until birth.
8553 Assessment of the same dose groups at PND22 did not show any changes. However, lower doses in
8554 this study (25 and 250 µg/kg bw per day), which were also assessed at PND22, showed an increase in
8555 mammary gland growth at 25 µg/kg bw per day. No effects were reported on mammary glands in
8556 males in the NTP Clarity Report (2018)/Camacho et al. (2019) [RefID 11370]. The CEP Panel judged
8557 the male mammary gland effects as ALAN.

8558 Females: Non-neoplastic findings on female rat mammary gland were observed in six Tier 1 (Kass et
8559 al., 2015 [RefID 3402]; Grassi et al., 2016 [RefID 2387]; NTP Clarity Report, 2018/Camacho et al.,
8560 2019 [RefID 11370]; Montévil et al., 2020 [RefID 13788]; Tucker et al., 2018 [RefID 13275]; Mandrup
8561 et al., 2016 [RefID 4831]) studies and one Tier 3 study (Leung et al., 2017 [RefID 3990]). Delayed
8562 ductal growth was observed at PND30 after s.c. application of 250 µg/kg bw per day (equal to 8925
8563 µg/kg bw per day, GD7–23, Kass et al., 2015 [RefID 3402]). In another study, increased branching
8564 score and number of terminal ducts (TDs) and TEB+TDs are induced by 25 µg/kg bw per day (but not

8565 at 250 µg/kg bw per day) on PND21 after exposure from GD10 until GD21 (Grassi et al., 2016 [RefID
8566 2387]). This finding is supported by a Tier 3 study (Leung et al., 2017 [RefID 3990]) showing also
8567 increased numbers of TEBs at 2.5 and 25 µg/kg bw per day. A comprehensive Tier 1 study (NTP Clarity
8568 Report, 2018/Camacho et al., 2019 [RefID 11370], doses applied: 2.5, 25, 250, 2500 and 25000 µg/kg
8569 bw per day from GD6 to PND0 and from PND1 to PND21 (stop-dose group of that study)) showed a
8570 decreased incidence of ductal dilatation in F1 females at 1 year (trend, significant at 250 and 25000
8571 µg/kg bw per day) and significant in all doses at 2 years. Furthermore, Montévil et al. (2020) [RefID
8572 13788] (Tier 1), who assessed samples from the CLARITY study (NTP Clarity Report, 2018/Camacho et
8573 al., 2019 [RefID 11370]), reported several effects at single doses in female F1 pups mammary glands:
8574 there were increases of gland density, Fractal dimension 3D, Dim 3 (third dimension from Principle
8575 Component Analysis) and angles of branches between beginning and end with a breaking point between
8576 25 and 250 µg/kg bw per day, of thickness of the epithelium and of variation of ductal thickness at 25
8577 µg/kg bw per day and an increased average branch length at 250 µg/kg bw per day. In addition, there
8578 were decreases of the gland depth, of the proportion of the small and very small branches, of the
8579 maximum branch length and topological asymmetry, of the lateral branching at 250 µg/kg bw per day
8580 and of the aspect ratio at 2.5 and 250 µg/kg bw per day. All effects with indications of a dose–response
8581 for which individual data were available (gland density, Dimension 3D and angles of branches between
8582 beginning and end, thickness of epithelium, variation of ductal thickness, aspect ratio) were statistically
8583 re-analysed by the CEP Panel. This re-analysis revealed that a formal dose–response could not be
8584 identified by fitting flexible biologically based functions or polynomials that are commonly used to
8585 describe biological systems except for ductal thickness and aspect ratio, for which a potential non-
8586 monotonic dose–response was identified statistically.

8587 Within another Tier 1 study (Tucker et al., 2018 [RefID 13275]) pups exposed twice daily to 500, 5000
8588 and 50000 µg/kg bw per day from GD10.5 to 17.5 showed increased branching density at low dose,
8589 whereas increased length of TEBs, mammary gland development score and number of TEBs are
8590 increased at mid dose. All effects occurred only at PND20 and not at later time points assessed (PND28,
8591 35, 56). These non-neoplastic findings are thus not consistent over studies with different study designs.
8592 Pre-neoplastic findings in adult females (PND400) were seen in one Tier 1 study, Mandrup et al. (2016)
8593 [RefID 4831], showing an increased intraductal hyperplasia at 250 µg/kg bw per day after treatment
8594 by gavage from GD7 until birth. One Tier1 study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
8595 11370]) revealed a neoplastic effect, an increase in adenoma and adenocarcinoma in the lowest dose
8596 group (2.5 µg/kg bw per day, exposure period: GD6–PND21, assessed at terminal sacrifice after 2
8597 years. Four other Tier 1 studies with shorter timepoints for measuring or higher doses showed no pre-
8598 neoplastic effects. One neoplastic lesion was reported, i.e. stromal polyps, in the NTP Clarity Report,
8599 2018/Camacho et al., 2019 [RefID 11370]. For this endpoint, a decrease (no adverse effect) was seen
8600 in this Tier 1 study at the highest dose after 2 years. The CEP Panel concludes, based on the inconsistent
8601 findings of non-neoplastic effects as well as the unconfirmed neoplastic or pre-neoplastic effects, that
8602 effects on the female mammary gland are ALAN.

8603 Overall, the CEP Panel assigned a likelihood level of ALAN to the developmental toxicity effects of BPA
8604 in the developmental exposure period based on bone development, mammary gland histology and body
8605 weight. Therefore, none of the endpoints are taken forward for BMD analysis. However, the ALAN
8606 endpoints were considered in the uncertainty analysis (see Appendix D).

8607 Developmental and adult (pre-natal and post-natal in pups until adulthood)

8608 **AGD:** As only one single-dose study (Tier 3, Patel et al., 2013 [RefID 5697]) assessed the AGD after
8609 exposure from GD11.5 until the assessment at 4 months of age, there is Inadequate evidence for this
8610 endpoint.

8611 **Embryo development:** The only study available is a Tier 3 study (Dobrzynska et al., 2018 [RefID
8612 11837]), thus there is Inadequate evidence for this endpoint.

8613 **Bone development:** As only one single-dose study (Tier 2, Auger et al., 2013 [RefID 301]) is available,
8614 there is Inadequate evidence for this endpoint.

8615 **Mammary gland histology:** Two Tier 1 studies (Montévil et al., 2020 [RefID 13788]; NTP Clarity
8616 Report 2018/Camacho et al., 2019 [RefID 11370]) assessed the mammary glands of female rats at
8617 doses of 2.5, 25, 250, 2500 and 25000 µg/kg bw per day by continuous dosing from GD6 to PND90 or

8618 6months. In Montévil et al. (2020) [RefID 13788] an additional pilot study dosing 2.5, 25, 260 and
8619 2700 µg/kg bw per day from GD6 to terminal measurement at PND90 is reported. Non-neoplastic effects
8620 were only observed at single doses without any dose–response, apart for mammary gland scores,
8621 where the data were in line with the definition by the CEP Panel of indications for a NMDR. For Montévil
8622 et al. (2020) [RefID 13788] the CEP Panel re-evaluated the dose–response for gland density by fitting
8623 flexible biologically based functions or polynomials that are commonly used to describe biological
8624 systems and concluded that there is no dose–response.

8625 Among the non-neoplastic effects identified in this exposure group several changes were only reported
8626 for one dose group in female rats, i.e. an increase in lobular alveolar budding at 250 µg/kg bw per day
8627 at PND90 (Montévil et al., 2020 [RefID 13788]), changes in ductal dilatation (increased at 1 year ;
8628 decreased at 2 years; adversity unclear) and a decrease in lobular hyperplasia, both at 25 µg/kg bw
8629 per day (NTP Clarity Report2018/Camacho et al.2019 [RefID 11370]). The BPA-induced decreases are
8630 different from results with E2 treatment which resulted in clear increases in duct dilatation and lobular
8631 hyperplasia as well in adenocarcinomas. An increase in alveolar dilatation was only reported in males
8632 at the lowest dose after 2 years (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) while
8633 the effect was not significant in females in both studies. Therefore, no dose–response could be
8634 established for non-neoplastic effects. Whereas for neoplastic effects, NTP Clarity Report
8635 (2018)/Camacho et al. (2019) [RefID 11370] showed an increase in atypical foci and adenocarcinomas
8636 at 2.5 µg/kg bw per day. In addition, there were non-significant increases in atypical foci in the 25 and
8637 250 µg/kg bw per day dose groups (9% and 8%, respectively, vs 0% in controls) at 1 year (NTP Clarity
8638 Report, 2018/Camacho et al., 2019 [RefID 11370]). One neoplastic lesion was also reported in NTP
8639 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370], i.e. stromal polyps. For this endpoint,
8640 however, a significant dose trend towards increased incidence at the higher doses at 1 year was
8641 observed, while a negative trend (no adverse effect) was observed at 2 years in this Tier 1 study. The
8642 CEP Panel therefore considered this result biologically implausible.

8643 Based on the above results, histological effects on mammary gland induced by BPA were judged as
8644 ALAN by the CEP Panel.

8645 Overall, the CEP Panel assigned a likelihood level of ALAN to the developmental toxicity effects of BPA
8646 in the developmental and adult exposure period based on effects on body weight and mammary gland
8647 histology. Therefore, none of the endpoints was taken forward for BMD analysis. However, the ALAN
8648 endpoints were considered in the uncertainty analysis (see Appendix D).

8649 Growth phase/young age

8650 **Age at first oestrus:** Based on one Tier 1 study (Li et al., 2016 [RefID 4128]) in mice, the likelihood
8651 of changes in age at first oestrus was rated ALAN by the CEP Panel. The mice showed a decreased age
8652 at first oestrus at 60 and an increased age at first oestrus at 600 µg/kg bw per day with a micropipette
8653 application three times per day after 5 weeks of exposure starting at PND22.

8654 The CEP Panel assigned a likelihood of ALAN to the cluster of developmental toxicity of BPA in the
8655 growth phase/young age exposure period. Therefore, none of the endpoints was taken forward for
8656 BMD analysis. However, both body weight and age at first oestrus were considered in the uncertainty
8657 analysis (see Appendix D).

8658 Adult exposure (after puberty)

8659 **Blastocyst outgrowth and F1 embryo development:** There is Inadequate Evidence for these
8660 endpoints as both are only assessed in one single dose Tier 1 Study (Martinez et al., 2015 [RefID
8661 4902]).

8662 The CEP Panel assigned a likelihood level of Not Likely to the developmental toxicity effects of BPA in
8663 the adult exposure period based on body weight. Therefore, none of the endpoints were taken forward
8664 for BMD analysis.

8665 Indirect (germline) exposure

8666 **Bone development:** As only one single-dose study (Tier 2, Auger et al., 2013 [RefID 301]) is available,
8667 there is Inadequate Evidence for this endpoint to conclude on the likelihood.

8668 The CEP Panel assigned a likelihood level of Not Likely to the developmental toxicity effects of BPA in
8669 the indirect germline exposure period based on body weight. Therefore, this endpoint was not taken
8670 forward for BMD analysis.

8671 Overall cluster selection for endpoints/studies for BMD for developmental toxicity

8672 Overall, the CEP Panel assigned a likelihood level of ALAN, to the developmental toxicity effects of BPA
8673 in the exposure periods developmental, developmental and adult and growth phase/young age, and of
8674 Not Likely in the adult and indirect (germline) exposure.

8675 The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster
8676 developmental toxicity was ALAN.

8677 The CEP Panel considered that the evidence from the studies available showed ALAN effects of BPA for
8678 the endpoints bone development, mammary gland histology, body weight (developmental exposure),
8679 body weight and mammary gland histology (developmental and adult exposure) as well as body weight
8680 and age at first oestrus (growth phase/young age). These endpoints were therefore not brought
8681 forward for BMD analysis.

8682 **Female reproductive toxicity**

8683 Within the cluster female reproductive toxicity, 17 studies were on mice, of which seven studies had
8684 exposure during development until weaning, one had exposure during development until adulthood,
8685 one had exposure during the growth phase, seven were exposed as adults and four had indirect
8686 germline exposure (some studies tested multiple exposure periods). Of the 16 studies on rats, 11
8687 studies had exposure during development until weaning, three had exposure during development until
8688 adulthood, four were exposed as adults. There was one study in hamsters which had exposure during
8689 development until weaning. In addition, three studies were on sheep, two had exposure during the
8690 development until weaning and one as adults.

8691 The specific endpoints that were included for effects of BPA on female reproductive toxicity cluster were
8692 plasma/serum thyroid hormones, testosterone, oestrus cyclicity, age at first oestrus, fertilisation rate
8693 and implantation incidences, ovary weight and histology, uterus weight and histology.

8694 Developmental exposure (pre-natal and/or post-natal until weaning)

8695 **Plasma/serum thyroid hormones:** For this exposure period the following studies were identified.
8696 **T₃:** one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]), **T₄:** two Tier
8697 1 rat studies (Bansal and Zoeller, 2019 [RefID 13783]; NTP Clarity Report, 2018/Camacho et al., 2019
8698 [RefID 11370]), one Tier 1 sheep study (Guignard et al., 2017 [RefID 2451]) and one Tier 3 mouse
8699 study (Bodin et al., 2014 [RefID 623]), and **T₃/TT₄:** one Tier 1 sheep study (Guignard et al., 2017
8700 [RefID 2451]).

8701 No effect was seen on **T₃** in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
8702 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to
8703 PND0 and F1 pups from PND1 to PND21 at interim sacrifice (1 year).

8704 No effect was seen on **T₄** in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
8705 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to
8706 PND0 and F1 pups from PND1 to PND21. On PND15, no effect was observed in the Tier 1 rat study
8707 (Bansal and Zoeller, 2019 [RefID 13783]) in which females were dosed from GD6–PND15 with 2.5, 25,
8708 250, 2500 or 25000 µg/kg bw per day. No change in T₄ was observed in fetuses (GD28–132/134) in
8709 the sheep Tier 1 study (Guignard et al., 2017 [RefID 2451]) after s.c. dosing with 5, 50 or 5000 µg/kg
8710 bw per day (using respectively 125 and 37 as dose conversion factors to oral dose in sheep, the
8711 equivalent oral doses are 625, 6250 or 625000 µg/kg bw per day and 185, 1850 or 185000 µg/kg bw
8712 per day).

8713 No change was observed on the serum ratio **T₃/TT₄** in the sheep Tier 1 study (Guignard et al., 2017
8714 [RefID 2451]).

8715 During this exposure period the likelihood of changes in thyroid hormones (T₃, T₄, rT₃/TT₄) were judged
8716 as Not Likely by the CEP Panel as no effects were observed in Tier 1 and Tier 2 studies in rats and in a
8717 Tier 1 study in sheep.

8718 **Plasma/serum testosterone:** For this exposure period, only three Tier 3 studies in rats (Castro et
8719 al., 2018 [RefID 11674]; Leung et al., 2017 [RefID 3990]; Johnson et al., 2016 [RefID 3241]) and two
8720 Tier 3 studies (Mahalingam et al., 2017 [RefID 4779]; Tucker et al., 2018 [RefID 13275]) in mice were
8721 identified for this endpoint. The CEP Panel noted that as only Tier 3 studies were available, the likelihood
8722 for this endpoint could not be determined because of Inadequate evidence.

8723 **Oestrus cyclicity:** For this exposure period, four Tier 1 studies (Hass et al., 2016 [RefID 2610],
8724 Ferguson SA et al., 2014 [RefID 1998]; NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370];
8725 Franssen et al., 2016 [RefID 2073]) and one Tier 2 study (Santamaria et al., 2016 [RefID 6448]) in
8726 rats, one Tier 1 study (Tucker et al., 2018 [RefID 13275]) and one Tier 2 study (Acevedo et al., 2018
8727 [RefID 11434]) in mice and one Tier 2 study (Veiga-Lopez et al., 2014 [RefID 7421]) in sheep were
8728 identified. Furthermore, a Tier 3 rat study (Leung et al., 2017 [RefID 3990]) and a Tier 3 mouse study
8729 (Wang W et al., 2014 [RefID 7759]) were identified.

8730 In the Tier 1 rat study (Hass et al., 2016 [RefID 2610]), no change in oestrus cyclicity was observed
8731 when measured in F1 females at 12 months; female rats were treated at oral doses of 25, 250 µg, 5000
8732 µg or 50000 µg/kg bw per day GD7–PND22. In the Tier 1 rat study (Ferguson SA et al., 2014 [RefID
8733 1998]) no change in oestrus cyclicity was observed (PND82–109); dams were treated daily by gavage
8734 from GD6–21 and pups PND1–21 at doses of 2.5 or 25 µg/kg bw per day. In the Tier 1 rat study (NTP
8735 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) no change in oestrus cycle was found after
8736 1 year; dams were treated daily at doses of 2.5, 25, 250, 2500 or 25000 µg/kg bw per day from GD6
8737 to PND0 and F1 pups from PND1 to PND21. In the Tier 1 rat study (Franssen et al., 2016 [RefID 2073])
8738 no change in oestrus cycle was identified when female pups received a subcutaneous injection of 0.025
8739 µg, 25 µg or 5,000 µg/kg per day from PND1–5 or PND1–15 (equivalent to oral doses of 0.8925, 892.5
8740 or 178500 µg/kg bw per day); measured daily from PND25 to PND80.

8741 In a Tier 1 mouse study (Tucker et al., 2018 [RefID 13275]) no change in oestrus cyclicity (PND63–84)
8742 was observed in F1 females after exposure by gavage at doses of 500, 5000 or 50000 µg/kg bw per
8743 day, twice daily from GD10.5–GD17.5 of F0 dams. In a Tier 2 mouse study (Acevedo et al., 2018 [RefID
8744 11434]) only a decrease in oestrus cyclicity by 6 months at the lowest dose (0.025 µg/kg bw per day)
8745 in F1 females was seen. In this study F0 dams were exposed subcutaneously from GD8 to PND16
8746 through osmotic mini pumps to 0.025 µg, 0.250 µg or 25 µg/kg bw per day (equivalent to oral doses
8747 5.5, 55, 5550 µg/kg bw per day) and oestrus cycle was examined at 5.5 and 8.5 months of age (F1)
8748 for 2 weeks.

8749 In the Tier 2 study in sheep (Veiga-Lopez et al., 2014 [RefID 7421]) a change in oestrus cyclicity
8750 (decrease in follicular count trajectories) in all dose groups was observed. Pregnant sheep were exposed
8751 from GD30–GD90 by subcutaneous injections 50, 500 or 5000 µg/kg bw per day (using respectively
8752 125 and 37 as dose conversion factors to oral dose in sheep, the equivalent oral doses are 6250, 62500
8753 or 625000 µg/kg bw per day and 1850, 18500 or 185000 µg/kg bw per day). Time of examination for
8754 oestrus cyclicity was not described but considered at least after 8 weeks (weaning) and when the F1
8755 females weighed more than 40 kg.

8756 Apart from the decreased oestrus cyclicity in only the lowest dose and one timepoint in the Tier 2
8757 mouse study (Acevedo et al., 2018 [RefID 11434]) and a decrease in oestrus cyclicity (decrease in
8758 follicular count trajectories) in the sheep study (Veiga-Lopez et al., 2014 [RefID 7421]), no change in
8759 oestrus cycle was observed in the other five Tier 1 studies in rats and mice. Therefore, the CEP Panel
8760 considered that the likelihood of a change in oestrus cyclicity is Not Likely.

8761 **Ovary weight:** For this exposure period one Tier 1 study (NTP Clarity Report, 2018/Camacho et al.,
8762 2019 [RefID 11370]) and one Tier 2 study (Santamaria et al., 2016 [RefID 6448]) in rats and one Tier
8763 3 mouse study (Patel et al., 2013 [RefID 5697]) were identified.

8764 In the Tier 1 study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]), rats were dosed
8765 with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups from
8766 PND1 to PND21. A decrease was observed in the high-dose group with a trend in the other dose groups.
8767 Ovary weight was decreased in the F1 females at 7 weeks of age. In a Tier 2 rat study (Santamaria et
8768 al., 2016 [RefID 6448]) an approximately equal decrease was observed on ovary weight in F1 animals
8769 of both BPA-treated dose groups at PND90; F0 dams and F1 female offspring were given 0.5 µg and
8770 50 µg/kg bw per day (via drinking water) from GD9–PND21.

8771 The CEP Panel considered the likelihood of the decrease of ovary weight as Likely as there was a trend
8772 in the Tier 1 study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) supported by one
8773 Tier 2 study with effects at lower doses without dose–response (Santamaria et al., 2016 [RefID 6448]).

8774 **Ovary histology (follicle count, cellular hypertrophy, follicular cysts):** For this exposure period
8775 one Tier 1 study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]), one Tier 2 study
8776 (Santamaria et al., 2016 [RefID 6448]) and one Tier 3 study (Patel et al., 2017 [RefID 5708]) in rats
8777 and three Tier 3 studies in mice (Mahalingam et al., 2017 [RefID 4779]; Wang W et al., 2014 [RefID
8778 7759]; Berger et al., 2016 [RefID 524]) were identified.

8779 In the Tier 1 study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]), rats were dosed
8780 with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups from
8781 PND1 to PND21. No change was observed in cell hypertrophy. The incidence in follicular cysts was
8782 increased in the highest dose group and in all dose groups there was an increased trend in incidence
8783 of follicular cysts.

8784 In the Tier 2 (Santamaria et al., 2016 [RefID 6448]) rats a decrease in the number of growing follicles
8785 was observed in the BPA-treated groups on PND90; the decrease was comparable in both groups (oral
8786 (via drinking water) 0.5 µg and 50 µg BPA/kg bw per day; GD9–PND21).

8787 The CEP Panel considered for the exposure period developmental exposure (pre-natal and/or post-natal
8788 until weaning) the likelihood of histological changes in the ovary as Likely.

8789 **Uterus weight:** For this exposure period one Tier 1 study (NTP Clarity Report, 2018/Camacho et al.,
8790 2019 [RefID 11370]) in rats, one Tier 1 study in hamsters (Radko et al., 2015 [RefID 6046]) and one
8791 Tier 3 study in mice (Patel et al., 2013 [RefID 5697]) were identified.

8792 In the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]), animals were
8793 dosed with 2.5, 25, 250, 2500 and 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups
8794 from PND1 to PND21. No change in uterus weight was observed. In the Tier 1 hamster study uterus
8795 weight (wet and dry) on PND21 was statistically significantly increased at 160000 µg/kg bw per day;
8796 female hamster pups were dosed with three daily oral doses (PND18–PND20) of 8000; 40000 and
8797 160000 µg/kg bw per day.

8798 The effect on uterus weight was considered as ALAN as no effect was observed in the Tier 1 study in
8799 rats and an increase in weaning hamsters (uterotropic assay) was only seen at the highest dose (160000
8800 µg/kg bw per day).

8801 **Uterus histology** (cystic endometrial hyperplasia, uterine dilation, squamous metaplasia, apoptosis in
8802 the luminal epithelial cells in the endometrium, endometrial hyperplasia, luminal epithelial anomalies
8803 and glands with cellular anomalies): Two Tier 1 studies in rats (NTP Clarity Report, 2018/Camacho et
8804 al., 2019 [RefID 11370]; Vigezzi et al., 2015 [RefID 7472]) were identified for this exposure period.

8805 In the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]), animals were
8806 dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups
8807 from PND1 to PND21. In this study a statistically significant increase was observed in cystic endometrial
8808 hyperplasia at the interim sacrifice (1 year) in the highest dose group and at terminal sacrifice (2 years)
8809 in the two highest dose groups (2500 or 25000 µg/kg bw per day group). At interim sacrifice uterine
8810 dilation and squamous metaplasia were increased in the 250 µg/kg bw per day and in the 25,000 µg/kg
8811 bw per day dose groups, respectively. There was a non-significant increase observed in the incidence
8812 of apoptosis in the luminal epithelial cells in the endometrium in the high-dose group. No change was
8813 observed in endometrial hyperplasia in any of the dose groups in this study.

8814 In the other Tier 1 rat study (Vigezzi et al., 2015 [RefID 7472]) dams were administered with 0.5 or 50
8815 µg/kg bw per day in drinking water from GD9–PND21; F1 females were necropsied on PND90 and 360.
8816 No changes in squamous metaplasia and luminal epithelial anomalies were observed. At necropsy on
8817 PND360 an increase in glands with cellular anomalies was observed in the 50 µg/kg bw per day; on
8818 PND90 no effects were seen in the 50 µg/kg bw per day group or at both times of necropsy in the 0.5
8819 µg/kg bw per day.

8820 The CEP Panel considered the likelihood during this exposure period to be Likely for the endpoint uterus
8821 histology based on effects seen at histological examination of the uterus in two rat Tier 1 studies (NTP
8822 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]; Vigezzi et al., 2015 [RefID 7472]).

8823 During developmental exposure (pre-natal and/or post-natal until weaning), the CEP Panel assigned a
8824 likelihood level of Likely to the cluster female reproductive toxicity of BPA. Since the likelihood level is
8825 Likely for the endpoint ovary weight in Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019
8826 [RefID 11370]), for the endpoint ovary histology (follicle count and follicle cysts) in one rat study (NTP
8827 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370] (Tier1)) and the endpoint uterus histology in
8828 two Tier 1 rat studies (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]; Vigezzi et al.,
8829 2015 [RefID 7472]), these were taken forward for BMD analysis (see Chapter 3.2.1) and uncertainty
8830 analysis (see Appendix D).

8831 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

8832 **Plasma/serum thyroid hormones:** For this exposure period one Tier 1 rat study (NTP Clarity Report,
8833 2018/Camacho et al., 2019 [RefID 11370]) was identified. No effect was seen on **T₃ or T₄** in this study.
8834 Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and
8835 F1 pups from PND1 to 1 year (continuous-dose group).

8836 Based on this Tier 1 rat study, the CEP Panel considered an effect on thyroid hormones (T₃, T₄) as Not
8837 Likely.

8838 **Oestrus cyclicity:** For this exposure period one Tier 1 rat study (NTP Clarity Report, 2018/Camacho
8839 et al., 2019 [RefID 11370]) was identified. No effect was seen on oestrus cyclicity in this study (NTP
8840 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or
8841 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups from PND1 to interim (1 year) and
8842 terminal sacrifice (2 years) (continuous-dose group).

8843 Based on this Tier 1 rat study, the CEP Panel judged an effect on oestrus cyclicity as Not Likely.

8844 **Ovary weight:** for this exposure period one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et
8845 al., 2019 [RefID 11370]) and one Tier 3 mouse study (Patel et al., 2013 [RefID 5697]) were identified.
8846 No effect was seen on ovary weight in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al.,
8847 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams
8848 from GD6 to PND0 and F1 pups from PND1 to interim sacrifice (1 year) (continuous-dose group).

8849 Based on this Tier 1 rat study, the CEP Panel judged an effect on ovary weight as Not Likely.

8850 **Ovary histology** (interstitial cell hypertrophy and follicle cysts): For this exposure period one Tier 1
8851 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) was identified. In this Tier 1
8852 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) a statistically significant
8853 increase was observed in interstitial cell hypertrophy in the 2,500 and 25,000 µg/kg bw per day groups
8854 at interim sacrifice. Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from
8855 GD6 to PND0 and F1 pups from PND1 to 1 or 2 years (continuous-dose group). In the same study no
8856 change in follicular cysts was observed. The CEP Panel judged the effect on interstitial cell hypertrophy
8857 at a dose level of 2500 and 25000 µg/kg bw per day as Likely.

8858 **Uterus weight:** For this exposure period two Tier 1 rat studies (NTP Clarity Report, 2018/Camacho et
8859 al., 2019 [RefID 11370]; Leung et al., 2020 [RefID 13789]) and one Tier 3 mouse study (Patel et al.,
8860 2013 [RefID 5697]) were identified. No effect was seen on uterus weight in the Tier 1 rat study (NTP
8861 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or
8862 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups from PND1 to interim sacrifice (1
8863 year) (continuous-dose group). In addition, no effects were observed on uterus weight in the other
8864 Tier 1 study (Leung et al., 2020 [RefID 13789]); dams were exposed from GD6 to PND0 and pups from
8865 PND1 to 1 year (continuous-dose group) to 2.5, 25, 250, 2500 or 25000 µg/kg bw per day;
8866 measurements were performed at PND90, 6 months and 1 year.

8867 Based on these two Tier 1 rat studies, the CEP Panel judged an effect on uterus weight as Not Likely.

8868 **Uterus histology:** (squamous metaplasia, apoptosis, uterine dilation, endometrial hyperplasia,
8869 squamous metaplasia): For this exposure period two Tier 1 rat studies (Leung et al., 2020 [RefID
8870 13789]; NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) were identified. In the Tier 1

8871 rat study (Leung et al., 2020 [RefID 13789]) no effects were observed on squamous metaplasia and
8872 apoptosis; dams were exposed from GD6 to PND0 and pups from PND1 to 1 year (continuous-dose
8873 group) to 2.5, 25, 250, 2500 and 25000 µg/kg bw per day; measurements were performed at PND90,
8874 6 months and 1 year. In the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
8875 11370]) animals were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6
8876 to PND0 and F1 pups from PND1 to 1 or 2 years (continuous-dose group). In this Tier 1 study the
8877 following effects (statistically significant) were observed at sacrifice at interim sacrifice (1 year):
8878 increased uterine dilation (250 µg/kg bw per day), increased endometrial hyperplasia (2.5 or 250 µg/kg
8879 bw per day), apoptosis and squamous metaplasia (25,000 µg/kg bw per day) and a decreased cystic
8880 endometrial hyperplasia (2.5 µg/kg bw per day). No other statistically significant effects were observed
8881 at interim or terminal sacrifice in this study.

8882 During the exposure period developmental until adult, no effect was seen in squamous metaplasia and
8883 apoptosis in one Tier 1 rat study (Leung et al., 2020 [RefID 13789]). In another Tier 1 rat study (NTP
8884 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]), in which the exposure time and dose range
8885 (2.5, 25, 250, 2500 and 25000 µg/kg bw per day) was the same, only an effect was observed at the
8886 highest dose. The other histological effects were only seen at low concentrations (uterine dilation at
8887 250 µg/kg bw per day and cystic endometrial hyperplasia (2.5 µg/kg bw per day)). Therefore, the CEP
8888 Panel considered the likelihood for this endpoint to be ALAN.

8889 **Number of implantation sites:** Within a Tier 1 rat study (Boudalia et al., 2014 [RefID 670]) dams
8890 were exposed from GD1 to LD21 by micropipette with 5 µg/kg bw. The examination of the dams at
8891 LD/PND21 revealed no change in the number of implantation sites. As this study is a single-dose study
8892 the CEP Panel considered the available study data of Inadequate evidence for any further conclusions:

8893 During developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood), the
8894 CEP Panel assigned a likelihood level of Likely to the cluster female reproductive toxicity of BPA. As the
8895 likelihood level is Likely for the endpoint ovary histology (interstitial cell hypertrophy in the Tier 1 study
8896 (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370])); this study was taken forward for BMD
8897 analysis (see Chapter 3.2.1). Moreover, the Likely and ALAN endpoints were considered for uncertainty
8898 analysis (see Appendix D)

8899 Growth phase/young age exposure

8900 **Oestrus cyclicity:** For this exposure period one Tier 1 mouse study (Li et al., 2016 [RefID 4128]), in
8901 which the oestrus cyclicity was studied, was identified. Mice were dosed from PND22 for 5 weeks (3
8902 times per day) orally (via micropipette) with 0, 60 and 600 µg/kg bw per day of BPA. In the high-dose
8903 group, the oestrous cyclicity was decreased (the females spent less time in pro-estrus and oestrus and
8904 more time in met and diestrus). The CEP Panel judged this endpoint as ALAN.

8905 **Implantation rate:** The implantation incidence was decreased in a dose-dependent manner in a Tier
8906 1 mouse study (Li et al., 2016 [RefID 4128]), in which mice were fed split doses of BPA from PND22
8907 for 5 weeks (0, 60 and 600 µg/kg bw per day). The CEP Panel judged this endpoint as Likely.

8908 During the growth phase/young age, the CEP Panel assigned a likelihood level of Likely to the cluster
8909 of female reproductive toxicity based on one Tier 1 mouse study (Li et al., 2016 [RefID 4128]) in which
8910 the implantation incidence was decreased in a dose-dependent manner. The study (Li et al., 2016
8911 [RefID 4128]) was taken forward for BMD analysis (see Chapter 3.2.1). Moreover, the Likely and ALAN
8912 endpoints were considered for uncertainty analysis (see Appendix D)

8913 Adult exposure (after puberty)

8914 **Plasma/serum thyroid hormones:** For this exposure period one Tier 1 sheep study (Guignard et
8915 al., 2017 [RefID 2451]) and one Tier 2 rat study (Zhang J et al., 2017 [RefID 8770]) were identified.

8916 The female sheep in the Tier 1 study (Guignard et al., 2017 [RefID 2451]) were injected s.c. with 5, 50
8917 or 5000 µg/kg bw per day (equivalent to oral doses of 185/625; 1850/6250; 185000/625000 µg/kg bw
8918 per day depending on the conversion factor of 37 or 125). In this study no effect was observed on **TT₄**.
8919 A decrease was observed in **TT₃** at 50 µg/kg bw per day and in **FT₄** at 50 or 5000 µg/kg bw per day.
8920 Furthermore, an increase in **reverse T₃/T₄** was observed at a s.c. dose of at 50 µg/kg bw per day
8921 (equivalent to oral dose of 1850/6250 µg/kg bw per day).

8922 In the Tier 2 rat study (Zhang J et al., 2017 [RefID 8770]) the female rats were dosed with 250 and
8923 1000 µg/kg bw per day from 6 weeks of age for 64 weeks. In this study a statistically significant increase
8924 was seen on **FT₄** at 1000 µg/kg bw per day and no changes in **FT₃**.

8925 The effects on **T₃** were judged as Not Likely as at approximately the same dose no effects were
8926 measured in rats (Zhang J et al., 2017 [RefID 8770]), but an effect without dose relationship was seen
8927 in sheep (Guignard et al., 2017 [RefID 2451]); the effect in sheep is considered to be a variation.

8928 The effects on **T₄** were judged as Not Likely as no dose–response was seen in sheep (Guignard et al.,
8929 2017 [RefID 2451]) for **FT₄** and an effect in opposite direction (increase) in rats (Zhang J et al., 2017
8930 [RefID 8770]) at a similar dose was observed.

8931 In the sheep study (Guignard et al., 2017 [RefID 2451]), the increase on reverse **T₃/T₄** was only seen
8932 at 50 µg/kg bw per day, the mid dose. Therefore, this effect was judged as Not Likely.

8933 During this exposure period the likelihood of changes in **thyroid hormones (T₃, T₄, FT₄ rT₃/TT₄)**
8934 was considered as Not Likely by the CEP Panel as no consistent effects were observed in a Tier 2 study
8935 in rats and in a Tier 1 study in sheep.

8936 **Plasma/serum testosterone:** For this exposure period one Tier 3 rat study (Rashid et al., 2018
8937 [RefID 12963]) and two Tier 3 mouse studies (Hu et al., 2018 [RefID 11119]; Xu XH et al., 2015 [RefID
8938 8232]) were identified. Therefore, data were Inadequate to judge the likelihood of an effect on
8939 testosterone levels.

8940 **Fertilisation rate:** one Tier 1 mouse study (Moore-Ambriz et al., 2015 [RefID 5196]), in which
8941 fertilisation rate was determined, was identified for this exposure period. In adult female mice exposed
8942 from the day of the first oestrus until the completion of three oestrous cycles orally (via pipette) at a
8943 dose of 50 µg/kg bw per day a decreased fertilisation rate was observed. As only one single-dose Tier
8944 1 mouse study was available data were Inadequate to judge the likelihood of an effect on fertilisation
8945 rate.

8946 **Implantation rate:** As only one single-dose Tier 1 study (Boudalia et al., 2014 [RefID 670]) and two
8947 Tier 3 studies (Yuan et al., 2018 [RefID 13593]; Dobrzynska et al., 2018 [RefID 11837]) are available,
8948 there is Inadequate evidence to conclude on a likelihood of an effect.

8949 **Oestrus cyclicity:** For this exposure period one Tier 1 study (Moore-Ambriz et al., 2015 [RefID 5196])
8950 and one Tier 3 study (Cao et al., 2018 [RefID 11666]) in mice and one Tier 2 rat study (Zaid et al.,
8951 2014 [RefID 10261]) were identified.

8952 No effect on oestrous cyclicity was observed in adult female mice (Tier 1 study, Moore-Ambriz et al.,
8953 2015 [RefID 5196]) exposed orally (via pipette) from the day of the first oestrus until the completion
8954 of three oestrous cycles at a dose of 50 µg/kg bw per day. In the Tier 2 rat study (Zaid et al., 2014
8955 [RefID 10261]) the number of females which were in persistent diestrus was increased after daily
8956 dosing of 10000 µg/kg bw per day (single-dose level) from PND28 for 6 weeks.

8957 The effect on oestrous cyclicity was judged ALAN. No effect was seen in the lower dose (50 µg/kg bw
8958 per day) Tier 1 mouse study, but an effect at higher dose level, 10000 µg/kg bw per day was observed
8959 in the Tier 2 rat study; both studies were single-dose studies.

8960 **Ovary weight:** One Tier 2 rat study (Zaid et al., 2014 [RefID 10261]) was identified for this exposure
8961 period. No change in absolute ovary weight was observed after daily dosing female rats from PND28
8962 for 6 weeks with 10000 µg/kg bw per day (only dose tested). Therefore, data were Inadequate to judge
8963 the likelihood of an effect on ovary weight.

8964 **Ovary histology:** (follicles count, premature activation of primordial follicles, large antral-like and
8965 atretic cystic-like follicles): For this exposure period one Tier 1 study (Moore-Ambriz et al., 2015 [RefID
8966 5196]) and one Tier 2 study (Hu et al., 2018 [RefID 11119]) in mice and one Tier 2 rat study (Zaid et
8967 al., 2014 [RefID 10261]) were identified.

8968 No effect on follicle count was observed in adult female mice (Tier 1 study Moore-Ambriz et al., 2015
8969 [RefID 5196]) exposed orally (via pipette) from the day of the first oestrus until the completion of three
8970 oestrous cycles at a dose of 50 µg/kg bw per day. In the Tier 2 mouse study (Hu et al., 2018 [RefID
8971 11119]) in all dose groups, a dose-related decrease was observed in the number of primordial follicles

8972 and the premature activation of primordial follicles; in this study adult female mice were dosed orally
8973 for 28 days with 1 µg, 10 µg, 100 µg, 1000 µg and 10000 µg/kg bw per day. In the Tier 2 rat study
8974 (Zaid et al., 2014 [RefID 10261]) female rats were dosed orally from PND28 for 6 weeks with 10000
8975 µg/kg bw per day (only dose tested). In this study, the numbers of atretic follicles, atretic cystic-like
8976 and large antral-like follicles were increased in the BPA-treated group when compared with the controls.

8977 The effects on ovary histology were judged as Likely. As the Tier 2 rat study (Zaid et al., 2014 [RefID
8978 10261]) is a single-dose study, only the Tier 2 mouse study (Hu et al., 2018 [RefID 11119]) was taken
8979 forward for BMD analysis (see Chapter 3.2.1).

8980 **Uterus histology:** (gland nests density, gland nests): For this exposure period a Tier 1 study in CD1
8981 mice (Kendzioriski and Belcher, 2015 [RefID 3453]) and a Tier 3 study in C57Bl/6J mice (Kendzioriski
8982 and Belcher, 2015 [RefID 3453]) were identified. In the Tier 1 study, CD-1 mice were exposed for 12–
8983 15 weeks via the diet to doses equivalent to 4, 40, 400, 4000 and 40000 µg/kg bw per day. Gland nests
8984 density and the number of gland nests was increased in the high-dose group. The design of the Tier 3
8985 study in C57Bl/6J mice was identical. In this study no effect on the number of gland nests was observed
8986 and the gland nests density was increased in the 4, 4000 and 40000 µg/kg bw per day group.

8987 The likelihood of the effects on uterus histology was considered as ALAN as gland nest number and
8988 density showed inconsistent effects; in the Tier 1 study in CD-1 mice an increase in gland nest number
8989 and density was observed only at high dose, in the Tier 3 study with a low number of C57Bl/6J mice,
8990 varying effect were seen.

8991 The CEP Panel assigned a likelihood level of Likely to the female reproductive toxicity cluster in the
8992 exposure period adulthood. The likelihood for the endpoint ovary histology is Likely. In a Tier 2 mouse
8993 study (Hu et al., 2018 [RefID 11119]), a dose-related decrease in the number of primordial follicles and
8994 the premature activation of primordial follicles was observed. This study is taken forward for BMD
8995 analysis (see Chapter 3.2.1). In addition, an increase in follicle abnormalities was reported in a single-
8996 dose rat Tier 2 study (Zaid et al., 2014 [RefID 10261]). As this study tested only one dose, this study
8997 will not be taken forward for BMD analysis. The Likely and ALAN endpoints were also considered for
8998 uncertainty analysis (see Appendix D).

8999 Indirect (germline) exposure

9000 For this exposure period five studies were assessed: three Tier 3 mouse studies (Ziv-Gal et al., 2015
9001 [RefID 9143]; Berger et al., 2016 [RefID 524]; Mahalingam et al., 2017 [RefID 4779]), in which the F2
9002 and F3 generation were studied and two Tier 3 mouse studies (Dobrzynska et al., 2015 [RefID 1644];
9003 Mahalingam et al., 2017 [RefID 4779]) in which the F2 generation were studied. In the study by Ziv-
9004 Gal et al. (2015) [RefID 9143] the age at first oestrus, in the study by Dobrzynska et al. (2015) [RefID
9005 1644] the embryo implantation incidence and in the study by Berger et al. (2016) [RefID 524] and
9006 Mahalingam et al. (2017) [RefID 4779] the follicle count (ovary histology) were reported.

9007 The CEP Panel noted that since for indirect (germline) exposure only Tier 3 studies were available, the
9008 likelihood for this endpoint could not be determined because of Inadequate evidence.

9009 Overall cluster selection for endpoints/studies for BMD for female reproductive toxicity

9010 Overall, the CEP Panel assigned a likelihood level of Likely, to the female reproductive toxicity cluster
9011 in the exposure periods developmental (pre-natal and/or post-natal until weaning), developmental and
9012 adult (pre-natal and/or post-natal until adulthood) and growth phase/young age, and of Inadequate
9013 Evidence in the adult and indirect (germline) exposure periods.

9014 The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster female
9015 reproductive toxicity was Likely.

9016 The CEP Panel considered that the evidence from the studies available showed a Likely effect for ovary
9017 weight (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]), for uterus histology (NTP Clarity
9018 Report, 2018/Camacho et al., 2019 [RefID 11370] and Vigezzi et al., 2015 [RefID 7472]) and ovary
9019 histology (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) during the developmental
9020 exposure period, for ovary histology (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370])
9021 during developmental and adult exposure and for ovary histology (Hu et al., 2018 [RefID 11119]) during

9022 adult exposure and for decreased implantation incidence during the growth phase (Li et al., 2016 [RefID
9023 4128]). Therefore, these endpoints were taken forward for BMD analysis (see Chapter 3.2.1).

9024

9025 **Male reproductive toxicity**

9026 Within the cluster male reproductive toxicity, 15 studies were on mice, of which six studies had exposure
9027 during the development until weaning, two had exposure during development until adulthood, seven
9028 were exposed as adults and two had germline exposure (some studies tested multiple exposure
9029 periods). Of the 26 studies on rats, 14 studies had exposure during development until weaning, four
9030 had exposure during development until adulthood, five had exposure during the growth phase, five
9031 were exposed as adults. In addition, one study was on sheep, which had exposure during the
9032 development until weaning and one study in monkeys during adult exposure period.

9033 The specific endpoints that were included for effects of BPA on the male reproductive toxicity cluster
9034 were plasma/serum thyroid hormones, testosterone, epididymis weight and histology, prostate
9035 histology, seminal vesicle weight, sperm count/morphology/motility/viability, testis weight and
9036 histology.

9037 Developmental exposure (pre-natal and/or post-natal until weaning)

9038 **Plasma/serum thyroid hormones:** For this exposure period the following studies were identified.
9039 **T3:** one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]), **T4:** two Tier
9040 1 rat studies (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]; Bansal and Zoeller, 2019
9041 [RefID 13783]) and one Tier 1 sheep study (Guignard et al., 2017 [RefID 2451]) and **T3/TT4:** one Tier
9042 1 sheep study (Guignard et al., 2017 [RefID 2451]).

9043 No effect was seen on **T3** in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
9044 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to
9045 PND0 and F1 pups from PND1 to PND21 at interim sacrifice (1 year).

9046 Apart from a decrease at the highest dose level (25000 µg/kg bw per day), no effect was seen on **T4**
9047 in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). In this study,
9048 rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and
9049 F1 pups from PND1 to PND21 at interim sacrifice. On PND15, no effect was observed in **T4** in another
9050 Tier 1 rat study (Bansal and Zoeller, 2019 [RefID 13783]) in which females were dosed from GD6–
9051 PND15 with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day.

9052 No change in **T4** was observed in fetus (GD113–135) in the sheep Tier 1 study (Guignard et al., 2017
9053 [RefID 2451]) after s.c. dosing with 5, 50 or 5000 µg/kg bw per day (equivalent to oral doses of 185–
9054 625; 1850–6250; 185000–625000 µg/kg bw per day). In addition, no change was seen in this study in
9055 the ratio **T3/T4**.

9056 During this developmental exposure (pre-natal and/or post-natal until weaning) period changes in
9057 thyroid hormones (**T3**, **T4**, **rT3/TT4**) were judged as Not Likely by the CEP Panel.

9058 **Plasma/serum testosterone:** for this endpoint four Tier 3 studies in rats (Quan et al., 2017 [RefID
9059 6025]; Wang C et al., 2014 [RefID 7579]; Castro et al., 2018 [RefID 11674]; Johnson et al., 2016
9060 [RefID 3241]) and one Tier 3 mouse study (Shi et al., 2018 [RefID 13099]) were identified. Therefore,
9061 data were considered Inadequate to judge the likelihood of an effect of BPA on testosterone levels.

9062 **Epididymis weight:** for this endpoint one Tier 1 (NTP Clarity Report, 2018/Camacho et al., 2019
9063 [RefID 11370]), one Tier 2 study (Spörndly-Nees et al., 2018 [RefID 13164]), one Tier 3 rat study
9064 (Tarapore et al., 2017 [RefID 7128]) and one Tier 2 mouse study (Meng Y et al., 2018 [RefID 12707])
9065 were identified.

9066 No effect was seen on epididymis weight in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et
9067 al., 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0
9068 dams from GD6 to PND0 and F1 pups from PND1 to PND21 at interim sacrifice (at 1 year).

9069 No effect was seen on epididymis weight in the other Tier 2 rat study (Spörndly-Nees et al., 2018 [RefID
9070 13164]): in this study animals were exposed via the drinking water to nominal doses of 5 or 50 µg/kg

- 9071 per day (achieved doses 4 or 40 µg/kg per day) from GD3.5–PND22 and necropsied at PND35 or 12
9072 months.
- 9073 No effect was seen on epididymis weight in the Tier 2 mouse study (Meng Y et al., 2018 [RefID 12707]):
9074 in this study animals were exposed via the drinking water to doses equivalent to 18 or 180 µg/kg per
9075 day from GD6–PND21 and necropsied at PND50.
- 9076 As there is no effect in any Tier 1 or Tier 2 study, the likelihood for this endpoint was considered as
9077 Not Likely.
- 9078 **Epididymis histology** (non-neoplastic, inflammatory changes, inflammation): for this endpoint one
9079 Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) and one Tier 2 rat
9080 study (Spörndly-Nees et al., 2018 [RefID 13164]) were identified.
- 9081 No effect was seen on epididymis histology (non-neoplastic, inflammatory changes) in the Tier 1 rat
9082 study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). Rats were dosed with 2.5, 25,
9083 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups from PND1 to PND21
9084 at interim sacrifice (at 1 year).
- 9085 An increase in inflammatory changes was seen at 40 µg/kg bw per day in the other Tier 2 rat study
9086 (Spörndly-Nees et al., 2018 [RefID 13164]): in this study animals were exposed via the drinking water
9087 to doses of 4 or 40 µg/kg per day from GD3.5–PND22 and necropsied at PND35 or 12 months.
- 9088 No effects were seen for the endpoint epididymis histology (non-neoplastic, inflammatory changes,
9089 inflammation) in one Tier 1 study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) and
9090 an effect only at the highest dose was seen in a Tier 2 rat study (Spörndly-Nees et al., 2018 [RefID
9091 13164]). Therefore, the likelihood assigned to this endpoint is Not Likely.
- 9092 **Prostate histology:** for this endpoint four Tier 1 (NTP Clarity Report, 2018/Camacho et al., 2019
9093 [RefID 11370]; Bernardo et al., 2015 [RefID 533]; Prins et al., 2018 [RefID 13779]; Brandt et al., 2014
9094 [RefID 700]), one Tier 2 (Hass et al., 2016 [RefID 2610]) and one Tier 3 (Prins et al., 2017 [RefID
9095 5930]) studies in rats were identified. In these studies several histological effects were examined.
- 9096 No effect was seen on prostate histology (non-neoplastic proliferative lesions, hyperplasia of the ventral
9097 prostate, epithelium hyperplasia and inflammatory changes, inflammation, dorsal/lateral prostate
9098 histology, suppurative inflammation) in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al.,
9099 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams
9100 from GD6 to PND0 and F1 pups from PND1 to PND21 at interim sacrifice (at 1 year).
- 9101 At histological examination, an increase (same effect size) in the incidence of inflammatory changes,
9102 pre-neoplastic lesions (atypical hyperplasia), non-neoplastic proliferative lesions (reactive hyperplasia),
9103 in the prostate was seen in both doses tested in a Tier 1 rat study (Bernardo et al., 2015 [RefID 533]).
9104 In this study animals were given 25 or 250 µg/kg bw per day from GD10–21 and necropsied at PND180.
- 9105 No effect was seen on prostate histology (non-neoplastic proliferative lesions, hyperplasia of the ventral
9106 prostate and inflammatory changes, inflammation, dorsal/lateral prostate histology, suppurative
9107 inflammation) in the Tier 1 rat study (Prins et al., 2018 [RefID 13779]). Rats were dosed with 2.5, 25,
9108 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups from PND1 to PND21
9109 and necropsied at 1 year.
- 9110 In a Tier 1 study (Brandt et al., 2014 [RefID 700]) rats were administered 25 and 250 µg/kg bw per
9111 day from GD10–21. F1 pups/adults were sacrificed on PND21/180. At histological examination on
9112 PND21 an increase in proliferation and hyperplasia/dysplasia of the prostate was observed in the lowest
9113 dose and in apoptosis in the highest dose. At both doses an increase (effect the same size) of multifocal
9114 inflammation in the ventral prostate on PND180 was observed.
- 9115 In the Tier 2 rat study (Hass et al., 2016 [RefID 2610]), no change in prostate histology (interstitial
9116 inflammation, proliferation or epithelial atypical hyperplasia) was observed when examined in F1 males
9117 at 3 or 8 months; F0 dams were treated at oral doses of 25, 250, 5000 or 50000 µg/kg bw per day
9118 GD7–PND22.
- 9119 In two Tier 1 rat studies (Bernardo et al., 2015 [RefID 533]; Brandt et al., 2014 [RefID 700]) from the
9120 same laboratory, inflammatory effects and reactive hyperplasia in the prostate were reported at doses

9121 of 25 and 250 µg/kg bw per day GD10–GD21. This effect was not confirmed in two other Tier 1 rat
9122 studies (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]; Prins et al., 2018 [RefID
9123 13779]) at doses of 2.5, 25, 250, 2500 or 25000 µg/kg bw per day from GD6–PND21 or in a Tier 2 rat
9124 study (Hass et al., 2016 [RefID 2610]) at doses of 25, 250, 5000 or 50000 µg/kg bw per day GD7–
9125 PND22. Therefore, the likelihood for this endpoint was considered to be ALAN.

9126 **Seminal vesicle weight:** for this endpoint one Tier 1 (NTP Clarity Report, 2018/Camacho et al., 2019
9127 [RefID 11370]) and one Tier 2 study (Spörndly-Nees et al., 2018 [RefID 13164]) in rats and one Tier
9128 3 mouse study (Patel et al., 2013 [RefID 5697]) were identified.

9129 No effect was seen on seminal vesicle weight in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho
9130 et al., 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0
9131 dams from GD6 to PND0 and F1 pups from PND1 to PND21 at interim sacrifice (at 1 year).

9132 No effect was seen on seminal vesicle weight in the other Tier 2 rat study (Spörndly-Nees et al., 2018
9133 [RefID 13164]): in this study animals were exposed via the drinking water to doses of 4 or 40 µg/kg
9134 per day from GD3.5–PND22 and necropsied at PND35 or 12 months.

9135 No effect was observed on seminal vesicle weight in one Tier 1 (NTP Clarity Report, 2018/Camacho et
9136 al., 2019 [RefID 11370]) and one Tier 2 rat study (Spörndly-Nees et al., 2018 [RefID 13164]).
9137 Therefore, the likelihood assigned to this endpoint is Not Likely.

9138 **Sperm count:** for this endpoint one Tier 1 (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
9139 11370]) and one Tier 3 study (Hass et al., 2016 [RefID 2610]) in rats and two Tier 3 studies in mice
9140 (Rahman et al., 2017 [RefID 6060]; Shi et al., 2018 [RefID 13099]) were identified.

9141 No effect was seen on epididymal sperm count and count of testicular sperm heads in the Tier 1 rat
9142 study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). Rats were dosed with 2.5, 25,
9143 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups from PND1 to PND21
9144 at interim sacrifice (at 1 year).

9145 No effect was observed on epididymal sperm count and count of testicular sperm heads in one Tier 1
9146 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) at dose levels of 2.5 to 25000
9147 µg/kg bw per day GD6–PND22. Therefore, the likelihood assigned to this endpoint is Not Likely.

9148 **Sperm morphology:** for this endpoint one Tier 1 study (NTP Clarity Report, 2018/Camacho et al.,
9149 2019 [RefID 11370]) and one Tier 2 study (Spörndly-Nees et al., 2018 [RefID 13164]) in rats and one
9150 Tier 3 mouse study (Kalb et al., 2016 [RefID 3312]) were identified.

9151 No effect was identified on sperm morphology in the Tier 1 rat study (NTP Clarity Report,
9152 2018/Camacho et al., 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg
9153 bw per day; F0 dams from GD6 to PND0 and F1 pups from PND1 to PND21 (at 1 year interim sacrifice).

9154 No effect was seen on sperm morphology in the Tier 2 rat study (Spörndly-Nees et al., 2018 [RefID
9155 13164]): in this study animals were exposed via the drinking water to doses of 4 or 40 µg/kg per day
9156 from GD3.5–PND22 and necropsied at 12 months.

9157 No effect was seen on sperm morphology in one Tier 1 rat study (NTP Clarity Report, 2018/Camacho
9158 et al., 2019 [RefID 11370]) at dose levels of 2.5 to 25000 µg/kg bw per day GD6–PND22 and in a Tier
9159 2 rat study (Spörndly-Nees et al., 2018 [RefID 13164]) at 4 or 40 µg/kg per day from GD3.5–PND22.
9160 Therefore, the likelihood assigned to this endpoint is Not Likely.

9161 **Sperm motility:** for this endpoint one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019
9162 [RefID 11370]) and three Tier 3 studies in mice (Shi et al., 2018 [RefID 13099]; Kalb et al., 2016 [RefID
9163 3312]; Rahman et al., 2017 [RefID 6060]) were identified.

9164 No effect was examined on sperm motility in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho
9165 et al., 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0
9166 dams from GD6 to PND0 and F1 pups from PND1 to PND21 at interim sacrifice (at 1 year).

9167 No effect was examined on sperm motility in one Tier 1 rat study (NTP Clarity Report, 2018/Camacho
9168 et al., 2019 [RefID 11370]). Therefore, the likelihood assigned to this endpoint is Not Likely.

9169 **Sperm viability:** for this endpoint one Tier 3 mouse study (Rahman et al., 2017 [RefID 6060]) was
9170 identified. Therefore, data were Inadequate to judge the likelihood of an effect of BPA on sperm
9171 viability.

9172 **Testis weight:** for this endpoint two Tier 1 studies (NTP Clarity Report, 2018/Camacho et al., 2019
9173 [RefID 11370]; Cao et al., 2015 [RefID 831]), and one Tier 3 study (Tarapore et al., 2017 [RefID 7128])
9174 in rats and two Tier 2 studies (Meng Y et al., 2018 [RefID 12707]; Shi et al., 2018 [RefID 13099]) and
9175 one Tier 3 study (Patel et al., 2013 [RefID 5697]) in mice were identified.

9176 No effect was reported on testis weight in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et
9177 al., 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0
9178 dams from GD6 to PND0 and F1 pups from PND1 to PND21 at interim sacrifice (at 1 year).

9179 On PND120, an increase in testis weight was observed in a single-dose Tier 1 rat study (Cao et al.,
9180 2015 [RefID 831]); the F0 females were administered via the drinking water (2 mg BPA/L; equivalent
9181 to 100 µg/kg bw per day) and co-treated with soy in the diet from GD1–PND21. No change was
9182 observed in the BPA-treated group with a soy-free diet.

9183 No effect was seen on testis weight in a Tier 2 mouse study (Meng Y et al., 2018 [RefID 12707]): in
9184 this study animals were exposed via the drinking water to doses equivalent to 18 or 180 µg/kg per day
9185 from GD6–PND21 and necropsied at PND50.

9186 No effect was seen on testis weight in the other Tier 2 mouse study (Shi et al., 2018 [RefID 13099]):
9187 in this study animals were exposed via the drinking water to doses equivalent to 0.5, 20 or 50 µg/kg
9188 bw per day from GD11 to birth and necropsied at PND60.

9189 As no effect was observed in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
9190 11370]) and two Tier 2 mouse studies (Meng Y et al., 2018 [RefID 12707]; Shi et al., 2018 [RefID
9191 13099]), the likelihood was considered to be Not Likely for this endpoint.

9192 **Testis histology:** for this endpoint one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al.,
9193 2019 [RefID 11370]), two rat Tier 2 studies (Quan et al., 2017 [RefID 6025]; Spörndly-Nees et al.,
9194 [RefID 13164]), two Tier 2 mouse studies (Shi et al., 2018 [RefID 13099]; Xie et al., 2016 [RefID
9195 8130]) and one Tier 3 mouse study (Rahman et al., 2017 [RefID 6060]) were identified.

9196 At histological examination, increased incidence of testis (and pancreas) polyarteritis was seen in the
9197 Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) at a dose of 2500
9198 µg/kg bw per day. Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from
9199 GD6 to PND0 and F1 pups from PND1 to PND21 at interim sacrifice (at 1 year).

9200 In the Tier 2 rat study (Quan et al., 2017 [RefID 6025]) an increase was seen at all dose levels (no
9201 dose–response) in the testis (seminiferous tubular changes, cell-specific and/or stage-specific:
9202 degeneration, germ cell) at PND50 in the F1 rats. The F0 animals were dosed with 1000; 10000 or
9203 100000 µg/kg bw from GD14–21.

9204 At histological examination at necropsy at 12 months, an increase was seen in inflammatory changes
9205 in the testis of the low-dose group (4 µg/kg per day from GD3.5–PND22) in a Tier 2 rat study (Spörndly-
9206 Nees et al., [RefID 13164]). No such effects were seen at the other dose tested (40 µg/kg per day) or
9207 at both dose levels on other histological effects in the testis (seminiferous tubular changes, non-specific
9208 seminiferous epithelial height and seminiferous tubule diameter).

9209 At histological examination of the testis in a Tier 2 mouse study (Shi et al., 2018 [RefID 13099]) at
9210 PND60, a decrease of seminiferous tubular changes, cell and/or stage-specific, in stage VII seminiferous
9211 epithelial cells and an increase in stage VIII seminiferous epithelial cells was seen in the mid-dose group
9212 (20 µg/kg bw per day). On PND12 an increase without dose–response was seen in testicular apoptosis
9213 in the mid and high-dose groups. In this study mice were dosed with 0.5, 20 or 50 µg/kg bw per day
9214 from GD11 to birth.

9215 In another Tier 2 mouse study (Xie et al., 2016 [RefID 8130]) a dose-related increase was observed at
9216 histological examination of the testis (degeneration, germ cell). In this study male mice were s.c.
9217 injected 10, 100 or 5000 µg/kg bw per day (equivalent to oral doses of 2222; 22220 or 1111000 µg/kg
9218 bw per day).

9219 The Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) reported increased
9220 incidence of polyarteritis at 2500 µg/kg bw per day only (not at other doses in the range from 2.5–
9221 25000 µg/kg bw per day. The Tier 2 rat studies showed effects on inflammatory changes at only one
9222 dose (4 µg/kg bw per day) (Spörndly-Nees et al., [RefID 13164]), or effects (apoptosis) without dose–
9223 response at 1000–100000 µg/kg bw per day (Quan et al., 2017 [RefID 6025]). In the Tier 2 mouse
9224 study (Shi et al., 2018 [RefID 13099]) only the mid dose (20 µg/kg bw per day) showed effects on the
9225 testis (decrease in stage VII and decrease in stage VIII seminiferous epithelial cells) and apoptosis in
9226 the mid and high dose, 20 or 50 µg/kg bw per day, with no dose–response. In another Tier 2 mouse
9227 study (Xie et al., 2016 [RefID 8130]) with s.c. administration dose-related testicular findings (germ cell
9228 (apoptosis)) were observed at doses equivalent to oral doses of 2220; 22220 or 1111000 µg/kg bw per
9229 day. The likelihood of the histological changes in the testis were considered to be ALAN:

9230 During developmental exposure (pre-natal and/or post-natal until weaning), the CEP Panel assigned a
9231 likelihood level of ALAN to the cluster male reproductive toxicity of BPA. Hence, none of these endpoints
9232 were taken forward for BMD analysis. However, the Likely and ALAN endpoints were considered in the
9233 uncertainty analysis (see Appendix D).

9234 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

9235 **Plasma/serum thyroid hormones:** For this exposure period only one Tier 1 rat study was identified
9236 (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). In this study **T₃** and **T₄** were measured.
9237 Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and
9238 F1 pups from PND1 to interim sacrifice (at 1 year).

9239 No effect was seen on **T₃** in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
9240 11370]). According to the authors, **T₄** levels in the serum showed a significant trend, but the nature of
9241 the trend was not evident from inspection of the data. The likelihood of an effect on **T₄** was therefore
9242 considered as Not Likely (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]).

9243 During this exposure period the likelihood of changes in thyroid hormones (**T₃**, **T₄**) are considered as
9244 Not Likely by the CEP Panel as no effects were observed in one Tier 1 rat study.

9245 **Testosterone:** For this endpoint one Tier 3 rat study (Gonzalez-Cadavid, 2018 (NTP Grantee study)
9246 [RefID 13787]) was identified. Therefore, data were Inadequate to judge the likelihood of an effect on
9247 serum testosterone.

9248 **Epididymis weight:** For this exposure period two Tier 1 rat studies (Dere et al., 2018 [RefID 11815];
9249 NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) were identified.

9250 No change in epididymis weight was seen in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho
9251 et al., 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0
9252 dams from GD6 to PND0 and F1 pups from PND1 to PND21 to interim sacrifice (at 1 year).

9253 In the other Tier 1 rat study (Dere et al., 2018 [RefID 11815]), rats were dosed with 2.5, 25, 250, 2500
9254 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups from PND1 to PND90. In this
9255 study an extra satellite control and 250000 µg/kg bw per day was added. A decrease in epididymis
9256 weight was only seen in the 250000 µg/kg bw per day group when compared with the extra (satellite)
9257 control group. This satellite control group showed a higher epididymis weight than the other control
9258 group.

9259 The likelihood of an effect on epididymis weight was considered as Not Likely.

9260 **Epididymis histology:** For this exposure period the following Tier 1 rat study (NTP Clarity Report,
9261 2018/Camacho et al., 2019 [RefID 11370]) was identified; this study had two different times of sacrifice.

9262 In this study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) an increased change in
9263 exfoliated germ cells and inflammation was seen at histological examination of the epididymis in the
9264 high-dose group (25,000 µg/kg bw per day) at interim sacrifice (1 year); these effects were not
9265 observed at terminal sacrifice (2 years). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw
9266 per day; F0 dams from GD6 to PND0 and F1 pups from PND1 to PND21 to interim sacrifice (1 year)
9267 and terminal sacrifice (2 years).

9268 The likelihood of the changes in epididymis histology (exfoliated germ cells and inflammation) was
9269 considered to be Likely, although effects were only seen in the highest dose group (25,000 µg/kg bw
9270 per day) at interim sacrifice (1 year) and not at terminal sacrifice (2 years), i.e. the effect was apparently
9271 transient.

9272 **Prostate histology:** For this exposure period the following Tier 1 rat study (NTP Clarity Report,
9273 2018/Camacho et al., 2019 [RefID 11370]) was identified; this study had two different times of sacrifice.
9274 In addition, another set of animals of the same study was examined (Prins et al., 2018 [RefID 13779]).

9275 In the Tier one rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) animals were
9276 dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups
9277 from PND1 to PND21 to interim sacrifice (1 year) or terminal sacrifice (2 years). At interim sacrifice no
9278 change in hyperplasia of the epithelium (non-neoplastic, proliferative lesions) was observed and at
9279 terminal sacrifice only an increase was observed at 250 µg/kg bw per day. At interim sacrifice an
9280 increase without dose–response in inflammatory changes of the prostate was observed at 2.5, 250,
9281 2500 or 25000 µg/kg bw per day; no change was seen at 25 µg/kg bw per day. At terminal sacrifice
9282 only an increase in inflammatory changes of the prostate was seen in the lowest dose group.

9283 There was no change in inflammation of the prostate in the Tier 1 rat study (Prins et al., 2018 [RefID
9284 13779]): in this study animals were dose with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams
9285 from GD6 to PND0 and F1 pups from PND1 to PND21 to 1 year.

9286 The likelihood of the changes in prostate histology were considered to be Not Likely as effects were
9287 not seen in different sets of animals and were only examined at interim sacrifice.

9288 **Seminal vesicle weight:** For this exposure period one Tier 1 rat study (NTP Clarity Report,
9289 2018/Camacho et al., 2019 [RefID 11370]) and one Tier 3 mouse study (Patel et al., 2013 [RefID
9290 5697]) were identified.

9291 In the Tier 1 rat study animals were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0
9292 dams from GD6 to PND0 and F1 pups from PND1 to interim sacrifice (1 year). No effects on seminal
9293 vesicle weight were observed.

9294 The likelihood of an effect on seminal vesicle weight was considered as Not Likely.

9295 **Sperm count:** for this endpoint one Tier 1 (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
9296 11370]) was identified.

9297 No effect was seen on epididymal sperm count and count of testicular sperm heads in the Tier 1 rat
9298 study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). Rats were dosed with 2.5, 25,
9299 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups from PND1 to interim
9300 sacrifice (1 year, continuous arm).

9301 The likelihood of an effect on sperm count was considered as Not Likely.

9302 **Sperm morphology:** for this endpoint one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al.,
9303 2019 [RefID 11370]) and one Tier 3 mouse study (Dobrzynska et al., 2018 [RefID 11837]) were
9304 identified.

9305 No effect on sperm morphology was observed in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho
9306 et al., 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0
9307 dams from GD6 to PND0 and F1 pups from PND1 to interim sacrifice (1 year).

9308 The likelihood of an effect on sperm morphology was considered as Not Likely.

9309 **Sperm motility:** for this endpoint one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019
9310 [RefID 11370]) and one Tier 3 mouse study (Dobrzynska et al., 2018 [RefID 11837]) were identified.

9311 No effect was seen on sperm motility in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al.,
9312 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams
9313 from GD6 to PND0 and F1 pups from PND1 to interim sacrifice (1 year).

9314 The likelihood of an effect on sperm motility was considered as Not Likely.

9315 **Testis weight:** For this exposure period two Tier 1 studies (NTP Clarity Report, 2018/Camacho et al.,
9316 2019 [RefID 11370]; Dere et al., 2018 [RefID 11815]) and one Tier 3 mouse study (Patel et al., 2013
9317 [RefID 5697]) were identified.

9318 No change in testis weight was seen in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al.,
9319 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams
9320 from GD6 to PND0 and F1 pups from PND1 to PND21 to interim sacrifice (1 year).

9321 In the other Tier 1 rat study (Dere et al., 2018 [RefID 11815]), rats were dosed with 2.5, 25, 250, 2500
9322 or 25000 µg/kg bw per day; in this study extra satellite groups, 0 and 250000 µg/kg bw per day were
9323 added. F0 dams were exposed from GD6 to PND0 and F1 pups from PND1 to PND90. A decrease in
9324 testis weight was only seen in the 250000 µg/kg bw per day group when compared with the extra
9325 control group. This extra control group showed a higher testis weight than the other control group.

9326 The likelihood of an effect on testis weight was considered as Not Likely.

9327 **Testis histology:** For this exposure period two Tier 1 studies (NTP Clarity Report, 2018/Camacho et
9328 al., 2019 [RefID 11370]; Dere et al., 2018 [RefID 11815]) were identified.

9329 At histological examination in the testis (polyarteritis) of the Tier 1 rat study (NTP Clarity Report,
9330 2018/Camacho et al., 2019 [RefID 11370]) no changes were observed. Rats were dosed with 2.5, 25,
9331 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups from PND1 to PND21
9332 to interim sacrifice (1 year).

9333 In the other Tier 1 rat study (Dere et al., 2018 [RefID 11815]), rats were dosed with 2.5, 25, 250,
9334 2500, or 25000 µg/kg bw per day; in this study extra satellite groups, 0 and 250000 µg/kg bw per day
9335 were added. F0 dams were exposed from GD6 to PND0 and F1 pups from PND1 to PND90. At
9336 histological examination of the testis, no changes in apoptosis, sperm production and spermiation were
9337 observed.

9338 The likelihood of an effect on testis histology was considered as Not Likely:

9339 During developmental exposure (pre-natal and/or post-natal in pups until adulthood), the CEP Panel
9340 assigned a likelihood level of Likely to the cluster male reproductive toxicity of BPA. Since the likelihood
9341 level is Likely for the endpoint epididymis histology (exfoliated germ cells and inflammation) in the Tier
9342 1 study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]), this study was taken forward
9343 for BMD analysis (see Chapter 3.2.1) and uncertainty analysis (see Appendix D).

9344 Growth phase/young age

9345 **Testosterone:** For this endpoint three Tier 3 rat studies (Ullah et al., 2018a [RefID 13281]; Ullah et
9346 al., 2018b [RefID 13282]; Gurmeet et al., 2014 [RefID 2502]) were identified. In the Tier 3 rat study
9347 (Ullah et al., 2018a [RefID 13281]) intratesticular testosterone was measured in addition to serum
9348 testosterone. In the other studies only serum/plasma testosterone was measured.

9349 As only Tier 3 studies were identified, data were considered Inadequate to judge the likelihood of an
9350 effect on serum testosterone.

9351 **Epididymis weight:** For this endpoint two Tier 1 rat studies (Ogo et al., 2018 [RefID 11201]; Gurmeet
9352 et al., 2014 [RefID 2502]) and one Tier 3 rat study (Ullah et al., 2018b [RefID 13282]) were identified.

9353 No effect on epididymis weight was seen when rats were dosed with 20 or 200 µg/kg bw per day from
9354 PND36–66 (Ogo et al., 2018 [RefID 11201]). In another Tier 1 rat study (Gurmeet et al., 2014 [RefID
9355 2502]) no effects were observed on epididymis weight when rats were dosed with 1000, 5000 or 100000
9356 µg/kg bw per day from PND28–70.

9357 The likelihood was considered as Not Likely as no effect was observed on epididymis weight in two Tier
9358 1 rat studies.

9359 **Seminal vesicle weight:** For this endpoint one Tier 1 rat study (Gurmeet et al., 2014 [RefID 2502])
9360 and one Tier 3 rat study (Ullah et al., 2018b [RefID 13282]) were identified.

9361 No effect on seminal vesicle weight was observed when rats were administered BPA via the drinking
9362 water with doses equivalent to oral doses of 1000, 5000 and 100000 µg/kg bw per day from PND28–
9363 70 weeks (Gurmeet et al., 2014 [RefID 2502]).

9364 The likelihood was considered as Not Likely as no effect was seen on seminal vesicle weight in one Tier
9365 1 rat study.

9366 **Sperm count** (testis/epididymis): For this endpoint one Tier 1 rat studies (Ogo et al., 2018 [RefID
9367 11201]) and one Tier 3 rat study (testicular count, Ullah et al., 2018b [RefID 13282]) were identified.

9368 No effect on epididymal sperm count was observed when rats were dosed with 20 or 200 µg/kg bw per
9369 day from PND36–66 (Ogo et al., 2018 [RefID 11201]).

9370 The likelihood was considered as Not Likely as no effect was seen on epididymal sperm count in one
9371 Tier 1 rat study.

9372 **Sperm motility:** For this endpoint only one Tier 3 rat study (Ullah et al., 2018b [RefID 13282]) was
9373 identified. As only one Tier 3 rat study was identified, data were Inadequate to judge the likelihood of
9374 an effect of BPA on sperm motility.

9375 **Testis weight:** For this endpoint two Tier 1 rat studies (Ullah et al., 2018a [RefID 13281]; Gurmeet
9376 et al., 2014 [RefID 2502]), one Tier 2 rat study (Brouard et al., 2016 [RefID 734]) and one Tier 3 rat
9377 study (Ullah et al., 2018b [RefID 13282]) were identified.

9378 In the Tier 1 rat study (Ullah et al., 2018a [RefID 13281]) no effect on testis weight was observed. In
9379 this study rats were dosed for 4 weeks with 5000, 25000 or 50000 µg/kg bw per day from PND70–80.
9380 In another Tier 1 rat study (Gurmeet et al., 2014 [RefID 2502]), no effects were observed on testis
9381 weight when rats were dosed with 1000, 5000 or 100000 µg/kg bw per day from PND28–70. In a Tier
9382 2 study (Brouard et al., 2016 [RefID 734]) rats were exposed from PND15–PND30 by s.c. injection with
9383 a single dose of 50 µg/kg bw per day (equivalent to an oral dose of 1800 µg/kg bw per day) and testis
9384 weight was increased.

9385 The likelihood was considered to be Not Likely as no effects on testis weight were seen in two Tier 1
9386 studies via gavage (dose range 5000–50000 µg/kg bw per day rats of PND70–PND80 for 4 weeks and
9387 1000–100000 µg/kg bw per day from PND23 for 48 weeks) and only an effect was observed in the Tier
9388 2 single-dose rat study via the s.c. route after dosing from PND15–PND30.

9389 **Testis histology:** For this endpoint two Tier 2 rat studies (Gurmeet et al., 2014 [RefID 2502]; Brouard
9390 et al., 2016 [RefID 734]) and two Tier 3 rat studies (Ullah et al., 2018a [RefID 13281]; Ullah et al.,
9391 2018b [RefID 13282]) were identified.

9392 In the Tier 2 rat study (Gurmeet et al., 2014 [RefID 2502]), a decrease of the seminiferous tubule
9393 diameter was observed in the high-dose group; rats were dosed with 1000, 5000 or 100000 µg/kg bw
9394 per day from PND28–PND70. In another Tier 2 study (Brouard et al., 2016 [RefID 734]), rats were
9395 exposed from PND15–PND30 by s.c. injection with a single dose of 50 µg/kg bw per day (equivalent to
9396 an oral dose of 1800 µg/kg bw per day), and incidences of seminiferous tubules with lumen, with
9397 acrosomal vesicles and acrosome reaction were increased in the BPA-treated group.

9398 The likelihood of the effect was considered as Likely as in a Tier 2 rat study (Gurmeet et al., 2014
9399 [RefID 2502]) a decrease in seminiferous tubule diameter at dose level of 100000 µg/kg bw per day
9400 was observed. These effects were supported by testicular effects in another Tier 2 rat study (Brouard
9401 et al., 2016 [RefID 734]) at a s.c. dose equivalent to an oral dose of 1800 µg/kg bw per day. In this
9402 study, incidence of seminiferous tubules with lumen, acrosomal vesicles and acrosome reaction was
9403 increased. This single-dose study was not taken forward for BMD analysis:

9404 During the exposure during the growth phase, the CEP Panel assigned a likelihood level of Likely to the
9405 cluster male reproductive toxicity of BPA. Since the likelihood level is Likely for the endpoint testis
9406 histology (decrease in seminiferous tubule diameter) in the Tier 2 rat study (Gurmeet et al., 2014 [RefID
9407 2502]); this study was taken forward for BMD analysis (see Chapter 3.2.1) and uncertainty analysis
9408 (see Appendix D).

9409 Adult exposure (after puberty)

9410 **Plasma/serum testosterone:** For this endpoint four Tier 3 rat studies (Srivastava and Gupta, 2018
9411 [RefID 13167]; Wu et al., 2016 [RefID 8036]; Huang DY et al., 2018 [RefID 12167]; Rashid et al., 2018
9412 [RefID 12963]), three Tier 3 mouse studies (Xu XH et al., 2015 [RefID 8232]; Chouhan et al., 2015

9413 [RefID 1216]; Gao et al., 2018 [RefID 11972]) and one Tier 3 monkey study (Vijaykumar et al., [RefID
9414 7477]) were identified.

9415 As only Tier 3 studies were identified, data were considered Inadequate to judge the likelihood of an
9416 effect on testosterone.

9417 **Epididymis weight:** For this exposure period only one Tier 3 mouse study (Dobrzynska et al., 2014
9418 [RefID 1645]) was identified in which epididymis weight was measured.

9419 As only one Tier 3 mouse study was identified, data were considered Inadequate to judge the likelihood
9420 of an effect on epididymis weight.

9421 **Prostate histology:** For this exposure period one Tier 2 (Olukole et al., 2018 [RefID 12841]) and two
9422 Tier 3 studies (Huang DY et al., 2018 [RefID 12167]; Wu et al., 2016 [RefID 8036]) in rats were
9423 identified.

9424 In the Tier 2 study (Olukole et al., 2018 [RefID 12841]), rats were dosed with 10000 µg/kg bw per day
9425 for 14 days. The following effects were increased in prostate histology of the single-dose BPA-treated
9426 group: degenerative changes, atrophy (atrophic tubules); non-neoplastic proliferative lesion: functional
9427 hyperplasia, proliferative lesions, reduced glandular diameter, hyperplasia reactive (accompanied by
9428 inflammation); inflammatory changes: inflammation and vascular digestion; atypical hyperplasia.

9429 The likelihood of the effects on prostate histology was judged as Inadequate evidence as the effects
9430 on prostate histology were observed in a single-dose Tier 2 rat study (Olukole et al., 2018 [RefID
9431 12841]).

9432 **Seminal vesicle weight:** For this exposure period one Tier 3 mouse study (Chouhan et al., 2015
9433 [RefID 1216]) was identified.

9434 As only one Tier 3 mouse study was identified, data were considered Inadequate to judge the likelihood
9435 of an effect on seminal vesicle weight.

9436 **Sperm count:** For this exposure period one Tier 1 study (Wang HF et al., 2016 [RefID 7618]), two
9437 Tier 2 studies (Park et al., 2018 [RefID 12869]; Yin et al., 2017 [RefID 8510]) and three Tier 3 studies
9438 (Dobrzynska et al., 2014 [RefID 1645]; Gao et al., 2018 [RefID 11972]; Chouhan et al., 2015 [RefID
9439 1216]) in mice and one Tier 3 rat study (Srivastava and Gupta, 2018 [RefID 13167]) were identified.

9440 In the Tier 1 study (Wang HF et al., 2016 [RefID 7618]) mice were dosed for 8 weeks with 10, 50 or
9441 250 µg/kg per day; no effect on sperm count was noted. In the Tier 2 mouse study (Yin et al., 2017
9442 [RefID 8510]) no effect was observed on sperm count after 5 weeks dosing with 3000, 30000 or 300000
9443 µg/kg bw per day. In another Tier 2 mouse study (Park et al., 2018 [RefID 12869]) a decrease in sperm
9444 count was seen at the only dose tested (10000 µg/kg bw per day) for 12 weeks.

9445 The likelihood of the effect on sperm count was judged as Not Likely as one Tier 1 study (Wang HF et
9446 al., 2016 [RefID 7618]) and one Tier 2 study (Yin et al., 2017 [RefID 8510]) in mice were without
9447 effects (dose range 10–300000 µg/kg per day) and the decrease on sperm count was seen in a single-
9448 dose study (Park et al., 2018 [RefID 12869]) at 10000 µg/kg bw per day.

9449 **Sperm motility:** For this exposure period one Tier 1 study (Wang HF et al., 2016 [RefID 7618]), one
9450 Tier 2 study (Park et al., 2018 [RefID 12869]) and one Tier 3 study (Dobrzynska et al., 2014 [RefID
9451 1645]) in mice were identified.

9452 In the Tier 1 study (Wang HF et al., 2016 [RefID 7618]) mice were dosed for 8 weeks with 10, 50 or
9453 250 µg/kg bw per day; a dose-related decrease on sperm motility was observed. In the Tier 2 mouse
9454 study (Park et al., 2018 [RefID 12869]) a decrease in sperm motility was observed at the only dose
9455 tested (10000 µg/kg bw per day) for 12 weeks.

9456 The likelihood of the decrease in sperm motility was judged as Likely based on the dose-related
9457 decrease in the Tier 1 mouse study (Wang HF et al., 2016 [RefID 7618]) at doses 10, 50 or 250 µg/kg
9458 bw per day. This effect was supported by decrease in sperm motility seen in the single dose (10000
9459 µg/kg bw per day) in the Tier 2 study (Park et al., 2018 [RefID 12869]). This single-dose study was
9460 not brought forward for BMD analysis.

9461 **Sperm morphology:** For this exposure period one Tier 2 study (Park et al., 2018 [RefID 12869]) and
9462 one Tier 3 study (Dobrzynska et al., 2014 [RefID 1645]) in mice were identified.

9463 In the Tier 2 mouse study (Park et al., 2018 [RefID 12869]) an increase in abnormal sperm was
9464 observed at the only dose tested (10000 µg/kg bw per day) for 12 weeks.

9465 The likelihood of the increase of abnormal sperm was judged as Inadequate as the increase was
9466 observed in a single dose (10000 µg/kg bw per day) Tier 2 mouse study (Park et al., 2018 [RefID
9467 12869]).

9468 **Sperm viability:** For this exposure period one Tier 1 mouse (Wang HF et al., 2016 [RefID 7618])
9469 study was identified.

9470 In the Tier 1 study (Wang HF et al., 2016 [RefID 7618]) mice were dosed for 8 weeks with 10, 50 or
9471 250 µg/kg bw per day; a dose-related decrease on sperm motility was observed which was significant
9472 at the highest dose level and decreased not statistically significant also in the mid-dose group.

9473 The likelihood of the decrease in sperm viability was judged as Likely based on the dose-related
9474 decrease in the Tier 1 mouse study (Wang HF et al., 2016 [RefID 7618]) at doses 10, 50 or 250 µg/kg
9475 bw per day.

9476 **Sperm acrosome reaction:** For this exposure period one Tier 1 mouse study (Wang HF et al., 2016
9477 [RefID 7618]) was identified. In this Tier 1 study (Wang HF et al., 2016 [RefID 7618]) mice were dosed
9478 for 8 weeks with 10, 50 or 250 µg/kg bw per day. A dose-related decrease in acrosome reaction was
9479 seen at the two highest dose levels.

9480 The likelihood of the decrease in acrosome reaction was considered to be Likely.

9481 **Testis weight:** For this exposure period one Tier 1 study (Wang HF et al., 2016 [RefID 7618]) and
9482 three Tier 3 studies (Dobrzynska et al., 2014 [RefID 1645], Gao et al., 2018 [RefID 11972], Chouhan
9483 et al., 2015 [RefID 1216]) in mice were identified. In the Tier 1 study (Wang HF et al., 2016 [RefID
9484 7618]) mice were dosed for 8 weeks with 10, 50 or 250 µg/kg bw per day; no effect on testis weight
9485 was noted.

9486 The likelihood for an effect on testis weight was judged as Not Likely as no effect was observed in the
9487 Tier 1 mouse study.

9488 Overall, the CEP Panel assigned a likelihood of Likely to the cluster male reproductive toxicity during
9489 adult exposure. As the likelihood level for male reproductive toxicity is Likely for the endpoint sperm
9490 motility, viability and acrosome reaction in the Tier 1 mouse study (Wang HF et al., 2016 [RefID 7618])
9491 these data were taken forward for BMD analysis (see Chapter 3.2.1) and uncertainty analysis (see
9492 Appendix D).

9493 Indirect (germline) exposure

9494 For this exposure period two Tier 3 mouse studies (Dobrzynska et al., 2015 [RefID 1644]; Dobrzynska
9495 et al., 2018 [RefID 11837]), in which epididymis weight, sperm count and sperm motility were
9496 measured, were identified. In addition, in the Tier 3 mouse study (Dobrzynska et al., 2015 [RefID
9497 1644]) sperm morphology was examined and in the other Tier 3 study (Dobrzynska et al., 2018 [RefID
9498 11837]) testis weight was measured.

9499 The CEP Panel noted that as only Tier 3 studies were available, the evidence was considered Inadequate
9500 for this exposure period.

9501 Overall cluster selection for endpoints/studies for BMD analysis for male reproductive toxicity

9502 Overall, the CEP Panel assigned a likelihood level of ALAN to the male reproductive toxicity cluster in
9503 the developmental exposure period, of Likely in the developmental and adult, growth phase/young age
9504 and adult exposure periods, and of Inadequate evidence in the indirect (germline) exposure period.

9505 The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster male
9506 reproductive toxicity was Likely.

9507 The CEP Panel considered that the evidence from the studies available showed a Likely effect for
9508 epididymis histology (exfoliated germ cells and inflammation) in the Tier 1 rat study (NTP Clarity Report,

2018/Camacho et al., 2019 [RefID 11370]) 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 [RefID 2502]) and during adult exposure effects on sperm (motility; viability; acrosome reaction) were observed in a Tier 1 mouse study (Wang HF et al., 2016 [RefID 7618]). Therefore, these endpoints were taken forward for BMD analysis (see Chapter 3.2.1).

3.1.6.3 Integration of likelihoods from human and animal studies

Table 13 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.

AGD, bone development and breast development are endpoints assessed in two different clusters: in pubertal/endocrine in the human stream and in developmental toxicity in the animal stream. Overall, the animal and human clusters are not comparable. It is however 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. In the human stream the fetal and post-natal growth cluster contains results for body weight and femoral length being comparable 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. 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 of 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. 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.

Table 13: Integration of the human and animal studies for Reproductive and developmental toxicity.

Human stream	Animal stream	Integrated likelihood
Cluster: Developmental toxicity	Cluster: Developmental toxicity	
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) ALAN	
	Growth phase/young age exposure ALAN	
	Adult exposure (after puberty) Not Likely	
	Indirect (germline) exposure Not Likely	
	<i>Overall likelihood: ALAN</i>	<i>ALAN</i>
Cluster: Fetal and Post-natal Growth	Cluster: Fetal and Post-natal Growth	
Exposure during Pregnancy Not Likely	Not applicable	

<i>Overall likelihood:</i>	<i>Not Likely</i>		<i>Not Likely</i>
Cluster: Pubertal/Endocrine		Cluster: Pubertal/Endocrine	
Exposure during Pregnancy	ALAN	Not applicable	
Exposure during Childhood	Not Likely		
<i>Overall likelihood:</i>	<i>ALAN</i>		<i>ALAN</i>
Cluster: Female fertility		Cluster: Female reproductive toxicity	
Exposure during Adulthood	ALAN	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
<i>Overall likelihood:</i>	<i>ALAN</i>	<i>Overall likelihood:</i>	<i>Likely</i>
Cluster: Male fertility		Cluster: Male reproductive toxicity	
Exposure during Adulthood	Not Likely	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
<i>Overall likelihood:</i>	<i>Not Likely</i>	<i>Overall likelihood:</i>	<i>Likely</i>
Cluster: Prematurity		Cluster: Prematurity	
Exposure during Pregnancy	Not Likely	Not applicable	
<i>Overall likelihood:</i>	<i>Not Likely</i>		<i>Not Likely</i>
Cluster: Pre-eclampsia		Cluster: Pre-eclampsia	
Exposure during Adulthood	ALAN	Not applicable	
<i>Overall likelihood:</i>	<i>ALAN</i>		<i>ALAN</i>

9542

9543 3.1.6.4. *In vitro* and Mechanistic studies

9544 Regarding scoring of likelihood of effects in the WoE in the HoC Reproductive effects, a few
 9545 cluster/endpoints were scored Likely and several were scored ALAN, either in animals or human studies.
 9546 In the following, MoA studies for these clusters are considered.

9547 **Developmental toxicity**

9548 The overall likelihood of effects of BPA in animal studies for the cluster developmental toxicity was
 9549 scored ALAN.

9550 In humans, no study offered an understanding on putative MoA pathways related to developmental
 9551 toxicity following exposure to BPA.

9552 In animals, overall, 27 studies considered a MoA related to one of the ALAN effects in animals. For
 9553 males these effects are body weight (54 studies), mammary gland development (three studies), testis
 9554 descent (two studies), and bone development (three studies). For the females seven studies assessed

9555 the mammary gland development, three the bone development, one the age of first estrus, and 47
9556 body weight.

9557 Results from *in vitro* experiments were only related to mammary gland development (12 studies).

9558 Body weight changes and their related MoA are described in the Metabolic section (Chapter 3.1.4.4).

9559 The *in vivo* studies on mammary gland effects show evidence that stromal–epithelial interactions may
9560 play a crucial role in the BPA-induced developmental changes in the mammary gland. Epigenetic effects
9561 of BPA including changes in methylation patterns are frequently reported along with altered expression
9562 of genes involved in proliferation/apoptosis and other cellular functions. Results from two breast cancer
9563 models (i.e. xenograft mouse model and chemically (DMBA) induced cancer) suggest that BPA may
9564 enhance the susceptibility to carcinogenic stimuli. In addition, there is some evidence from *in vitro*
9565 studies with breast cancer cells which supports the hypothesis that BPA promotes changes in
9566 proliferation and pathological processes which ultimately could contribute to carcinogenesis in the
9567 mammary gland. More details are reported in Chapter 3.1.8.4, *In vitro* and Mechanistic studies on
9568 Carcinogenicity and mammary gland proliferative effects.

9569 Bone development studies reported both increased male femoral cortical thickness, male femoral
9570 diaphyseal cortex thickness and female femur length, and decreased male femur length (Lejonklou et
9571 al., 2016 [RefID 3974]; Lind et al., 2017 [RefID 4350]). One of these studies linked the differential
9572 expression of bone marrow gene expression to the sex-specific effects observed (Lind et al., 2017
9573 [RefID 4350]). No *in vitro* studies were available on bone development.

9574 **Female reproductive toxicity**

9575 After the integration of the human and animal evidence, the overall likelihood of effects of BPA for the
9576 cluster female reproductive toxicity was scored Likely.

9577 Two studies performed in human populations were deemed relevant to contribute to our understanding
9578 on the MoA for female reproductive toxicity (Caserta et al., 2013 [RefID 883]; Cao et al., 2018 [RefID
9579 11666]).

9580 Addressing general aspects of female reproductive toxicity, Caserta et al. (2013) [RefID 883] reported
9581 a positive association between BPA and expression of genes involved in hormone response pathways
9582 and in steroid biosynthesis (ER α , ER β , AR, AhR, PXR) in infertile women.

9583 **Effects on ovary**

9584 BPA appears to affect ovarian follicle development at low doses. BPA-induced oxidative stress leading
9585 to altered steroidogenesis is a plausible mechanism.

9586 In humans, Cao et al. (2018) [RefID 11666] found that anti-Müllerian hormone (AMH) and E2 levels in
9587 the follicular fluid of patients with decreased ovarian reserve (DOR) were lower than those of non-DOR
9588 patients and that the BPA concentration was correlated with the AMH and E2 levels in the follicular
9589 fluid.

9590 In animals, Thilagavathi et al. (2017) [RefID 9247] reported that the number of antral follicles in rats
9591 was decreased after BPA 10 $\mu\text{g}/\text{kg}$ bw per day for 12 weeks but increased after 50 and 100 $\mu\text{g}/\text{kg}$ bw
9592 per day. Based on their data, they proposed that BPA-induced oxidative stress leads to antioxidant
9593 depletion in ovary, which leads to elevated level of TBARS, which leads to overexpression of endothelial
9594 nitric oxide (eNOS), which prevents steroidogenic acute regulatory protein (StAR) transport from outer
9595 to inner mitochondria membrane, which inhibits CYP11A1, leading to downregulation of aromatase.

9596 Berger et al. (2016) [RefID 524] reported that in mice dosed during gestation with BPA 0.5–50 $\mu\text{g}/\text{kg}$
9597 bw per day, germ cell nest breakdown was directly inhibited, probably by altering gene expression for
9598 apoptosis, oxidative stress and autophagy. Preantral follicle numbers were decreased, potentially by
9599 causing death of these follicles at time points earlier than 3 months. They concluded that *in utero* BPA
9600 exposure has different effects on the gene expression of steroidogenic enzymes and these effects
9601 depend on the dose of BPA, age and the generation of the mice.

9602 Santamaria et al. (2016) [RefID 6448] reported reduced primordial to primary follicle transition and
9603 altered steroidogenesis in adult rats dosed during gestation and lactation with 0.5 or 50 $\mu\text{g}/\text{kg}$ bw per
9604 day. AR was increased in primordial follicles at 5 $\mu\text{g}/\text{kg}$ bw per day and decreased in primary follicles

9605 at 50 µg/kg bw per day. They concluded that BPA could affect ovulation through different mechanisms
9606 depending on dose but did not speculate on what was causing the altered steroidogenesis.

9607 Santamaria et al. (2017) [RefID 6449] reported reduced ovulatory response to exogenous
9608 gonadotrophins in pre-pubertal female rats dosed during gestation and lactation at 50 µg/kg bw per
9609 day. They suggested that different mechanisms might be leading the follicles to atresia and that loss
9610 of AR in the later stages of follicular development might be part of a paracrine mechanism that affords
9611 protection against premature luteinisation and atresia.

9612 Cao et al. (2018) [RefID 11666] reported that BPA decreased the ovarian reserve in adult mice dosed
9613 with 5–500 µg/kg bw per day for 28 days. They hypothesised that BPA may reduce ovarian granulosa
9614 cell activity and accelerate its apoptosis, leading to the decreased synthesis of AMH. They did not
9615 speculate on upstream mechanisms.

9616 Soleimani Mehranjani and Mansoori (2016) [RefID 5007] reported that adult rats dosed with BPA 60
9617 µg/kg bw per day for 20 days had reduced antral follicles and increased atretic follicles. Concomitant
9618 vitamin C ameliorated these adverse effects. They did not propose a MoA, but antagonism of BPA-
9619 induced oxidative stress is a plausible possibility.

9620 Ganesan and Keating (2016) [RefID 2141] reported that *in vitro* incubation of perinatal rat ovary with
9621 very high dose of BPA (440 µM) reduced primary and secondary follicle numbers after 2 days, followed
9622 by a reduction in primordial follicle numbers after four days and induced ovarian DNA damage. They
9623 concluded that BPA, via biotransformation, may be converted to a DNA alkylating agent. It is noted
9624 that the concentration tested was likely many orders of magnitude higher than achievable *in vivo* levels.

9625 Overall, at lower doses, oxidative stress leading to steroid alterations is a plausible mechanism for
9626 reduced follicle development. DNA damage is only reported at a much higher dose/concentration.

9627 **Effects on uterus**

9628 The possible mechanism of BPA for the effects on uterus in animals (uterus weight and uterus histology,
9629 including non-neoplastic lesions, pre-neoplastic lesions, neoplastic lesions, proliferation and apoptosis
9630 are described here and are also described in Chapter 3.1.8.4 (MoA on Carcinogenicity).

9631 Uterus weight

9632 The CEP Panel considered the likelihood for an increase in uterus weight ALAN during developmental
9633 exposure. No MoA study specially addressed this outcome.

9634 *Uterus histology (non-neoplastic changes)*

9635 No human studies were available addressing these effects, while several non-neoplastic changes were
9636 evaluated in animal studies. These changes can be included in the broad categories of hyperplasia and
9637 metaplasia-

9638 Some MoA studies have investigated *in vitro* the role of BPA in proliferation of uterine cells derived from
9639 tumour cell lines or endometrial biopsies from woman. Wang GH et al. (2013) [RefID 7675] showed
9640 that BPA increased human uterine myoma tissue-derived mesenchymal stem cells (hUM-MSCs)
9641 proliferation in a dose-dependent manner. The same group (Wang KH et al., 2015 [RefID 7676])
9642 showed that BPA increased growth rate efficiency in a dose-dependent manner in human endometrial
9643 carcinoma cells. According to Mannelli et al. (2015) [RefID 4841], BPA demonstrated a proliferative
9644 effect at a concentration of 100 nM after 48 h in *in vitro* decidualised endometrial stromal cells (ESCs)
9645 from hysterectomy specimens. On the contrary, in another study (Forte et al., 2016 [RefID 2056]) BPA
9646 did not affect cell proliferation on human ESCs, derived from endometrial biopsies from woman not
9647 affected by endometriosis.

9648 In conclusion, most MoA studies indicate that BPA may increase the proliferative rate of different types
9649 of uterine cells.

9650 Uterus histology (pre-neoplastic lesions)

9651 No pre-neoplastic lesions were evaluated in human or animal studies and no MoA study specifically
9652 addressed this outcome.

9653 *Uterus histology (neoplastic lesions)*

9654 No neoplastic lesions were evaluated in human studies. Based on evidence from animal (rats and mice)
9655 studies, the overall likelihood of effects of BPA for uterine neoplastic lesions was considered Not Likely.

9656 Uterus histology (proliferation and apoptosis)

9657 Proliferation or apoptosis in uterus were not evaluated in human studies. In animals, apoptosis was
9658 evaluated with standard histology in one study in rats (NTP Clarity Report, 2018/Camacho et al., 2019
9659 [RefID 11370]) that reported an increase (trend) at the highest dose (25000 µg/kg body weight (bw)
9660 per day) at 1 year. However, no effect was demonstrated in the related study (Leung et al., 2020 [RefID
9661 13789]). Based on these divergent results from animal (rat) studies, the overall likelihood of effects of
9662 BPA for uterine proliferative and apoptotic changes was ALAN for apoptosis in the developmental until
9663 adult exposure period. *In vitro* MoA studies do not support/explain the increase of apoptosis observed
9664 in BPA-treated animals in one *in vivo* study.

9665 Chou et al. (2017) [RefID 1210] suggested that BPA exposure might alter miRNA expression to activate
9666 the hedgehog signalling pathway for promoting cell proliferation and decreasing apoptosis in
9667 endometrial tumorigenesis. Ponniah et al. (2015) [RefID 5876] showed that treatment of BeWo cells
9668 with BPA during stress-induced paradigms led to a reduction in apoptosis. Wang KH et al. (2015) [RefID
9669 7676] reported that BPA increases cell migration and invasion ability in human endometrial carcinoma
9670 cell line possibly through MAPK pathway-dependent upregulation of COX-2 gene expression. Wang KH
9671 et al. (2015) [RefID 7676] reported that BPA induces upregulation of COX-2 gene expression that can
9672 involve multiple physiological functions including resistance to apoptosis.

9673 In conclusion, MoA studies do not support/explain the increase of apoptosis observed in BPA-treated
9674 animals in one *in vivo* study.

9675 **Implantation incidence**

9676 As indicated in the WoE chapter, the CEP Panel considered that the evidence from the studies available
9677 indicated a Likely effect of BPA on implantation incidence. No human evidence on mechanisms of action
9678 was available.

9679 In animals, the *in vivo* studies showed an effect of BPA on the number of embryo implantations and
9680 implantation loss (Li et al., 2016 [RefID 4128]; Yuan et al., 2018 [RefID 13593]). BPA caused a change
9681 in tight junction (TJ) proteins in the uterine epithelium during early pregnancy (Martínez-Peña et al.,
9682 2013 [RefID 4909]). Exposure to BPA interfered with ERα-mediated and PGR-mediated signalling
9683 pathways in the uterus during early pregnancy. In *in vitro* studies BPA can downregulate SGK1 and
9684 ENaCa protein expression through ERs in Ishikawa cells which may lead to embryo implantation failure
9685 through the ER/SGK1/ENaCa signalling pathway (Yuan et al., 2018 [RefID 13593]). BPA did not affect
9686 cell proliferation, but arrested ESCs at G2/M phase of cell cycle enhancing cell migration (Forte et al.,
9687 2016 [RefID 2056]). BPA also increased gene expression and protein levels of some decidualisation
9688 markers, such as insulin growth factor binding protein 1 (IGFBP1) and prolactin (PRL), amplifying the
9689 effect of progesterone alone. Data suggest that BPA might alter human endometrium physiology,
9690 hence, affecting fertility and pregnancy outcome. In another *in vitro* study (Olson et al., 2017 [RefID
9691 5518]) BPA impaired steroid-hormone receptor expression at very high concentrations but not at lower
9692 concentrations. Cell proliferation and cyclin D2 expression in the high-dose groups was decreased
9693 compared with controls. These findings demonstrate that BPA disrupts *in vitro* decidualisation of uterine
9694 stromal fibroblasts by altering steroid-hormone receptor expression at higher concentrations but not at
9695 lower physiological doses. BPA treatment could reduce the invasion ability of BeWo human endometrial
9696 cells (model mimicking embryo implantation) and alter the expression level of E-cadherin, DNMT1,
9697 TIMP-1, TIMP-2, MMP-2 and MMP-9 (Wang ZY et al., 2015 [RefID 7852]). BPA activated ERK through
9698 nuclear and membrane ERs and inhibited CXCL8 expression in DSCs, thereby affecting their regulation
9699 of trophoblast invasion (Li X et al., 2018 [RefID 11157]).

9700 Overall, BPA interfered with ERα-mediated and PGR-mediated signalling pathways in the uterus during
9701 early pregnancy *in vivo*. *In vitro* BPA interfered with ERα-mediated and PGR-mediated signalling
9702 pathways in the uterus during early pregnancy BPA, can alter expression of genes associated with
9703 decidualisation, cell cycle regulation and several proliferative markers.

9704 **Male reproductive toxicity**

9705 After the integration of the human and animal evidence, the overall likelihood of effects of BPA for the
9706 cluster male reproductive toxicity was scored ALAN.

9707 **Effects on prostate**

9708 No evidence was available regarding the MoA of BPA for prostate effects in the human studies. In
9709 animals, for histology, the following subclusters were considered if available: non-neoplastic changes,
9710 pre-neoplastic lesions, neoplastic lesions, proliferation and apoptosis.

9711 Seventeen MoA studies specifically addressing carcinogenic effects on the prostate were considered,
9712 five within the mammalian stream and 12 in the *in vitro* stream. MoA studies from the *in vitro* stream
9713 often used prostate cancer cell lines (in seven studies) and in particular LNCaP, PC3 and C4-2 cell lines,
9714 in some cases stem cells (in four studies) and normal cells (in 2 studies). Other studies related to non-
9715 neoplastic changes have also been taken in consideration.

9716 Prostate histology (*in vivo* studies)

9717 Multifocal inflammation and reactive hyperplasia in the ventral prostate were reported in two single-
9718 dose-level Tier 1 rat studies (Bernardo et al., 2015 [RefID 533] and Brandt et al., 2014 [RefID 700],
9719 both from the same institution).

9720 Bernardo et al. (2015) [RefID 533] suggested there was 'stimulated growth of the fetal/neonatal
9721 prostate' and reported that AR expression was increased on PND21 in the ventral prostate at the lower
9722 but not the higher dose (25 not 250 µg/kg bw per day); they suggested that this was consistent with
9723 pre-natal BPA-induced androgen sensitivity leading to an increased expression of AR in the prostate in
9724 pubertal and young adults rodents. Brandt et al. (2014) [RefID 700] suggested that prostate
9725 morphological changes seen on PND21 (but not PND180) after gestational BPA exposure may be related
9726 to delayed prostate morphogenesis, but also that the data are consistent with stimulated growth of the
9727 fetal/neonatal prostate.

9728 Bernardo et al. (2015) [RefID 533] reported that co-treatment with genistein attenuated the BPA effects
9729 and suggested that a mechanism might be ERβ-mediated antagonism of AR function and AR-dependent
9730 proliferation. Brandt et al. (2014) [RefID 700] reported attenuation by co-treatment with indole-3-
9731 carbinol and suggested that this might possibly be related to suppressed responsiveness to oestrogen
9732 and decreased expression of oestrogen receptor alpha (ERα). A detailed rationale for choice of these
9733 bioactive plant compounds and for the doses used was not provided in the papers, but the data do
9734 suggest that oestrogen/androgen balance is involved in BPA early developmental effects.

9735 Mechanisms involved in oestrogen/androgen balance are explored in a number of studies, including
9736 gene and protein expression related to oestrogen/androgen balance in the adult rodent prostate and
9737 developing rodent epididymis:

9738 Castro et al. (2018) [RefID 11674] reported increased plasma E2 and aromatase expression in prostate,
9739 leading to an increase in intraprostatic E2, in juvenile rats after gestational exposure to BPA 10 µg/kg
9740 bw per day.

9741 Wong et al. (2015) [RefID 7997] reported that after neonatal (PND1, 3 and 5) BPA exposure to 2, 10
9742 or 50 µg/kg bw per day, adult rats had upregulated expression of the secretoglobin polypeptide family
9743 member Scgb2a1, with a parallel increase in histone H3 lysine 9 acetylation (H3K9Ac). They noted that
9744 *Scgb2a1* is an AR-regulated gene that exhibits androgenic responsiveness in *in vitro* reporter assays.
9745 but that functional consequence of BPA-mediated developmental reprogramming of Scgb2a1 in the
9746 prostate is not clearly defined.

9747 Wu et al. (2016) [RefID 8036] concluded that low-dose BPA (10–90 µg/kg bw per day) may induce
9748 proliferation of ventral prostate in adult rats by increasing the oestrogen-to-androgen ratio and
9749 upregulating expression of prostaglandin D2 synthase (Ptgds) to promote production of
9750 dihydrotestosterone.

9751 Huang DY et al. (2017) [RefID 2869] concluded that BPA 0.1–1 nM directly promotes the in-vitro-
9752 proliferation of rat prostate cells through increasing the expression of ERs, reducing the expression of
9753 ARs and thus decreasing apoptosis-induced cell death.

9754 Huang DY et al. (2018) [RefID 12167] reported that BPA increased the oestrogen-to-androgen ratio
9755 and upregulated ER α and AR expression, and subsequently further induced the occurrence of epithelial–
9756 mesenchymal transition.

9757 It seems plausible that at low doses, reported BPA effects on developing prostate could involve
9758 oestrogen/androgen balance in some way. However, explicit upstream mechanisms have not been
9759 proposed.

9760 Olukole et al. (2018) [RefID 12841] reported rat prostate hyperplasia and inflammation after high-dose
9761 BPA (10000 μ g/kg bw per day for 14 days). Effects were ameliorated by melatonin, which is considered
9762 to be an antioxidant substance (endogenous free radical scavenger, e.g. Hu C et al. 2021). These
9763 findings are consistent with generation of oxidative stress as a mechanism for high-dose BPA effects
9764 on adult prostate.

9765 At lower doses, there may be other mechanisms. Wu et al. (2016) [RefID 8036] and Huang DY et al.
9766 (2018) [RefID 12167] (from the same laboratory) reported prostate proliferation after 10, 30 and 90
9767 μ g/kg bw per day for 1 or 3 months, respectively. Wu et al. (2016) [RefID 8036] reported that 90 μ g/kg
9768 bw per day BPA had less effects on prostate than 10 μ g/kg bw per day. They concluded that BPA
9769 increased the oestrogen-to-androgen ratio and upregulated prostaglandin D2 synthase (Ptgds)
9770 expression to transport testosterone into the prostate and convert into dihydro-testosterone, which
9771 would cause the prostatic epithelium to proliferate. They noted that it is unclear how BPA affects Ptgds
9772 gene expression, but this is a downstream effect.

9773 *In vivo* studies on mechanisms for rodent developmental BPA-induced prostate inflammation and
9774 reactive hyperplasia have explored a number of endpoints, including alterations in ER-related and AR-
9775 related gene and protein expression in the prostate. Proposed mechanisms include increased expression
9776 of AR, decreased expression of ER α , increased prostate aromatase expression leading to increased
9777 intraprostatic E2, and altered expression of other androgen-related genes (e.g. Scgb2a1, Ptgds).

9778 In conclusion, despite the relatively large amount of data available, there is no consensus on
9779 mechanisms underlying developmental BPA-induced prostate effects, in particular whether effects are
9780 upstream or downstream.

9781 Prostate histology (*in vitro* studies)

- 9782 • Prostate histology (non-neoplastic changes)

9783 No evidence was available regarding the MoA of BPA for this effect in the human studies. Two
9784 histological non-neoplastic findings (inflammation and hyperplasia) were evaluated in animal studies:
9785 based on the inconsistencies in the outcomes and the diversity of the effects, the CEP Panel judged the
9786 prostatic non-neoplastic changes as ALAN.

9787 Regarding inflammation, the results of two Tier 1 related studies (NTP Clarity Report, 2018/Camacho
9788 et al., 2019 [RefID 11370]; Prins et al., 2018 [RefID 13779]) indicated no effect on inflammation. An
9789 increase in multifocal inflammation was observed in the two other related Tier 1 studies (Bernardo et
9790 al., 2015 [RefID 533]; Brandt et al., 2014 [RefID 700]) and a decrease in lymphocytic infiltration was
9791 reported in another study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). Regarding
9792 hyperplasia, two Tier 1 studies (Bernardo et al., 2015 [RefID 533]; Brandt et al., 2014 [RefID 700])
9793 showed an increase, whereas one Tier 1 study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
9794 11370]) showed a decrease. Another related Tier 1 study (Prins et al., 2018 [RefID 13779]) showed no
9795 effect.

9796 Some lines of research have pointed to a potential role for inflammation in prostatic carcinogenesis and
9797 tumour progression in humans. The cause of prostatic inflammation is often unknown, but might be
9798 caused by infection, exposure to chemical, physical trauma, hormonal variations and/or exposures, or
9799 dietary factors. The resultant epithelial cellular injury might cause a loss of tolerance to normal prostatic
9800 antigens, resulting in a self-perpetuating autoimmune reaction. No MoA studies have specifically
9801 investigated *in vitro* the role of BPA in inducing prostate inflammation.

9802 Regarding hyperplasia, a small number of *in vitro* MoA studies indicate that BPA can enhance
9803 proliferation of prostate (see the subcluster proliferation and apoptosis).

9804 In conclusion, *in vitro* MoA studies indicate that BPA may increase the proliferative rate of different
9805 types of prostate cells while no data are available for inflammation.

9806

- Prostate histology (pre-neoplastic lesions)

9807 No evidence was available regarding the MoA of BPA for this effect in the human studies. Two
9808 histological pre-neoplastic findings (atypical hyperplasia and dysplasia) were evaluated in animal studies
9809 in the developmental exposure period and were judged as ALAN.

9810 No *in vitro* MoA study specifically addressed these outcomes.

9811

- Prostate histology (neoplastic lesions)

9812 No evidence was available regarding the MoA of BPA for this effect in the human studies. In animal
9813 studies, no neoplastic lesions of prostate were reported.

9814 Sex hormones play a role in prostate cancer development. Prostate cancer is initially androgen-
9815 dependent and relies on AR activation. Increasing evidence has demonstrated that *in utero* exposure
9816 to oestrogenic compounds may have a significant role decade after these initial exposures in promoting
9817 prostate cancer development through a synergistic effect with testosterone. However, all prostate
9818 cancers eventually become androgen-independent with the participation of many other signalling
9819 molecules.

9820 Some MoA studies have focused their attention on androgen and oestrogen signalling. Prins et al.
9821 (2014) [RefID 5929] assessed whether BPA has oestrogen-like effects on human prostate stem-
9822 progenitor cells. They demonstrated that BPA increases stem-progenitor cell self-renewal and
9823 expression of stem-related genes as well as signalling activation of Akt ERK. The authors proposed
9824 that early-life perturbations in oestrogen signalling, including exposure to BPA, can amplify and modify
9825 the stem-progenitor cell populations of the prostate gland and increase the hormone-dependent cancer
9826 risk. Tarapore et al. (2014) [RefID 7129] showed that BPA can disrupt the centrosome duplication cycle
9827 and maturation, which underlies genomic instability, neoplastic transformation and cancer progression
9828 in the prostate. It was also suggested from the changes in microtubulin dynamics that in the non-
9829 tumorigenic cells, BPA may initiate or promote prostate cancer progression by interfering with AR
9830 function. Ho et al. (2017) [RefID 2727] investigated BPA effects on centrosome amplification, showing
9831 that BPA disrupts centrosome function and microtubule organisation, likely mediated via ER 1 signalling.
9832 Burton et al. (2015) [RefID 763] looked at the effect of E2 and BPA on the expression of histone
9833 modifying enzymes in prostate cancer cell lines. BPA exposure resulted in differential expression, and
9834 the use of an ER antagonist reversed the effects, indicating that BPA can regulate gene expression in
9835 prostate cancer via ER signalling.

9836 Other *in vitro* MoA studies considered epigenetic events or other mechanisms such as modulation of
9837 gene expression, protein expression, signalling pathways, stem cell homeostasis or centrosome activity.
9838 Ho et al. (2015) [RefID 2726] determined whether epigenetic events mediating the action of BPA on
9839 human prostatespheres (PS) enriched in epithelial stem-like/progenitor cells are linked to prostate cancer.
9840 A set of non-coding small nucleolar RNAs with C/D motifs, known as SNORDs, were identified to be
9841 repressed by BPA. The authors considered the action of BPA to be mediated by histone modification of
9842 the exonic/regulatory sequences of the affected SNORDs and not by aberrant methylation of SNORD-
9843 specific CpG sites which might be relevant to prostate cancer carcinogenesis. Prins et al. (2018) [RefID
9844 13779] reported that chronic low-dose BPA exposure in a model of epithelial stem and progenitor cells
9845 isolated by PS culture reprogrammes adult rat prostate stem cell homeostasis with increases in stem
9846 cell numbers and a shift in lineage commitment toward basal progenitor cells, while reducing luminal
9847 progenitor cells. This may underpin the increased carcinogenic risk with ageing. Calderon-Gierszal and
9848 Prins (2015) [RefID 796] reported effects of BPA in a model of prostatic organoids. Budding structures
9849 increased with 1 nM but decreased with 10 nM with a parallel increase in degraded structures. Vimentin
9850 and 963 mRNA expression were also increased at 10 nM as were prostate epithelial cell stemness genes
9851 NANOG, CD49 and OCT4. The authors concluded that nanomolar levels of BPA could modify embryonic
9852 prostate morphogenesis and disrupt prostate stem cell homeostasis in the maturing prostate. Such
9853 stem cell perturbations were considered to potentially increase the risk of human prostate cancer with
9854 ageing. Derouiche et al. (2013) [RefID 1548] reported that environmentally relevant concentrations of
9855 BPA (1–10 nM) induce migration of prostate cancer cells by modulating the cell calcium signalling.

9856 In conclusion, data obtained from *in vitro* MoA studies indicate that BPA can enhance prostate cancer
9857 susceptibility, while its direct carcinogenicity is still debated. However, the results of studies carried out
9858 in laboratory rodents do not demonstrate a clear and direct evidence of tumorigenic activity of BPA.

- 9859 • Prostate histology (proliferation and apoptosis)

9860 No evidence was available regarding the MoA of BPA for this effect in the human studies. In animal
9861 studies carried out in rats, the CEP Panel considered proliferation and apoptosis in the developmental
9862 exposure period ALAN. Brandt et al. (2014) [RefID 700] showed an increase in proliferation at the dose
9863 of 25 µg/kg/bw from GD10 to GD21. This result was not supported by a Tier 2 study (Hass et al., 2016
9864 [RefID 2610]). Brandt et al. (2014) [RefID 700] showed an increase in apoptosis at the highest dose
9865 (250 µg/kg/bw from GD10 to GD21).

9866 A BPA-related increase of proliferation was supported by a few *in vitro* MoA studies. Prins et al. (2018)
9867 [RefID 13779] reported that stem cells, assessed by PS number, doubled with chronic 2.5 µg BPA/kg
9868 bw per day exposures. Moreover, PS size, reflecting progenitor cell proliferation, was greater at 25 and
9869 250 µg BPA doses. Wu et al. (2013) [RefID 8034] conclude that BPA is the androgenic effectors of cell
9870 proliferation. Huang DY et al. (2017) [RefID 2869] investigated the proliferative effect and possible
9871 mechanisms of action of nanomolar BPA on the prostatic epithelium from rats. The results from BPA
9872 doses of 0.1–1 nM showed increased proliferation and upregulated ER but downregulated AR
9873 expression. It was concluded that BPA promotes the proliferation of prostate cells through increasing
9874 the expression of ERs, reducing the expression of ARs.

9875 Regarding apoptosis, Urriola-Muñoz et al. (2018) [RefID 13285] showed that BPA induces apoptosis in
9876 prostate and ovary cancer cell lines, in a process dependent on ADAM17 activation. By contrast, lower
9877 rates of apoptosis were reported by Huang DY et al. (2017) [RefID 2869] on the prostatic epithelium
9878 from rats.

9879 In conclusion, data obtained from a small number of *in vitro* MoA studies indicate that BPA can enhance
9880 proliferation of the prostate supporting the weak evidence gathered from studies in animals. Data from
9881 *in vitro* MoA studies regarding apoptosis do not shed light on this effect.

9882 **Effects on testis**

9883 Chevalier et al. (2015) [RefID 1148] reported a case–control study undertaken with 52 cases of
9884 cryptorchidism (26 transient, 26 persistent) in young boys born after 34 weeks of gestation compared
9885 with 128 healthy young boys matched for gestational age, birthweight and, when possible, parental
9886 geographical origin. Insulin-like peptide 3 (INSL3), a major regulator of testicular descent, was
9887 decreased in idiopathic undescended testis and inversely related, in the whole population of newborn
9888 males, to umbilical cord BPA concentrations. This negative correlation provides indirect evidence for an
9889 impact of BPA on INSL3 Leydig production during fetal development. It suggests that INSL3 is a possible
9890 target of fetal exposure to BPA. However, the deleterious impact of BPA on fetal testicular descent, via
9891 the disturbance of the INSL3 pathway, has yet to be demonstrated directly.

9892 At relatively high dose (>1,000 µg/kg bw per day), NTP Clarity Report (2018)/Camacho et al. (2019)
9893 [RefID 11370] reported polyarteritis in adult rat testis (and in pancreas) after dosing from GD6 at 2,500
9894 µg/kg bw per day only (not at lower or higher doses). They did not comment on the isolated finding;
9895 it is unclear whether this is a treatment effect or a random variation.

9896 Quan et al. (2017) [RefID 6025] reported increased apoptosis in testis at all doses 1,000–100000 µg/kg
9897 bw per day, without dose–response. The proposed mechanism was BPA-induced ROS which attacks
9898 cells in testes directly and induces cell apoptosis.

9899 Xie et al. (2016) [RefID 8130] reported spermatogenic arrest at the spermatocyte level and dose-
9900 related increased germ cell apoptosis, at high subcutaneous doses equivalent to oral 2220, 22220 and
9901 1111000 µg/kg bw per day. In the same study they reported elevated expression levels of ERα and
9902 ERβ and proposed that this likely contributed to the abnormal proliferation of germ cells which then
9903 triggered increased apoptosis. They made no reference to ROS.

9904 At lower doses, Shi et al. (2018) [RefID 13099] reported increased apoptosis at 20 and 50 µg BPA/kg
9905 bw per day with no dose–response, and decreased testis stages VII and VIII seminiferous epithelial
9906 cells only at 20, not at 0.5 or 50 µg/kg bw per day, in neonatal mice. They also measured markers of

9907 oxidative stress and methyltransferases for DNA methylation and histone modification. It is not entirely
9908 clear from their discussion, but it appears that they concluded that increased ROS from damaged
9909 mitochondria was a mechanism in the BPA effects.

9910 Gurmeet et al. (2014) [RefID 2502] reported reduced seminiferous tubule diameter at 100000 but not
9911 at 5,000 or 1,000 $\mu\text{g}/\text{kg}$ bw per day in rats dosed from PND28–70. The study also reported significant
9912 lower level of free plasma testosterone and $17\beta\text{-E}_2$ in the BPA-treated animals; they speculated that
9913 the low testosterone level might have caused failure of spermatogenesis and disruption of the
9914 seminiferous epithelium, and that the low plasma testosterone level in BPA-treated animals was
9915 probably due to interference of proliferative activity and development of Leydig cells. No upstream
9916 mechanism was proposed.

9917 Brouard et al. (2016) [RefID 734] reported increased incidences of seminiferous tubules with lumen,
9918 acrosomal vesicles and acrosome reaction after subcutaneous BPA equivalent to oral 1,800 $\mu\text{g}/\text{kg}$ bw
9919 per day (the only dose tested) PND15–30. They suggested that BPA could be promoting spermatocyte
9920 and spermatid formation via activation of receptors in testis other than classical ERs, such as androgen
9921 (AR), oestrogen-related ($\text{ERR}\gamma$) and $\text{PPAR}\gamma$ receptors. They also reported decreased expression of genes
9922 encoding for blood-testis barrier (BTB) proteins, thus, suggested that BPA could be disrupting the BTB,
9923 presumably as a downstream effect.

9924 Given the relatively high doses in Gurmeet et al. (2014) [RefID 2502] and Brouard et al. (2016) [RefID
9925 734] ($>1,000$ $\mu\text{g}/\text{kg}$ bw per day), it is possible that BPA-induced oxidative stress was involved in
9926 inducing Leydig cell and androgen/oestrogen changes.

9927 However, also at lower doses, BPA-induced oxidative stress is implicated in testis effects.

9928 Ullah et al. (2018a) [RefID 13281] reported reduced rat testis epithelial height and increased peroxidase
9929 in testis of rats dosed 50000 but not 5,000 or 25,000 $\mu\text{g}/\text{kg}$ bw per day for 28 days. Although they
9930 reported that BPA did not significantly induce signs of oxidative stress *in vitro* (1, 10 or 100
9931 ng/mL = 4.4, 44 or 440 pM), the authors concluded that *in vivo* BPA induces oxidative stress, which
9932 reduces testosterone secretion and thus spermatogenesis.

9933 Ullah et al. (2018b) [RefID 13282] reported that markers of oxidative stress in the testis were
9934 significantly elevated and sperm motility and daily sperm production were reduced after dosing 50 but
9935 not 25 or 5 $\mu\text{g}/\text{L}$ in drinking water (at oral dose ~ 2.5 , but not at 1.25 or 0.25 $\mu\text{g}/\text{kg}$ bw per day) from
9936 PND23 for 48 weeks. They also reported reductions in LH, FSH and testosterone, and concluded that
9937 low concentrations of BPA suppress gonadotropin secretion from pituitary, has oestrogenic and
9938 antiandrogenic effects, induces oxidative stress in the testis and affects spermatogenesis by causing
9939 arrest at the spermatogonial and spermatid stage (but the exact mechanism of action is unknown).

9940 **Effects on sperm**

9941 Two publications coming from the same study assessed whether occupational BPA exposure is
9942 associated with altered LINE-1 methylation bearing inconclusive results. Miao et al. (2014) [RefID 5073]
9943 investigated 77 male factory workers routinely exposed to BPA compared with 72 male workers not
9944 routinely exposed, to investigate if BPA exposure is associated with LINE-1 methylation changes in
9945 spermatozoa. The researchers concluded that LINE-1 hypomethylation does not appear to play a major
9946 role for the reported associations between BPA exposure and poor semen quality. In a different analysis,
9947 Tian et al. (2018) [RefID 13256] used 72 male workers routinely exposed via their employment
9948 compared with 86 unexposed workers and found a statistically significant association with LINE-1
9949 methylation changes in spermatozoa. Finally, Zheng et al. (2017) [RefID 8992] considered if BPA
9950 exposure is associated with alteration in DNA hydroxymethylation, a marker for epigenetic modification,
9951 in human sperm and also used a case–control approach with male workers routinely exposed to BPA
9952 as part of their employment compared with those that were not. It was reported that BPA exposure
9953 likely interferes with gene expression via affecting DNA hydroxymethylation in a way partially dependent
9954 on trimethylation of histone 3 in human spermatogenesis.

9955 Park et al. (2018) [RefID 12869] reported decreased sperm motility and increased abnormal sperm in
9956 mice at a single-dose-level (10000 μg BPA/kg bw per day) and increased serum markers of oxidative
9957 stress. BPA effects were ameliorated by a plant extract (*Lespedeza cuneata*) which has been reported
9958 to have anti-oxidative properties.

9959 Dobrzynska et al. (2014) [RefID 1645] reported reduced mouse sperm counts at 5,000–20000 µg
9960 BPA/kg bw per day. They speculated that the mechanism might be oxidative stress disrupting cell
9961 junctions and adhesion between Sertoli and germ cells, leading to decreased number or dysfunction of
9962 Leydig and Sertoli cells.

9963 These effects were seen at relatively high BPA doses (>1,000 µg/kg/d) but other studies reported
9964 effects on sperm at lower doses.

9965 Wang HF et al. (2016) [RefID 7618] reported dose-related reduction in sperm motility in mice dosed
9966 10, 50 or 250 µg BPA/kg bw per day for 8 weeks. They also reported that BPA dose-relatedly reduced
9967 CatSper expression and selectively and transiently inhibited CatSper currents in in mature mouse
9968 spermatozoa (maximum inhibition at about 100 pM). They therefore proposed that the mechanism
9969 involved the CatSper channel, either by downregulating the expression of CatSper or by inhibiting
9970 CatSper currents, although they could not exclude the involvement of other receptors such as ER. They
9971 also reported a dose-related decrease in sperm acrosome reaction at 50 and 250 µg BPA/kg bw per
9972 day. This is likely to be a downstream effect, since the acrosome reaction is dependent on CatSper
9973 (Tamburrino et al., 2014).

9974 Rahman et al. (2015) [RefID 6061] reported that BPA at up to 1,000 nM did not produce significant or
9975 partial toxic effects on spermatozoa; however, at very high concentration (100 µM) BPA inhibited sperm
9976 motility and reduced intracellular ATP levels in spermatozoa, and increased levels of glycolysis/electron
9977 transport chain enzymes (GAPDHS/SDHB), for which the authors could not find a clear mechanistic
9978 explanation.

9979 Rahman et al. (2016) [RefID 6062] reported that BPA 1–100 µM negatively affected *in vitro* mouse
9980 spermatozoa motility, viability, mitochondrial functions and intracellular ATP levels by activating the
9981 MAPK, phosphatidylinositol 3-kinase (PI3K) and protein kinase-A (PKA) pathways. They concluded that
9982 BPA adversely affects sperm function by activating several kinase pathways.

9983 Skibinska et al. (2016) [RefID 6776] reported that BPA increased sperm mitochondrial membrane
9984 potential significantly at 1 nM but significantly decreased at 1,000 nM, with no change in mitochondrial
9985 superoxide formation, sperm vitality or phosphatidylserine membrane translocation. They concluded
9986 that BPA may target mitochondria by increasing the intracellular calcium ion concentration which may
9987 activate mitochondrial protein phosphorylation and in turn dephosphorylates cytochrome *c* oxidase.
9988 Consequently, membrane mitochondrial potential and ROS may increase.

9989 Liu C et al. (2014) [RefID 4378] reported that in the testes of adult male rats dosed oral BPA 20 µg/kg
9990 bw per day daily for 60 days, initiation of meiosis (programmed DNA DSBs) was delayed in the early
9991 meiotic stage (spermatids), and chromosomal abnormalities and meiotic DSBs accumulated in the late
9992 meiotic stage (pachytene spermatocytes), suggesting that spermiation was inhibited and
9993 spermatogenesis was disrupted. The authors had no explanation for the spermiation delay but
9994 suggested that meiotic DSB accumulation in spermatocytes might be caused by BPA-induced inhibition
9995 of DNA break repair (by some unknown mechanism).

9996 Chen et al. (2017) [RefID 1121] reported that testis of adult rats dosed BPA 50 µg/kg bw per day for
9997 35 weeks had altered histone acetylation and upregulated ERβ with no obvious change in ERα or GPER.
9998 They concluded that ERβ is likely involved, at least in part, in the BPA-induced epigenetic changes in
9999 testis tissue (cell type(s) unknown).

10000 Li Y et al. (2018a) [RefID 12491] reported *in vitro* DNA methylation and histone responses to BPA in
10001 spermatogonial cells (mouse cell line GC-1, immortalised by transformation with the plasmid pSV3-neo
10002 in type B spermatogonia). Expression of DNMT1 (DNA methylation enzyme) was increased 1.5-fold at
10003 1 and 10 ng/mL (4.4 and 44 nM); decreased DNMT1 and changes in histone and DNA methylation were
10004 seen only at higher concentrations at which cell growth was inhibited.

10005 **Effects on Epididymis**

10006 NTP Clarity Report (2018)/Camacho et al. (2019) [RefID 11370] reported increased epididymis
10007 exfoliated germ cells and inflammation at 25,000 µg/kg bw per day at interim sacrifice (1 year) but not
10008 at terminal sacrifice (2 years); lower doses (2.5–2,500 µg/kg bw per day) did not affect these endpoints.
10009 This is a high-dose effect; possible mechanisms include both oestrogenic effects and oxidative stress.

10010 Cluster overview for Reproductive and developmental toxicity

10011 BPA effects are reported over a huge range of effective concentrations/doses (low nM and µg/kg bw
10012 per day to high µM and >1,000 µg/kg). This always needs to be considered; there may be qualitative
10013 as well as quantitative differences in mechanisms, depending on dose.

10014 It is clear that many of the reported effects of BPA on male reproductive endpoints are measuring
10015 downstream intermediate pleiotropic effects (e.g. altered expression of many genes and proteins, DNA
10016 methylation and histone changes, activation of MAPK, PI3K, PKA, Akt/mTOR pathways, INSL3, StAR,
10017 etc.).

10018 Details of upstream mechanisms, which are more likely to be BPA specific, are unclear in many cases.
10019 For example, mechanisms for BPA-induced sperm mitochondrial membrane potential changes could
10020 include altered ion channels, receptors (and receptor cross-talk) and ROS.

10021 Receptor interactions are an obvious candidate mechanism for early BPA effects (AR, ER α , ER β , GPER,
10022 ERR γ , PPAR γ , etc.). Some studies have reported receptor-dependency of BPA effects, but the results
10023 are difficult to generalise, because steroid receptor properties and interactions are dynamic; substance
10024 effects can vary by e.g. tissue, life-stage and dose. In fact, much of the complexity of steroid receptor
10025 mechanisms has been revealed by research with BPA.

10026 A plausible upstream mechanism for BPA effects is oxidative stress generation, at both low and high
10027 doses.

10028 In summary, it seems plausible that BPA-induced oxidative stress (with modulation of androgen and
10029 oestrogen pathways, and downstream inflammation) is an early key event in the adverse effects of BPA
10030 on adult male and female reproductive organs.

**10031 3.1.6.5. Conclusion on hazard identification for Reproductive and developmental toxicity
10032 of BPA**

10033 In the previous opinion on BPA assessment (EFSA CEF Panel, 2015), the CEF Panel concluded that the
10034 evidence is not sufficient to infer a causal link between BPA exposure and reproductive and
10035 developmental effects in humans but re-confirmed that BPA is a reproductive toxicant in experimental
10036 animal studies at high doses (above a HED of 3.6 mg/kg bw per day, corresponding to the NOAEL HED
10037 for General toxicity). The CEF Panel assigned a likelihood level of ALAN to reproductive and
10038 developmental effects of BPA in animals at low doses (below HED 3.6 mg/kg bw per day).

10039 In adult animals exposed at doses lower than 3.6 mg/kg bw per day, reproductive effects were
10040 considered to be ALAN; the data suggested that low-dose BPA may have adverse effects on testis
10041 function, especially various measures of spermatogenesis, although these effects were modest, and in
10042 several multigeneration studies no effects were observed at dose levels from as low as 3 µg/kg bw per
10043 day up to at least 50 mg/kg bw per day. There was less evidence that BPA will significantly impair testis
10044 morphology or reproductive endocrinology, especially in the longer term.

10045 In animals exposed *in utero* at doses lower than 3.6 mg/kg bw per day, reported effects on reproductive
10046 function were contradictory and highly variable between studies. A likelihood level of Likely was
10047 assigned to BPA-induced proliferative changes in the mammary gland. The CEF Panel established a
10048 tolerable daily intake which was designated as temporary (t-TDI), pending the outcome of a long-term
10049 study in rats involving pre-natal as well as post-natal exposure to BPA then being undertaken by
10050 NTP/FDA.

10051 Based on the human data, none of the reproduction clusters was considered Likely. Female fertility and
10052 pre-eclampsia after adult exposure, pubertal development after exposure during pregnancy were
10053 considered ALAN. Male fertility after exposure during adulthood, prematurity, fetal and post-natal
10054 growth after exposure during pregnancy and pubertal development after exposure during childhood
10055 were considered Not Likely.

10056 In the animal studies, the likelihood of reproductive effects was assessed by WoE in three clusters
10057 (developmental toxicity, male reproductive toxicity, female reproductive toxicity), each subdivided
10058 according to exposure periods (developmental, developmental and adult, growth phase/young age,
10059 adult and indirect (germline) exposure).

10060 Based on the animal data, several endpoints in both female and male reproductive toxicity clusters
10061 were judged as Likely.

10062 In the female reproductive toxicity cluster, there were Likely effects on ovary weight and histology after
10063 developmental exposure, on implantation rate after growth phase/young age exposure and on follicle
10064 counts after adult exposure. Therefore, these endpoints were taken forward for BMD analysis (see
10065 Chapter 3.2.1).

10066 In the male reproductive toxicity cluster, there were Likely effects on epididymis (exfoliated germ cells
10067 and inflammation) after developmental and adult exposure, on testis histology (increased seminiferous
10068 tubules with lumen and acrosomal vesicles) after growth phase/young age exposure and effects on
10069 sperm motility, morphology, viability and acrosome reaction after adult exposure. Therefore, these
10070 endpoints were taken forward for BMD analysis (see Chapter 3.2.1).

10071 In all three clusters studied in animal studies (developmental toxicity, male reproductive toxicity, female
10072 reproductive toxicity), several endpoints were rated ALAN.

10073 In the Developmental Toxicity cluster, mammary gland and bone development (both sexes) and body
10074 weight (described in Metabolic hazard identification section) after developmental exposure, and also
10075 for age at first oestrus and body weight effects after exposure during growth phase, were judged as
10076 ALAN.

10077 In the Female reproductive toxicity cluster, effects on uterus histology (increase in apoptosis and
10078 squamous metaplasia) after developmental and adult exposure, and on oestrus cyclicity after adult
10079 exposure, were judged as ALAN.

10080 In the Male reproductive toxicity cluster, effects on prostate (inflammation, reactive hyperplasia and
10081 apoptosis) and testis (polyarteritis, inflammation, reduced stage VIII seminiferous epithelial cells and
10082 increased germ cell degeneration) after developmental exposure were judged as ALAN.

10083 After the integration of the human and animal evidence, the overall likelihood of BPA effects was
10084 considered Likely for the clusters Female reproductive toxicity and Male reproductive toxicity, and ALAN
10085 for Developmental toxicity.

10086 Based mainly on the animal data and in reasonable agreement with the human data, a female
10087 reproduction hazard is identified in terms of likely effects on ovary weight and histology after
10088 developmental exposure, on implantation rate after growth phase/young age exposure and on follicle
10089 counts after adult exposure.

10090 Male reproductive toxicity effects identified from the animal data as Likely were epididymis (exfoliated
10091 germ cells and inflammation) after developmental and adult exposure, testis histology (increased
10092 seminiferous tubules with lumen and acrosomal vesicles) after growth phase/young age exposure and
10093 sperm (motility, morphology, viability and acrosome reaction) after adult exposure. This is broadly in
10094 agreement with the previous EFSA conclusion (EFSA CEF Panel, 2015) that doses of BPA below 3.6
10095 mg/kg bw per day may have modest adverse effects on testis function, especially on various measures
10096 of spermatogenesis.

10097 Mechanisms of action for the identified BPA reproductive toxicity endpoints have been non-
10098 systematically explored in the literature. They include oestrogen and AR interactions and associated
10099 downstream and cross-stream effects, including epigenetic changes. Other possible mechanisms,
10100 including notably BPA-induced generation of oxidative stress, have been less explored.

10101

10102 **3.1.7. Cardiotoxicity**

10103 **3.1.7.1. Epidemiological studies**

10104 For the HOC Cardiotoxicity, no information on endpoints related to this HOC was found in the case-
10105 control or cohort studies considered.

10106 The endpoints for each study identified as relevant in this opinion are reported in Annex C. Myocardial
10107 infarction, coronary artery stenosis and peripheral artery disease were key endpoints in the 2015 EFSA
10108 opinion (EFSA CEF Panel, 2015, see Section 3.6.4, WoE of Cardiovascular effects of BPA in humans).

10109 *Identification of the clusters to be considered for WoE*

10110 On the basis on the approach described in Chapter 2.3.2 'Definition of Health Outcome Categories and
10111 Clusters', no clusters related to Cardiotoxicity were identified.

10112 *WoE of the relevant clusters*

10113 No information on endpoints related to Cardiotoxicity was found in the case-control or cohort studies
10114 considered.

10115 Overall conclusions

10116 On the basis of the above, the CEP Panel concluded that the evidence for a positive association between
10117 BPA exposure and Cardiotoxicity is Inadequate.

10118 *Cross-sectional studies*

10119 One cross-sectional study was identified (Xiong et al., 2015 [RefID 8164]) that determined serum BPA
10120 concentrations in patients with dilated cardiomyopathy (DCM, n = 88) and a group of 88 healthy
10121 individuals. BPA levels in the DCM group were significantly higher compared with that in the controls
10122 (6.9 ± 2.7 ng/mL vs. 3.8 ± 1.9 ng/mL, $p < 0.001$).

10123 **3.1.7.2. Animal studies**

10124 For the HOC Cardiotoxicity, a total of 22 studies were appraised by the CEP Panel. The details of the
10125 appraisals (internal and external validity) are reported in Annex E.

10126 The endpoints for each study identified as relevant are reported in Annex F.

10127 *Identification of clusters of relevant endpoints*

10128 In the HOC cardiotoxicity, several clusters were determined and extracted from the available literature
10129 after assessment by the experts: absolute and relative heart weight, incidence of cardiac lesions, cardiac
10130 structural changes as measured by echocardiography, effects on cardiac function as measured by
10131 echocardiography, blood pressure and atherosclerotic lesions.

10132 Elevated blood pressure is the starting event, which if persistent over long time and having a large
10133 effect size will eventually lead to hypertrophy of ventricular myocardium and increased heart weight.
10134 Hypertrophy of ventricular myocardium due to high blood pressure will cause changes of left ventricular
10135 structures, (measured by echocardiography) as well as cardiomyopathy (diagnosed by histopathology).
10136 Both may be the underlying cause of a failing heart function, which can be measured by
10137 echocardiography. Persistent elevated blood pressure is a well known and accepted risk factor for a
10138 number of serious and potentially life-threatening cardiovascular diseases in humans, such as stroke,
10139 heart failure, reduced kidney function and vascular dementia. Persistent elevated blood pressure is also
10140 contributing to the development of atherosclerotic lesion and is a known risk factor for it whereby the
10141 mechanism by which elevated blood pressure contributes to the development of atherosclerosis is not
10142 fully understood.

10143 Oxidative stress as could be discussed as a common underlying molecular mechanism for the described
10144 effects. Oxidative stress is an inducer of cardiac lesions by initiating inflammatory processes which may
10145 lead to atherosclerosis, fibrosis and cardiomyopathy. The link to cardiac hypertrophy, heart weight
10146 increase, cardiac structural changes and increased blood pressure are, however, less clearly
10147 understood. Details of the involved pathways and their messengers can be taken from Elahi et al.
10148 (2009). However, the relevance of this mechanism for humans, especially for females, is currently
10149 unclear (Reckelhoff et al., 2019).

10150 *WoE of the clusters of relevant endpoints*

10151 The main information extracted from the studies addressing relevant endpoints in the HOC
10152 Cardiotoxicity are summarised in Annex G. The outcome of the weight of the evidence is described in
10153 the text below and presented in a tabulated format in Annex H.

10154 The clusters of the effects of BPA on cardiotoxicity considered for this assessment were the following:

- 10155 • Blood pressure
- 10156 • Absolute and relative heart weight
- 10157 • Incidence of cardiac lesions
- 10158 • Cardiac structural changes (as measured by echocardiography)
- 10159 • Effects on cardiac function (as measured by echocardiography)
- 10160 • Atherosclerotic lesions.

10161

10162 **Blood pressure**

10163 Developmental exposure (pre-natal and/or post-natal until weaning)

10164 No studies were available for this exposure period.

10165 Developmental and adult (pre-natal and post-natal in pups until adulthood)

10166 For this exposure period, two studies, one in rats, Desai et al. (2018) [RefID 11817] (Tier 2), and one
10167 in mice, Patel et al. (2013) [RefID 5697] (Tier 3), were identified. In the rat study, an increase in blood
10168 pressure was seen in male rats only, however, no effect was observed in female rats at the single dose
10169 administered of 600 µg/kg bw per day. In the mouse study, the systolic blood pressure was increased
10170 at a dose of 5 µg/kg bw per day but not at 0.5 µg/kg bw per day and not at 200 µg/kg bw per day with
10171 females. Furthermore, in female mice, the diastolic blood pressure was increased at the doses of 0.5,
10172 5, and 200 µg/kg bw per day. However, no dose–response could be identified. In this study, no effect
10173 on blood pressure was observed in male animals. The effect in female animals might be related to the
10174 fact that in female controls, both systolic and diastolic blood pressure was lower than in the male
10175 controls.

10176 Because of the inconsistent results, effects in males in one study and not in the second study, and the
10177 reverse for females and the inconsistency in the dose–response, overall, the CEP Panel considered the
10178 evidence Inadequate to assign a likelihood level to the effect of BPA on blood pressure in the
10179 developmental and adult exposure period, so, this endpoint was not taken forward for BMD analysis.

10180 Growth phase/young age exposure

10181 No studies were available for this exposure period.

10182 Adult exposure (after puberty)

10183 For this exposure period three studies, one in rats (Jiang et al., 2015 [RefID 3189], Tier 2), and two in
10184 mice (Saura et al., 2014 [RefID 6494]; Belcher et al., 2015 [RefID 490], both Tier 3), were identified.

10185 In rats, no effect on the blood pressure was observed in the single-dose study of 50 µg/kg bw per day.
10186 In one of the mouse studies, Saura et al. (2014) [RefID 6494] (sex of the animals not given), the
10187 systolic and the diastolic blood pressure was elevated at 16, 164, 1641 and 16416 µg/kg bw per day.
10188 In the second mouse study, Belcher et al. (2015) [RefID 490], male animals had a decreased systolic
10189 blood pressure at doses of 4.3, 43, 430, 4490 and 44900 µg/kg bw per day, whereas no effect was
10190 seen on the diastolic blood pressure. In female animals, the systolic blood pressure decreased with a
10191 dose of 44,900 µg/kg bw per day and no effect on the diastolic blood pressure was seen. Thus, whereas
10192 no effect was seen on the blood pressure in rats, contradictory results are reported for the two mouse
10193 studies with overlapping dosing, which is considered as unexplained inconsistency.

10194 The CEP Panel assigned a likelihood level of Not Likely to the effect of BPA on blood pressure in the
10195 adult exposure period, so, this endpoint was not taken forward for BMD analysis.

10196 Indirect (germline) exposure

10197 No studies were available for this exposure period.

10198

10199 Overall cluster selection of the endpoints/studies for BMD analysis for Blood pressure

10200 Overall, the CEP Panel assigned a likelihood level of Inadequate evidence/Not Likely to the effects of
10201 BPA on the cardiovascular function (blood pressure) in the exposure periods developmental and adult
10202 and adulthood (after puberty), respectively.

10203 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
10204 Likely effect of BPA in any exposure period, therefore, none of the endpoints was taken forward for
10205 BMD analysis.

10206

10207 **Absolute and relative heart weight**10208 Developmental exposure (pre-natal and/or post-natal until weaning)

10209 For this exposure period, five studies in rats were identified, three in Tier 1 (Cao et al., 2015 [RefID
10210 831]; Gear et al., 2017 [RefID 2229]; NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]),
10211 and two in Tier 2 (Lejonklou et al., 2017 [RefID 3975]; Dunder et al., 2018 [RefID 11866]).

10212 The doses tested encompass five doses in rats in the dose range between 2.5 and 25,000 µg/kg bw
10213 per day in rats (Gear et al., 2017 [RefID 2229] and NTP Clarity Report, 2018/Camacho et al., 2019
10214 [RefID 11370]) and 0.5 and 50 µg/kg bw per day in the studies of Lejonklou et al. (2017) [RefID 3975]
10215 and Dunder et al. (2018) [RefID 11866]. Cao et al. (2015) [RefID 831] investigated one single dose of
10216 240 µg/kg bw per day. Male and female rats were tested.

10217 As no effects were noted at any dose in any study, the CEP Panel assigned a likelihood level of Not
10218 Likely to the effect of BPA on relative heart weight during the developmental exposure period, so, this
10219 endpoint was not taken forward for BMD analysis.

10220 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

10221 For this exposure period, five studies in rats were identified, three in Tier 1 (Cao et al., 2015 [RefID
10222 831]; Gear et al., 2017 [RefID 2229]; NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]),
10223 one in Tier 2 (Dunder et al., 2018 [RefID 11866]) and one in Tier 3 (Patel et al., 2013 [RefID 5697]).
10224 The doses tested encompass five doses in the dose range between 2.5 and 25000 µg/kg bw per day in
10225 rats (Gear et al., 2017 [RefID 2229] and NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370])
10226 and 0.5 and 50 µg/kg bw per day in the studies of Lejonklou et al. (2017) [RefID 3975] and Dunder et
10227 al. (2018) [RefID 11866]. Cao et al. (2015) [RefID 831] investigated one single dose of 240 µg/kg bw
10228 per day. Male and female animals were tested. In the Tier 3 study Patel et al. (2013) [RefID 5697] in
10229 mice, inconsistent results were obtained with a decrease at 5 µg/kg bw per day and no effect at the
10230 others with doses tested of 0.5, 5 and 50 µg/kg bw per day.

10231 As no effects at any dose in any study were noted, the CEP Panel assigned a likelihood level of Not
10232 Likely to the effect of BPA on relative heart weight during the developmental and adult exposure period,
10233 so, this endpoint was not taken forward for BMD analysis.

10234 Growth phase/young age exposure

10235 No studies were available for this exposure period.

10236 Adult exposure (after puberty)

10237 For this exposure period, two studies in rats were identified, both in Tier 2 (Hu et al., 2016 [RefID
10238 2843]; Jiang et al., 2015 [RefID 3189]). Furthermore, results from one Tier 3 study in mice (Belcher et
10239 al., 2015 [RefID 490]) were available.

10240 In Jiang et al. (2015) [RefID 3189] (Tier 2 study) in rats with testing of a single dose (50 µg/kg bw per
10241 day), no effect was observed after 24 weeks but a marginal increase of relative heart weight was seen
10242 after exposure for 48 weeks. In the other Tier 2 study in rats (Hu et al., 2016 [RefID 2843]), a
10243 statistically non-significant increasing trend (doses of 20000 and 100000 µg/kg bw per day) was seen.
10244 In the Tier 3 study in mice, inconsistent results were obtained with a increase in two middle doses

10245 without a clear dose–response with doses tested of 4.3, 43, 430, 4490 and 44900 µg/kg bw per day
10246 (Belcher et al., 2015 [RefID 490]).

10247 The CEP Panel assigned a likelihood level of Not Likely to the effect of BPA on relative heart weight
10248 during the adult exposure (after puberty) period, so, this endpoint was not taken forward for BMD
10249 analysis.

10250 Indirect (germline) exposure

10251 No studies were available for this exposure period.

10252

10253 Overall cluster selection of the endpoints/studies for BMD analysis for absolute and relative heart weight

10254 Overall, the CEP Panel assigned a likelihood level of Not Likely to the effects of BPA on relative heart
10255 weight in the exposure periods developmental, developmental and adult and adulthood (after puberty).

10256 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
10257 Likely effect of BPA in any exposure period, therefore, none of the endpoints was taken forward for
10258 BMD analysis.

10259

10260 **Incidence of cardiac lesions**

10261 Developmental exposure (pre-natal and/or post-natal until weaning)

10262 For this exposure period two studies in rats were identified, all in Tier 1. Increases at 2.5 and 25 µg/kg
10263 bw per day in males only and no effect of the other three doses in the study of NTP Clarity Report
10264 (2018)/Camacho et al. (2019) [RefID 11370] (Tier 1) were observed. No effect in females was noted.
10265 The doses ranged between 2.5 and 25000 µg/kg bw per day. In the other Tier 1 study (Gear et al.,
10266 2018 [RefID 2229]) no effect was shown in males, but an increase in females at 2.5, 25 and 250 µg/kg
10267 bw per day. This effect was judged not treatment related.

10268 Because of the inconsistency of the results, the CEP Panel assigned a likelihood level of Not Likely to
10269 the effect of BPA on the incidence of cardiac lesions in the developmental exposure period, so, none of
10270 the endpoints was taken forward for BMD analysis.

10271 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

10272 In this exposure period, two studies in rats were identified, both in Tier 1 (NTP Clarity Report,
10273 2018/Camacho et al., 2019 [RefID 11370]; Gear et al., 2017 [RefID 2229]). The doses tested were the
10274 same as described above (2.5, 25, 250, 2500, 25000 µg/kg bw per day in both sexes).

10275 No effect was seen in both sexes in both Tier 1 studies over a dose range between 2.5 and 25000
10276 µg/kg bw per day.

10277 The CEP Panel assigned a likelihood level of Not Likely to the effect of BPA on the incidence of cardiac
10278 lesions in the developmental and adult exposure period, so, none of the endpoints was taken forward
10279 for BMD analysis.

10280 Growth phase/young age exposure

10281 No studies were available for this exposure period.

10282 Adult exposure (after puberty)

10283 For this exposure period, two studies in rats (Hu et al., 2016 [RefID 2843]; Jiang et al., 2015 [RefID
10284 3189]) were identified, both in Tier 2, and one study in rabbits (Fang et al., 2014 [RefID 1914])
10285 allocated to Tier 2.

10286 In one rat study (Hu et al., 2016 [RefID 2843]), an increase in fibrosis was seen at doses of 20000 and
10287 100000 µg/kg bw per day, but there was no effect at the only dose (5000 µg/kg bw per day) below
10288 the cut-off (10000 µg/kg bw per day). In this study, only male animals were tested. In the other rat
10289 study (Jiang et al., 2015 [RefID 3189]), cardiac hypertrophy was seen male animals, the only sex
10290 tested, at the only dose tested of 50 µg/kg bw per day. In the rabbit study (Fang et al., 2014 [RefID

10291 1914]), fibrosis was increased at the only dose tested of 400 µg/kg bw per day in male animals. Female
10292 animals were not tested.

10293 As no response was seen in the relevant dose range below the cut-off of 10000 µg/kg bw per day or
10294 only Inadequate evidence was available from single-dose studies, the CEP Panel assigned a likelihood
10295 level of Not Likely to the effect of BPA on the incidence of cardiac lesions in the adult exposure (after
10296 puberty) period, so, none of the endpoints was taken forward for BMD analysis.

10297 Indirect (germline) exposure

10298 No studies were available for this exposure period.

10299 Overall cluster selection of the endpoints/studies for BMD analysis for incidence of cardiac lesions

10300 Overall, the CEP Panel assigned a likelihood level of Not Likely to the effects of BPA on incidence of
10301 cardiac lesions in the exposure periods developmental, developmental and adult and adulthood (after
10302 puberty).

10303 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
10304 Likely effect of BPA in any exposure period, therefore, none of the endpoints was taken forward for
10305 BMD analysis.

10306

10307 **Cardiac structural changes**

10308 Developmental exposure (pre-natal and/or post-natal until weaning)

10309 For this exposure period, one study in rats was identified, allocated to Tier 1 (Gear et al., 2017 [RefID
10310 2229]). The endpoint measured was left ventricular wall thickness (LVWT). Male and female animals
10311 were exposed since GD6 at doses of 2.5, 25, 250, 2500, 25000 µg/kg bw per day. Examination took
10312 place on PND21.

10313 As no effect was observed at any doses in both sexes in the only study available, the CEP Panel assigned
10314 a likelihood level of Not Likely to the effect of BPA on cardiac structural changes (i.e. LVWT) in the
10315 developmental exposure period, so, this endpoint was not taken forward for BMD analysis.

10316 Developmental and adult (pre-natal and post-natal in pups until adulthood)

10317 In this exposure period, two studies were identified, one study in rats allocated to Tier 1 (Gear et al.,
10318 2017 [RefID 2229]) and one study in mice allocated to Tier 3 (Patel et al., 2013 [RefID 5697]).

10319 In Gear et al. (2017) [RefID 2229], the endpoint measured was LVWT. Doses of 2.5, 25, 250, 2500,
10320 25000 µg/kg bw per day were investigated. Exposure was in male and female animals from GD6 to
10321 parturition followed by observational period until termination at 2 years (called stop group) and
10322 exposure GD6 until termination at 2 years (called continuous group). Examination took place at PND90
10323 and 6 months. Male animals had an increase at 250 µg/kg bw per day and female animals had a decrease
10324 at PND90 at 2.5 µg/kg bw per day. No consistent effects were seen in both sexes.

10325 Patel et al. (2013) [RefID 5697] measured left ventricular internal dimension, at diastole (LVIDd) and
10326 found no effect in males and females at doses of 0.5, 5 and 200 µg/kg bw per day. Mice were treated
10327 with BPA 0.5 or 5 µg/kg bw per day from GD11.5 until euthanasia at 4 months. A separate group was
10328 treated with BPA 200 µg/kg bw per day from GD11.5 until weaning. After weaning of the progeny,
10329 dams were treated with vehicle water. In this study relative wall thickness (RWT) was increased in
10330 male animals at all doses, but the effect was in the order 5 > 0.5 > 200 µg/kg bw per day; in female
10331 animals 0.5 µg/kg bw per day had no effect, at 5 µg/kg bw per day an increase was observed and at
10332 200 µg/kg bw per day, again no effect was observed.

10333 The CEP Panel assigned a likelihood level of Not Likely to the effect of BPA on cardiac structural changes
10334 (LVW, LVIDd, RWT) in the developmental and adult exposure period, so, none of the endpoints was
10335 taken forward for BMD analysis.

10336 Growth phase/young age exposure

10337 No studies were available for this exposure period.

10338 Adult exposure (after puberty)

10339 For this exposure period, two studies (Hu et al., 2016 [RefID 2843]; Jiang et al., 2015 [RefID 3189])
10340 in rats were identified, both allocated to Tier 2. One further study in mice, Tier 3 (Belcher et al., 2015
10341 [RefID 490]), was also found. The endpoints measured were indices of diameters of wall or calculated
10342 derived parameters (in systole or in diastole) such as LVWT, interventricular septum (IVS), left
10343 ventricular internal dimension at diastole (LVIDd), left ventricular internal dimension at systole (LVIDs),
10344 left ventricle wall thickness (LVPW) measured by echocardiography.

10345 In the two Tier 2 rat studies, inconsistent effects (only in one dose) or at high doses were observed.
10346 In the study by Hu et al. (2016) [RefID 2843], doses of 5000, 20000 and 100000 µg/kg bw per day
10347 were given for 30 days to male animals. IVS was increased at 100000 µg/kg bw per day (above the
10348 cut-off dose), but no effect was observed at 5000 and 20000 µg/kg bw per day. No effect was shown
10349 for LVIDd and LVIDs, whereas left ventricle posterior wall thickness, at diastole (LVPWd) was increased
10350 at 20000 µg/kg bw per day (above the cut-off dose), no effect of 5000 and 100000 µg/kg bw per day
10351 were observed. In Jiang et al. (2015) [RefID 3189], male animals were dosed with 50 µg/kg bw per
10352 day for 48 weeks. No other doses were tested. LVIDd was increased at 48 weeks with 50 µg/kg bw per
10353 day, but not at 24 weeks; the same findings were described for LVIDs and LVPW with increase at 48
10354 weeks, but not at 24 weeks.

10355 In the Tier 3 mouse study of Belcher et al. (2015) [RefID 490], doses of 50, 500, 5000 and 50000
10356 µg/kg bw per day did not show statistically significant effects on LW thickness at PND90.

10357 The CEP Panel assigned a likelihood level of Not Likely to the effect of BPA on cardiac structural changes
10358 in the adult exposure period, so, none of the endpoints was taken forward for BMD analysis.

10359 Indirect (germline) exposure

10360 No studies were available for this exposure period.

10361

10362 Overall cluster selection of the endpoints/studies for BMD analysis for cardiac structural changes as
10363 **measured by echocardiography**

10364 Overall, the CEP Panel assigned a likelihood level of Not Likely to the effects of BPA on cardiac structural
10365 changes in the exposure periods developmental, developmental and adult and adulthood (after
10366 puberty).

10367 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
10368 Likely effect of BPA in any exposure period, therefore, none of the endpoints was taken forward for
10369 BMD analysis.

10370

10371 Effects on cardiac function**10372 Developmental exposure (pre-natal and/or post-natal until weaning)**

10373 No studies were available for this exposure period.

10374 Developmental and adult (pre-natal and post-natal in pups until adulthood)

10375 No studies were available for this exposure period.

10376 Growth phase/young age exposure

10377 No studies were available for this exposure period.

10378 Adult exposure (after puberty)

10379 Two studies, both allocated to Tier 2 (Hu et al., 2016 [RefID 2843]; Jiang et al., 2015 [RefID 3189]),
10380 were available in which ejection fraction (EF) and fractional shortening (FS) were measured by
10381 echography in rats.

10382 In one of the studies (Jiang et al., 2015 [RefID 3189]), a single dose of 50 µg/kg bw per day,
10383 administered daily in feed to male rats from PND22 to 24 weeks or 48 weeks, respectively, resulted in

10384 no effect at 24 weeks, and a small, clinically not relevant effect, at 48 weeks, on the EF and the FS.
10385 The study was performed in male animals only. Females were not investigated.

10386 In the second study (Hu et al., 2016 [RefID 2843]), at 30 days no effects were observed at doses of
10387 5000 and 20000 µg/kg bw per day and a small effect, a clinically not relevant decrease on EF, was
10388 observed at 100000 µg/kg bw per day. This study was also performed in male animals only.

10389 In the second study, an indication of a small but clinically not relevant decrease in FS and EF is
10390 described. However, no effects at the cumulative dose (dose × duration of exposure) of 8400 µg/kg
10391 (at 24 weeks) and of 150000 µg/kg and 600000 µg/kg (at 30 days) were seen. At cumulative doses of
10392 16,600 µg/kg (48 weeks) and of 3000 mg/kg (at 30 days) a decrease of both related endpoints was
10393 seen.

10394 When taking together the evidence from the two studies, using the cumulative doses, no clear dose-
10395 response was identified; the effect size was small and clinically not relevant.

10396 Given the fact that the effect size was small and clinically not relevant, the CEP Panel assigned a
10397 likelihood level of Not Likely to the effect of BPA on cardiac function in the adult exposure period, so,
10398 none of the endpoints was taken forward for BMD analysis.

10399 Indirect (germline) exposure

10400 No studies were available for this exposure period.

10401 Overall cluster selection of the endpoints/studies for BMD analysis for effects on cardiac function as 10402 measured by echocardiography

10403 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
10404 Likely effect of BPA in any exposure period, therefore, none of the endpoints was taken forward for
10405 BMD analysis.

10406

10407 **Atherosclerotic lesions**

10408 Developmental exposure (pre-natal and/or post-natal until weaning)

10409 No studies were available for this exposure period.

10410 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

10411 No studies were available for this exposure period.

10412 Growth phase/young age exposure

10413 No studies were available for this exposure period.

10414 Adult exposure (after puberty)

10415 One study in rabbits was allocated to Tier 2 (Fang et al., 2014 [RefID 1914]). As the study which found
10416 an increase in atherosclerotic lesions in the aorta at 400 µg/kg bw per day was a single-dose study not
10417 supported by findings in other studies, the CEP Panel considered the evidence Inadequate to assign a
10418 likelihood level to the effect of BPA on atherosclerotic lesions in the adult exposure period, so, this
10419 endpoint was not taken forward for BMD analysis.

10420 Indirect (germline) exposure

10421 No studies were available for this exposure period.

10422 Overall cluster selection of the endpoints/studies for BMD analysis for atherosclerotic lesions

10423 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
10424 Likely effect of BPA in any exposure period, therefore, the endpoint atherosclerotic lesion (arch, thoracic
10425 and abdominal) was not taken forward for BMD analysis.

10426 Overall evidence on Cardiotoxicity

10427 After evaluating the risk of bias of the studies and performing a WoE analysis, the evidence for an
10428 adverse effect was graded in all clusters as Not Likely. Hence, none of the endpoints were carried
10429 forward for BMD analysis.

10430 **3.1.7.3. Integration of likelihoods from human and animal studies**

10431 The integration of the likelihood per cluster from both the human and animal studies available showed
10432 an overall likelihood as Not Likely.

10433 **3.1.7.4. *In vitro* and Mechanistic studies**

10434 Considering that the evidence of effects of BPA on the cardiotoxicity clusters in human and animal
10435 studies were judged as Not Likely or as Inadequate Evidence, the section on *in vitro* and mechanistic
10436 studies was not considered for this HOC.

10437 **3.1.7.5. Conclusions on hazard identification for Cardiotoxicity of BPA**

10438 In the 2015 EFSA opinion (EFSA CEF Panel, 2015), BPA attenuation of repeated and acute exposure to
10439 BPA was demonstrated in adult female rats on cardio-respiratory reflexes elicited by phenylbiguanide
10440 (PBG) (Pant et al., 2012). In the acute experiment, suggesting an influence of BPA on vagal nerve
10441 afferent activity, an extremely high dose of BPA (35 mg/kg bw) was given intravenously. In the 2010
10442 EFSA opinion (EFSA CEF Panel, 2010), two cross-sectional studies reporting associations between BPA
10443 exposure and cardiovascular outcomes. Cardiovascular endpoints were therefore considered relevant
10444 for the new evaluation.

10445 There were indications in the 2015 EFSA opinion (EFSA CEF Panel, 2015) from one prospective study
10446 that BPA exposure may be associated with cardiovascular effects, but it could not be ruled out that the
10447 effect was confounded by diet or other concurrent exposure factors. This association did not provide
10448 sufficient evidence to infer a causal link between BPA exposure and cardiovascular effects in humans.
10449 Potential effects were considered to be ALAN.

10450 In the current assessment, no case-control or cohort studies were available on BPA cardiotoxicity; only
10451 one cross-sectional study was identified (Xiong et al., 2015 [RefID 8164]) that determined serum BPA
10452 concentrations in patients with dilated cardiomyopathy (DCM, n = 88) and a group of 88 healthy
10453 individuals. BPA levels in the DCM group were significantly higher compared with that in the controls.
10454 On the basis of this finding, the CEP Panel concluded that the evidence for an adverse association
10455 between BPA exposure and cardiotoxicity in human is Inadequate.

10456 As for the animal evidence, overall the likelihood for an adverse effect was graded in most the
10457 cardiotoxicity clusters as Not Likely and the others as Inadequate evidence. Hence, none of the
10458 endpoints were brought forward for BMD analysis.

10459 In accordance with the results from 2015, in the current risk assessment of BPA, no clear causal
10460 association was found for cardiotoxicity effects of BPA based on WoE of animal data.

10461

10462 **3.1.8. Carcinogenicity and mammary gland proliferative effects**

10463 **3.1.8.1. Epidemiological studies**

10464 Two human studies were available addressing effects of BPA on carcinogenicity: Tse et al. (2017)
10465 [RefID 7312] on prostate and Costas et al. (2015) [RefID 10171] on lymphoid tissue.

10466 However, the criteria to bring forward to WoE analysis the clusters/exposure periods were not met: i.e.
10467 there were not clusters/exposure periods for which at least two studies were available and at least one
10468 was showing a statistically significant effect for one of the endpoints measured.

10469 3.1.8.2. Animal studies

10470 For the HOC Carcinogenicity and mammary gland proliferative effects a total of 46 studies were
10471 appraised by the CEP Panel. The details of the appraisals (internal and external validity) are reported
10472 in Annex E.

10473 The endpoints for each study identified as relevant are reported in Annex F.

10474 *Identification of clusters of relevant endpoints*

10475 BPA-induced proliferative effects in the mammary gland were already considered key in the uncertainty
10476 analysis in the 2015 EFSA opinion (EFSA CEF Panel, 2015, Section 4.3.2) For more details see Annex
10477 A, Section 2.5.

10478 From the newly available BPA literature, data on mammary gland weight and histology, on uterus
10479 weight and histology and on prostate gland histology were identified as relevant endpoints. As a general
10480 approach, histological changes were subdivided into the following subclusters:

- 10481 • Non-neoplastic changes: that includes endpoints such as hyperplasia, inflammation and other
10482 endpoints which might be related to pathological conditions that ultimately could result in
10483 neoplasia.
- 10484 • Pre-neoplastic lesions: that includes endpoints such as atypical hyperplasia or dysplasia.
- 10485 • Neoplastic lesions: which includes both benign and malignant tumours as endpoints.
- 10486 • Proliferation and apoptosis: which considers data obtained by the quantitative evaluation of
10487 histological sections stained with specific markers of proliferation (e.g. Ki67, proliferating cell
10488 nuclear antigen (PCNA)) or apoptosis (e.g. caspase 3, terminal deoxynucleotidyl transferase
10489 dUTP nick end labeling (TUNEL)).

10490 Even if the endpoints taken into consideration have been divided into different subclusters, it must be
10491 considered that many of these are related/connected to each other (e.g. hyperplasia seen with standard
10492 histology and proliferation detected with immunohistochemistry) or biologically (dysplasia that can
10493 progress in a tumour).

10494 For the histologic evaluation, also whole mount techniques were described in some studies. These
10495 techniques consist in the microscopic examination of an entire organism or organ small enough or thin
10496 enough to be placed directly onto a slide and then stained. Along with histology and molecular biology
10497 techniques, the whole mount evaluation of mammary glands has proven useful in detecting changes
10498 on the entire 3D epithelial structure of the mammary gland related to the treatment with endocrine
10499 disrupting chemicals in rodents (Mandrup et al., 2015).

10500 Effects on Mammary gland weight

10501 In addition to physiological changes during pregnancy and lactation, pathological processes or
10502 xenobiotic-induced increases may affect mammary gland weights (Greaves, 2007). An abnormal
10503 increase of mammary gland weight is a parameter that can be related to proliferative processes and
10504 especially neoplasia (Sacco et al., 2000).

10505 Effects on Mammary gland histology

10506 The following relevant endpoints were retrieved for the cluster mammary gland effects:

- 10507 • non-neoplastic changes;
- 10508 • pre-neoplastic lesions;
- 10509 • neoplastic lesions;
- 10510 • proliferation and apoptosis.

10511 Given the similarities in alterations in growth and differentiation induced by environmental chemicals in
10512 mammary gland development and carcinogenesis in humans and experimental animals, rodent models
10513 can be used as reasonable surrogates for human mammary gland development (Rudel et al., 2011).
10514 Transient or persistent developmental effects can be induced by such chemicals depending on their

10515 dose and the exposure period (Fenton, 2006). Undifferentiated mammary gland structures in particular
10516 TEBs are considered sensitive to chemical carcinogens and eventually may give rise to carcinomas,
10517 whereas benign neoplastic lesions such as adenomas arise from more differentiated structures. TEBs
10518 are characterised by a high rate of cell proliferation which decreases progressively towards the ductal
10519 portions, the more differentiated alveolar buds and lobes (Russo, 2015). An increased number of TEBs
10520 and ducts due to lateral branching as well as increased gland density are associated with an increased
10521 risk of breast cancer (Muñoz-de-Toro et al., 2005). Therefore, non-neoplastic findings detected in
10522 mammary glands of rodents following BPA treatment (such as changes in TEBs, TDs, lobular
10523 hyperplasia, longitudinal growth, branching, gland density and ductal dilatation) were considered
10524 relevant endpoints for human risk assessment of BPA.

10525 Usually, long-term studies have to be conducted to detect the induction of mammary neoplastic lesions
10526 by non-genotoxic chemicals in rodents which also spontaneously develop such lesions during ageing.
10527 Adenocarcinomas are known to arise either spontaneously or be induced by chronic administration of
10528 estrogens and xenobiotics in rodents (Greaves, 2007; Russo, 2015). Therefore, a chronic study with
10529 BPA included in the current evaluation was particularly relevant for detecting pre-neoplastic (atypical
10530 foci) and neoplastic lesions (adenomas and adenocarcinomas). Epithelial foci with unusual growth
10531 patterns are considered as pre-neoplastic lesions which may develop into adenocarcinomas (Russo,
10532 2015). Adenomas are glandular neoplastic lesions without atypical cytological features of malignancy
10533 while adenocarcinomas infiltrate the surrounding tissue in rodents (Greaves, 2007).

10534 For the carcinogenic process the ratio of proliferation to apoptosis is important. The administration of
10535 genotoxic carcinogens in rodents is known to induce excessive proliferation of the glandular epithelium
10536 (Russo, 2015), the intraductal proliferation with multiple layers of epithelial cells is considered a pre-
10537 neoplastic lesion. For BPA, marked decreases of apoptotic cells in TEBs was reported (Muñoz-de-Toro
10538 et al., 2005). Thus, both processes proliferation and reduced apoptosis may result in hyperplastic
10539 changes in the mammary gland.

10540 **Effects on Prostate histology**

10541 The following relevant endpoints were retrieved for the cluster prostate effects:

- 10542 • non-neoplastic changes;
- 10543 • pre-neoplastic lesions;
- 10544 • proliferation and apoptosis.

10545 Among non-neoplastic changes, inflammation was reported as a relevant endpoint in a number of
10546 studies carried out on rats. In some cases, the type or the distribution of the inflammatory process was
10547 specified as 'suppurative', 'lymphocytic infiltration', 'multifocal' or 'reactive atypia'. The findings were
10548 different depending on the anatomical part of the prostate gland considered (ventral vs dorsolateral).
10549 Exposure to chemicals and hormonal exposures are considered among the numerous causes of
10550 prostatitis in humans. Moreover, a potential role for inflammation in prostatic carcinogenesis and
10551 tumour progression in humans has been proposed. Hyperplasia was also reported as a relevant
10552 endpoint in a number of studies carried out on rats. In some cases, this finding was further specified
10553 as 'epithelial' or 'reactive' or 'functional'. Reactive hyperplasia is a reparative process characterised by
10554 hyperplasia of acinar epithelium in response to degeneration and inflammation, which is generally
10555 caused by an infection (Creasy et al., 2012). Functional hyperplasia is an enlargement of the prostate
10556 gland caused by hypertrophy and hyperplasia of the epithelial cells in response to increased demand
10557 or hormonal stimulation (Creasy et al., 2012). Other endpoints were reported in a single study: atrophic
10558 tubules, glandular diameter and vascular congestion.

10559 Pre-neoplastic lesions were considered as relevant endpoints in few rat studies. In this subcluster
10560 atypical hyperplasia and dysplasia (that can be considered synonym of atypical hyperplasia (Creasy et
10561 al., 2012) were included. Atypical hyperplasia is considered a pre-neoplastic lesion in mouse and rat
10562 prostate cancer models since there appears to be a morphologic continuum between atypical
10563 hyperplasia and benign adenoma of the prostate and the distinction between the two is not always
10564 clear (Creasy et al., 2012).

10565 Proliferation and programmed cell death (apoptosis) were investigated and reported in some studies.
10566 Dysregulations if these processes can create a favourable environment for tumour development and

10567 progression. An increase in the proliferation rate is a key event in the following, already mentioned
10568 endpoints: all types of hyperplasia (including atypical hyperplasia/dysplasia) and neoplasia.

10569 **Effects on Uterus weight**

10570 Uterus weight was considered as relevant endpoint in a number of studies in rats, mice and in one
10571 study in hamsters. An increase of uterus weight is a sensitive method to detect ER agonists. This can
10572 be used to evaluate the ability of a test chemical to elicit biological activities consistent with oestrogenic
10573 agonists (uterotrophic assay) (Marty and O'Connor, 2014).

10574 **Effects on Uterus histology**

10575 The following relevant endpoints were retrieved for the cluster uterus histology:

- 10576 • non-neoplastic changes;
- 10577 • neoplastic lesions;
- 10578 • proliferation and apoptosis.

10579 Among non-neoplastic changes, squamous metaplasia was reported as a relevant endpoint. Squamous
10580 metaplasia is characterised by the presence of stratified squamous epithelium that is replacing the
10581 normal uterine columnar lining epithelium. Squamous metaplasia often develops in the rat and mouse
10582 uterus under oestrogen dominance, for example in response to elevated endogenous oestrogen level
10583 or when treated with high doses of oestrogenic compounds (Dixon et al., 2014). Endometrial (cystic)
10584 hyperplasia is characterised by an increase of proliferating endometrial cells. Prolonged oestrogen
10585 excess or administration of xenobiotics with oestrogenic effects can induce endometrial hyperplasia
10586 (Dixon et al., 2014). Dilation of the uterine horns is a common finding in laboratory rodents and is due
10587 to the accumulation of watery fluid in the uterine lumen. This change is a physiological feature that
10588 occur during the pro-estrus and estrus phases under the influence of oestrogen. In *in vivo* studies, an
10589 increased incidence of luminal dilation can indicate a drug-induced alteration of the hormonal status
10590 (e.g. relative oestrogen dominance) (Dixon et al., 2014).

10591 A neoplastic lesion, stromal polyps, was considered as relevant endpoint in one study in rats. Stromal
10592 polyps are polypoid masses protruding into the uterine lumen; the predominant bulk of the lesion is
10593 composed of stromal spindle-shaped or stellate cells. Growth is expansive without invasion (Dixon et
10594 al., 2014). Stromal polyps are a common spontaneous uterine lesion in rats. There is limited information
10595 concerning the aetiology and significance of these polyps as an endpoint in toxicology and
10596 carcinogenicity studies. Uterine endometrial polyps that occur in women and the uterine stromal polyps
10597 that occur in rodents have distinct characteristics; both lesions are benign (Davis, 2012).

10598 Proliferation and programmed cell death (apoptosis) were investigated and reported in some studies
10599 using different histological techniques: 5-bromo-2'-deoxyuridine (BrdU) incorporation for proliferation,
10600 standard histology or TUNEL for apoptosis. Dysregulations of these processes can promote the
10601 development and progression of pre-neoplastic and neoplastic lesions.

10602

10603 *WoE of the clusters of relevant endpoints*

10604 The clusters of the effects of BPA on Carcinogenicity and mammary gland proliferation effects in this
10605 assessment were the following:

- 10606 • mammary gland weight
- 10607 • mammary gland histology
- 10608 • prostate histology;
- 10609 • uterus weight;
- 10610 • uterus histology.

10611 For histology, the following subclusters were considered if available:

- 10612 • non-neoplastic changes;

- 10613 • pre-neoplastic lesions;
- 10614 • neoplastic lesions;
- 10615 • proliferation and apoptosis (immunohistochemistry).

10616

10617 **Effects on mammary gland weight**

10618 Developmental exposure (pre-natal and/or post-natal until weaning)

10619 For effects on mammary gland weight in this exposure period only one Tier 1 study in rats was identified
10620 (Montévil et al., 2020 [RefID 13788]).

10621 The mammary gland weight in female rats was determined at PND90 (Montévil et al., 2020 [RefID
10622 13788]). Based on the authors' description it appears that a data-driven approach was used for
10623 identifying a NMDR by defining a step function around the doses of 25 and 250 µg/kg bw per day.
10624 However, when using more conventional approaches for risk assessment, e.g. modelling the data in
10625 PROAST (Hill and Exponential models) or using spline and polynomial fit (without overfitting the data),
10626 no dose–response was identified by the CEP Panel. The CEP Panel considered that alternative
10627 interpretations of the data may be plausible, e.g. that a significant difference between the dose group
10628 with 25 µg/kg bw per day and the controls, in the absence of dose–response, may be likely explained
10629 by random fluctuations and variability in the data. These considerations also apply to other NMDRs for
10630 histological findings in this study.

10631 Developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood)

10632 No studies were available for this exposure period.

10633 Growth phase/young age exposure

10634 No studies were available for this exposure period.

10635 Adult exposure (after puberty)

10636 No studies were available for this exposure period.

10637 Indirect (germline) exposure

10638 No studies were available for this exposure period.

10639

10640 Overall cluster selection of the endpoints/studies for BMD analysis for mammary gland weight effects:

10641 The CEP Panel assigned a likelihood level of Not Likely to the mammary gland weight effects of BPA in
10642 the developmental exposure period.

10643 The CEP Panel considered that the evidence from the study available did not indicate a Likely or Very
10644 Likely effect of BPA in any exposure period, therefore this endpoint was not taken forward for BMD
10645 analysis.

10646

10647 **Effects on mammary gland histology**

10648 Developmental exposure (pre-natal and/or post-natal until weaning)

10649 For histological effects in this exposure period seven studies were identified, i.e. one Tier 1 study in
10650 mice (Tucker et al., 2018 [RefID 13275]), five Tier 1 studies in rats (Grassi et al., 2016 [RefID 2387];
10651 Kass et al., 2015 [RefID 3402]; Mandrup et al., 2016 [RefID 4831]; NTP Clarity Report, 2018/Camacho
10652 et al., 2019 [RefID 11370]; Montévil et al., 2020 [RefID 13788] and one Tier 3 study in rats (Leung et
10653 al., 2017 [RefID 3990]).

10654 Numerous histological non-neoplastic changes were evaluated using the whole mount technique. No
10655 change in mammary gland density was seen at PND30 in female and male rats by Kass et al. (2015)
10656 [RefID 3402]. Montévil et al. (2020) [RefID 13788], who assessed samples from the CLARITY study
10657 (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]), reported several effects at single doses
10658 in female F1 pups mammary glands: there were increases of gland density, Fractal dimension 3D, Dim
10659 3 (third dimension from Principal Component Analysis) and angles of branches between beginning and

10660 end with a breaking point between 25 and 250 µg/kg bw per day, thickness of the epithelium and
10661 variation of ductal thickness at 25 µg/kg bw per day and an increased average branch length at 250
10662 µg/kg bw per day. In addition, there were decreases of the gland depth, the proportion of the small
10663 and very small branches, the maximum branch length and topological asymmetry of the epithelial trees
10664 (i.e. they were more symmetric in the 250 µg BPA group) the lateral branching at 250 µg/kg bw per
10665 day and the aspect ratio at 2.5 and 250 µg/kg bw per day (smaller AR means that the glands were
10666 rounder). Some of these results (gland density, Dimension 3D and angles of branches between
10667 beginning and end, thickness of epithelium, variation of ductal thickness, aspect ratio) were re-analysed
10668 by the CEP Panel. This re-analysis revealed that a formal dose–response is not identified by fitting
10669 flexible biologically based functions or polynomials that are commonly used to describe biological
10670 systems. Without further biological explanations justifying a NMDR instead of a deviation due to chance,
10671 it would be reasonable to conclude that there is no dose–response but rather single-dose effects, apart
10672 for variation for ductal thickness and aspect ratio where a potential NMDR may occur.

10673 In the CLARITY study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) independent
10674 changes in mammary glands of female rats were observed at 1 year at some doses only, such as
10675 decreased incidences of ductal dilatation and lobular hyperplasia, which was not considered adverse.
10676 However, no effect on ductal dilatation (3, 8 and 14 months) was found in female mice (Tucker et al.,
10677 2018 [RefID 13275]) and no lobular hyperplasia was observed by Montévil et al. (2020) [RefID 13788]
10678 at PND90 in female rats with the same treatment as used in the CLARITY study.

10679 No effects on lobuloalveolar structures were found in another Tier 1 study (Mandrup et al.,
10680 2016 [RefID 4831]) in female rats but a decrease was seen in male rats at PND100 along with a
10681 potential increase in the tubuloalveolar pattern ('in very few structures in each gland') indicating a
10682 female-like morphology at high doses (5000 µg and 50000 µg/kg bw per day). At the lowest dose (25
10683 µg/kg bw per day) used in this study, mammary glands of males at PND22 showed a higher longitudinal
10684 growth and a lower distance to lymph node. A reduction of the distance between lymph node and final
10685 edge (ductal growth) was reported at PND30 in male rats exposed to 64 µg/kg bw per day via drinking
10686 water (average oral dose) (Kass et al., 2015 [RefID 3402]). Additionally, in Kass et al. (2015) [RefID
10687 3402] male pups were exposed to BPA by s.c. injection of 25 and 250 µg/kg bw per day (converted to
10688 the correspondent oral doses 892.5 and 8,925 µg/kg bw per day) from GD9 until GD23, resulting in an
10689 increased ductal growth at PND5 (transient effect) and a delay in ductal growth when assessed on
10690 PND30, both at the higher dose. No changes in ductal length were observed at PND21 in female rats
10691 when exposed to 25 or 250 µg/kg bw per day from GD10–GD21 (Grassi et al., 2016 [RefID 2387]). No
10692 changes in longitudinal growth at PND20 or in lobuloalveolar hyperplasia at 8 and 14 months were
10693 reported in female mice exposed to high doses (500, 5000 or 50000 µg/kg bw per day) during gestation
10694 (Tucker et al., 2018 [RefID 13275]). In the same study, there was an increase in branching density at
10695 the low dose at PND20. The branching score in female rats on PND21 increased at a low dose (25
10696 µg/kg bw per day; Grassi et al., 2016 [RefID 2387]) while no changes in branching occurred in females
10697 at PND100 (Mandrup et al., 2016 [RefID 4831]). At 6 months reduced lateral branching was observed
10698 at 250 µg/kg bw per day in female rats (Montévil et al., 2020 [RefID 13788]). In female mice increases
10699 in TEB length and TEB counts along with higher developmental scores were observed in the mid-dose
10700 group (Tucker et al., 2018 [RefID 13275]). An increase in the number of TEB was also reported in
10701 female rats fed a high butter fat (HBF) diet at low BPA doses (2.5 and 25 µg/kg bw per day) compared
10702 with HBF controls in a Tier 3 study (Leung et al., 2017 [RefID 3990]). However, decreases in the
10703 number of TEBs were reported (at PND15 and PND30) along with a reduced ductal growth (at PND30)
10704 in male but not in female rats exposed via drinking water to 64 µg/kg bw per day (Kass et al., 2015
10705 [RefID 3402]), possibly indicating a delayed gland development in male rats. At PND21 the number of
10706 TDs increased in female rats at a low dose (25 µg/kg bw per day) without changes in the number of
10707 TEBs (Grassi et al., 2016 [RefID 2387]). In summary, for three non-neoplastic endpoints (ductal
10708 dilatation, TEBs and effects on branching/branches) significant effects were observed in females but
10709 not with consistent findings in studies with different study designs. Additionally, in two Tier 1 studies
10710 on male rats effects (ductal growth and alveolar morphology) were observed (Mandrup et al., 2016
10711 [RefID 4831]; Kass et al., 2015 [RefID 3402]). While no effects in alveolar dilatation were reported on
10712 mammary glands in males in the NTP Clarity Report (2018)/Camacho et al. (2019) [RefID 11370].
10713 Taking the diversity of the effects and their time and dose dependency into account, the CEP Panel
10714 judged the induction of non-neoplastic changes in mammary gland of female and male rats as ALAN.

10715 **Pre-neoplastic lesions** in mammary glands were examined in two Tier 1 studies with female rats: a
10716 significant increase in intraductal hyperplasia at the second lowest dose (250 µg/kg bw per day) at
10717 PND400 was reported by Kass et al. (2015) [RefID 3402] but no changes in pre-neoplastic lesions
10718 including atypical foci were observed at 1 or 2 years in the CLARITY study (NTP Clarity
10719 Report, 2018/Camacho et al., 2019 [RefID 11370]). Based on the outcomes in these two studies the
10720 CEP Panel judged the induction of pre-neoplastic lesions in mammary glands in female rats as Not Likely
10721 in this exposure period.

10722 **Neoplastic lesions** were reported in female rats at 2 years in the CLARITY study (NTP Clarity
10723 Report, 2018/Camacho et al., 2019 [RefID 11370]). In the lowest dose group (2.5 µg/kg bw per day)
10724 the incidence of adenocarcinomas (22% vs 6% in controls) and of combined adenomas and
10725 adenocarcinomas (24% vs 8%) was significantly increased, while incidences of adenocarcinomas of
10726 10%, 14%, 18% and 11% were observed at 25, 250, 2500 and 25000 µg/kg bw per day, respectively,
10727 and did not differ significantly from controls. No adenomas or adenocarcinomas were observed at 1
10728 year. Also at 6 months, there were neither adenomas nor adenocarcinomas in females while ductal
10729 carcinomas *in situ* (in 2 out of 10 females) were reported at an early timepoint of PND90 in the mid-
10730 dose group treated with 250 µg/kg bw per day (Montévil et al., 2020 [RefID 13788]). Also, no
10731 carcinomas were found in any dose group of female mice at 14 months (Tucker et al., 2018 [RefID
10732 13275]). Based at the age-dependent findings of adenomas or adenocarcinomas at 2 years the CEP
10733 Panel judged the neoplastic lesions in female rats as Likely.

10734 Immunohistochemistry results did not indicate significant changes in proliferation in TEBs of mammary
10735 glands in female rats at PND21 ($p = 0.056$ only at 25 µg/kg bw per day: Grassi et al., 2016 [RefID
10736 2387]) or in glands of female and male rats at PND30 (Kass et al., 2015 [RefID 3402]). Based on these
10737 studies the CEP Panel judged the induction of proliferation in mammary gland of female and male rats
10738 as Not Likely.

10739 The CEP Panel assigned a likelihood level of ALAN to the mammary gland histological effects of BPA in
10740 the developmental exposure period, based on the non-neoplastic histological findings in female and
10741 male rats (ALAN) and neoplastic (Likely) histological findings in studies with female rats, while no clear
10742 evidence of proliferation (immunohistochemistry) and pre-neoplastic outcomes was reported
10743 Consequently, none of these endpoints was taken forward for BMD analysis. However, the Likely and
10744 ALAN endpoints were considered in the uncertainty analysis (see Appendix D).

10745 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

10746 For histological effects in this exposure period two Tier 1 studies in rats were identified (NTP Clarity
10747 Report, 2018/Camacho et al., 2019 [RefID 11370]; Montévil et al., 2020 [RefID 13788]).

10748 Among the non-neoplastic effects identified in this exposure group several changes were only reported
10749 for one dose group in female rats, i.e. an increase in lobular alveolar budding at 250 µg/kg bw per day
10750 at PND90 (Montévil et al., 2020 [RefID 13788]), changes in ductal dilatation (increase at 1 year;
10751 decrease at 2 years) and a decrease in lobular hyperplasia, both at 25 µg/kg bw per day (NTP Clarity
10752 Report, 2018/Camacho et al., 2019 [RefID 11370]); therefore, no dose–response could be established.
10753 An increase in alveolar dilatation was only reported in males at the lowest dose after 2 years (NTP
10754 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) while the effect was not significant in females
10755 in both studies. Montévil et al. (2020) [RefID 13788] reported a NMDR for mammary gland scores at
10756 2.5 (accelerated gland development) and 25 µg/kg bw per day (no significantly increased proliferation)
10757 on PND90 in rats from a 90-day pilot study (Delclos et al., 2014) for females in oestrus. No changes in
10758 lobular hyperplasia were observed at any dose on PND90 in Montévil et al. (2020) [RefID 13788]. The
10759 same authors reported significant decreases in average gland density in the rostral area (area 1) and
10760 in the middle of the gland (area 2) at 250 µg/kg bw per day on PND90 which were reported to be
10761 significant when compared with controls. Montévil et al. (2020) [RefID 13788] reported that gland
10762 density in the anterior area (area 3) showed a NMDR with a breaking point between 25 and 250 µg/kg
10763 bw per day. However, these data were re-analysed by the CEP Panel, revealing that a formal dose–
10764 response was not identified by fitting flexible biologically based functions or polynomials that are
10765 commonly used to describe biological systems. Without further biological explanations justifying a
10766 NMDR instead of a deviation due to chance, it would be reasonable to conclude that there is no dose–
10767 response but single-dose effects. Regarding mammary gland score, the data were in line with the
10768 definition by the CEP Panel of indications for a NMDR. Taking this and the diversity of the effects into

10769 account the CEP Panel judged the induction of non-neoplastic changes in mammary gland of female
10770 and male rats as ALAN.

10771 Regarding pre-neoplastic lesions the only reported effect was a significant increase in atypical foci in
10772 the 2.5 µg/kg bw per day dose group of females after one and 2 years of treatment. In addition, there
10773 were non-significant increases in the 25 and 250 µg/kg bw per day dose groups (9% and 8%,
10774 respectively, vs 0% in controls) at 1 year (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
10775 11370]). The CEP Panel judged the lesions in this exposure group as ALAN.

10776 No significant effects on neoplastic lesions were observed in the two studies. The incidences of
10777 adenocarcinomas in the two lowest dose groups (2.5 and 25 µg/kg bw per day) were both 4%
10778 compared with 0% in controls at 1 year and 18–20% compared with 12% in controls at 2 years (NTP
10779 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]). At 6 months no adenomas or
10780 adenocarcinomas were observed in any dose group (Montévil et al., 2020 [RefID 13788]). Therefore,
10781 the CEP Panel judged the neoplastic effects in this exposure group as Not Likely.

10782 The CEP Panel assigned a likelihood level of ALAN to the mammary gland effects of BPA during the
10783 developmental and adult exposure period, based on some non-neoplastic changes in female and male
10784 rats (ALAN) and pre-neoplastic findings in female rats (ALAN). Hence, none of the endpoints was taken
10785 forward for BMD analysis. However, they were considered in the uncertainty analysis (see Appendix D).

10786 Growth phase/young age exposure

10787 No studies were available for this exposure period.

10788 Adult exposure (after puberty)

10789 No studies were available for this exposure period.

10790 Indirect (germline) exposure

10791 No studies were available for this exposure period.

10792 Overall cluster selection of the endpoints/studies for BMD analysis for mammary gland histological 10793 effects:

10794 The CEP Panel noted the inconsistency in the likely pre-neoplastic lesions (ALAN) in the absence of
10795 neoplastic lesions during developmental to adult exposure, while in the developmental exposure period
10796 there was only evidence of neoplastic lesions (Likely) but not of pre-neoplastic lesions. Therefore, the
10797 overall likelihood in the cluster mammary gland effects across all exposure periods was ALAN.

10798 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
10799 Likely effect of BPA in the cluster mammary gland histological effects in any exposure period, therefore,
10800 none of the endpoints was taken forward for BMD analysis.

10801

10802 **Effects on prostate gland histology**

10803 Developmental exposure (pre-natal and/or post-natal until weaning)

10804 For effects on prostate following developmental exposure of BPA, four Tier 1 studies in rats (Bernardo
10805 et al., 2015 [RefID 533]; Brandt et al., 2014 [RefID 700]; NTP Clarity Report, 2018/Camacho et al.,
10806 2019 [RefID 11370]; Prins et al., 2018 [RefID 13779]), one Tier 2 study in rats (Hass et al., 2016 [RefID
10807 2610]) and one Tier 3 study in rats (Prins et al., 2017 [RefID 5930]) were identified.

10808 Two histological non-neoplastic changes were evaluated: inflammation and hyperplasia. Regarding
10809 inflammation, the results of a Tier 1 (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370])
10810 for suppurative inflammation and of a Tier 1 Grantee study (Prins et al. (2018) [RefID 13779]), were
10811 supported by a Tier 2 study (Hass et al., 2016 [RefID 2610]) and by a Tier 3 study (Prins et al., 2017
10812 [RefID 5930]) indicating no effects on inflammation. An increase in multifocal inflammation was
10813 observed at PND18 in the other two Tier 1 studies from the same laboratory with two doses tested, i.e.
10814 25 and 250 µg/kg bw per day (Bernardo et al., 2015 [RefID 533]; Brandt et al., 2014 [RefID 700]). In
10815 addition, an increase in inflammatory reactive atypia was observed in the ventral prostate at 25 (not

10816 statistically significant) and 250 µg/kg bw per day (Brandt et al., 2014 [RefID 700]). In another study
10817 a decrease in lymphocytic infiltration was reported (NTP Clarity Report, 2018/Camacho et al., 2019
10818 [RefID 11370]); this decrease was not considered to be adverse. Regarding hyperplasia, two Tier 1
10819 studies (Bernardo et al., 2015 [RefID 533]: reactive hyperplasia; Brandt et al., 2014 [RefID 700]:
10820 hyperplasia/dysplasia) showed an increase at the lowest dose only or at the two doses tested with
10821 same effect size; it should be mentioned that in the study of Brandt et al. (2014) [RefID 700], data on
10822 incidence of hyperplasia and dysplasia were reported together as one endpoint. One Tier 1 study (NTP
10823 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) showed a decrease of hyperplasia in the
10824 animals treated with 2.5 µg/kg bw from GD6 to PND21 at PND730. A Grantee Tier 1 study (Prins et al.,
10825 2018 [RefID 13779]) showed no effect. Based on the inconsistencies in the outcomes and the diversity
10826 of the effects, the CEP Panel judged the prostatic non-neoplastic changes in the developmental
10827 exposure period as ALAN.

10828 Two histological **pre-neoplastic lesions** were evaluated: atypical hyperplasia and dysplasia.
10829 Regarding atypical hyperplasia, one Tier 1 study (Bernardo et al., 2015 [RefID 533]) showed an increase
10830 at the lowest dose only (25 µg/kg bw per day from GD10 to GD21). This result was not supported by
10831 a Tier 2 study (Hass et al., 2016 [RefID 2610]) and a Tier 3 study (Prins et al., 2017 [RefID 5930]) that
10832 showed no effect on this endpoint. Regarding dysplasia, one Tier 1 study (Brandt et al., 2014 [RefID
10833 700]) showed an increase at the lowest dose only (25 µg/kg bw per day from GD10 to GD21) but it
10834 should be mentioned that in this study data on incidence of hyperplasia and dysplasia were reported
10835 as together as one endpoint. Based on these results, the CEP Panel judged the pre-neoplastic lesions
10836 in prostate in the developmental exposure period as ALAN.

10837 **Proliferation** was evaluated in two studies. One Tier 1 study (Brandt et al., 2014 [RefID 700]) showed
10838 an increase at the lowest dose only (25 µg/kg bw per day from GD10 to GD21). This result was not
10839 supported by a Tier 2 study (Hass et al., 2016 [RefID 2610]) that showed no effect on this endpoint.
10840 Apoptosis was evaluated in one study (Brandt et al., 2014 [RefID 700]) that showed an increase at the
10841 highest dose only (250 µg/kg bw per day from GD10 to GD21). Based on these results, the CEP Panel
10842 judged the prostatic changes regarding proliferation and apoptosis in the developmental exposure
10843 period as ALAN.

10844 The CEP Panel assigned a likelihood level of ALAN to the prostate histological effects of BPA during the
10845 developmental exposure period, hence, none of the endpoints was taken forward for BMD analysis.
10846 However, they were considered in the uncertainty analysis (see Appendix D).

10847 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

10848 For effects on prostate histology following developmental and adult exposure, two Tier 1 studies (NTP
10849 Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]; Prins et al., 2018 [RefID 13779]) in rats were
10850 identified.

10851 Two histological non-neoplastic changes were evaluated: inflammation and hyperplasia. Regarding
10852 inflammation, the results of one study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370])
10853 showed two opposite effects for dorso-lateral (increase) and for ventral prostate (decrease, not
10854 adverse) both for suppurative inflammation and lymphocytic infiltration. The second study showed no
10855 effect (Prins et al., 2018 [RefID 13779]). Regarding hyperplasia, the results of one study (NTP Clarity
10856 Report, 2018/Camacho et al., 2019 [RefID 11370]) showed an effect at one intermediate dose only
10857 (250 µg/kg bw per day) out of five doses at PND730. The related study showed no effect (Prins et al.,
10858 2018 [RefID 13779]).

10859 Based on the inconsistencies in the outcomes, the diversity of the effects and no clear MDR or NMDR,
10860 the CEP Panel assigned a likelihood level of Not Likely to the prostate histological effects of BPA during
10861 the developmental and adult exposure period, hence, none of the endpoints was taken forward for
10862 BMD analysis.

10863 Growth phase/young age exposure

10864 No studies were available for this exposure period.

10865 Adult exposure (after puberty)

10866 For effects on prostate histology following adult exposure, one Tier 2 study (Olukole et al., 2018 [RefID
10867 12841]) and two Tier 3 studies (Wu et al., 2016 [RefID 8036]; Huang DY et al., 2018 [RefID 12167])
10868 in rats were identified.

10869 The following non-neoplastic changes were increased in a Tier 2 study (Olukole et al., 2018 [RefID
10870 12841]) in which a single-dose was used (10000 µg/kg bw per day; 14 days of treatment, starting at
10871 16 weeks of age): inflammation, hyperplasia (reactive), hyperplasia (functional), atrophic tubules,
10872 glandular diameter and vascular congestion. Proliferation was evaluated in two Tier 3 studies (Wu et
10873 al., 2016 [RefID 8036]; Huang DY et al., 2018 [RefID 12167]) from the same research group. Both
10874 studies showed an increase in all BPA dose groups (10, 30, 90 µg/kg bw per day for 3 months in Huang
10875 DY et al., 2018 [RefID 12167] and for 1 month in Wu et al., 2016 [RefID 8036]).

10876 Among pre-neoplastic lesions, atypical hyperplasia was observed in a Tier 2 study (Olukole et al., 2018
10877 [RefID 12841]) in which a single dose was used (10000 µg/kg bw per day; 14 days of treatment,
10878 starting at 16 weeks of age).

10879 Based on the limited data from one Tier 2 single-dose study and two Tier 3 studies, the CEP Panel
10880 considered the evidence on prostatic histological changes in the adult exposure period as Inadequate
10881 to assign a likelihood of the effects. Therefore, none of the endpoints was taken forward for BMD
10882 analysis.

10883 Indirect (germline) exposure

10884 No studies were available for this exposure period.

10885 Overall cluster selection of the endpoints/studies for BMD analysis for effects on prostate gland histology

10886 Overall, the CEP Panel assigned a likelihood level of ALAN to the prostate gland histological effects of
10887 BPA in the developmental exposure period, Not Likely in the developmental and adult exposure, and
10888 considered the evidence Inadequate for the adult exposure period, based on non-neoplastic changes,
10889 pre-neoplastic lesions and proliferation/apoptosis reported in some studies but with no clear dose-
10890 response and/or inconsistent results between different studies.

10891 Therefore, the overall likelihood across all exposure periods, i.e. the highest likelihood given in the
10892 cluster prostate gland histology, was ALAN.

10893 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
10894 Likely effect of BPA in any exposure period, therefore, none of the endpoints was taken forward for
10895 BMD analysis.

10896

10897 **Effects on uterus weight**

10898 Developmental exposure (pre-natal and/or post-natal until weaning)

10899 For this exposure period one Tier 1 study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
10900 11370]) in rats, one Tier 1 study in hamsters (Radko et al., 2015 [RefID 6046]) and one Tier 3 study
10901 in mice (Patel et al. (2013) [RefID 5697]) were identified.

10902 In the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]), animals were
10903 dosed with 2.5, 25, 250, 2500 and 25000 µg/kg bw per day and no change in uterus weight was
10904 observed. In the Tier 1 hamster study uterus weight (wet and dry) on PND21 was statistically
10905 significantly increased at 160000 µg/kg bw per day; female hamster pups were dosed with three daily
10906 oral doses (PND18–20) of 8000, 40000 and 160000 µg/kg bw per day.

10907 The CEP Panel assigned a likelihood level of ALAN to the uterus weight effects of BPA during the
10908 developmental exposure period, as no effect was observed in the Tier 1 in (young) adult rats and an
10909 increase in weanling hamsters (uterotropic assay) was only seen at the highest dose (160000 µg BPA/kg
10910 bw per day). Therefore, this endpoint was not taken forward for BMD analysis. However, it was
10911 considered in the uncertainty analysis (see Appendix D).

10912 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

10913 For this exposure period two Tier 1 rat studies (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
10914 11370]; Leung et al., 2020 [RefID 13789]) and one Tier 3 mouse study (Patel et al., 2013 [RefID 5697])
10915 were identified. No effect was seen on uterus weight in the Tier 1 rat study (NTP Clarity Report,
10916 2018/Camacho et al., 2019 [RefID 11370]). Rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg
10917 bw per day 1 year. In addition, no effects were observed on uterus weight in the other Tier 1 study
10918 (Leung et al., 2020 [RefID 13789]) at PND90, 6 months and 1 year.

10919 Based on these two Tier 1 rat studies, the CEP Panel assigned a likelihood level of Not Likely to the
10920 effects on uterus weight. Therefore, this endpoint was not taken forward for BMD analysis.

10921 Growth phase/young age exposure

10922 No studies were available for this exposure period.

10923 Adult exposure (after puberty)

10924 No studies were available for this exposure period.

10925 Indirect (germline) exposure

10926 No studies were available for this exposure period.

10927 Overall cluster selection of the endpoints/studies for BMD analysis for effects on uterus weight

10928 Overall, the CEP Panel assigned an overall likelihood level of ALAN to effects of BPA on uterus weight
10929 during the developmental exposure period, and Not Likely during developmental and adult exposure
10930 period. Therefore, the overall likelihood across all exposure periods, i.e. the highest likelihood given in
10931 the cluster effects on uterus weight, was ALAN.

10932 The CEP Panel considered that the evidence from the studies available did not show a Likely or Very
10933 Likely effect of BPA in any exposure period, therefore, this endpoint was not taken forward for BMD
10934 analysis.

10935

10936 **Effects on uterus histology**

10937 Developmental exposure (pre-natal and/or post-natal until weaning)

10938 For effects on uterus histology following developmental exposure of BPA, two Tier 1 studies in rats
10939 (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]; Vigezzi et al., 2015 [RefID 7472]) were
10940 identified.

10941 Several **non-neoplastic** changes were evaluated: uterine dilatation, luminal epithelium anomalies,
10942 gland cell anomalies, cystic endometrial hyperplasia, endometrial hyperplasia and squamous
10943 metaplasia. Regarding uterine dilatation, an increase was observed in one Tier 1 study at an
10944 intermediate dose out of five doses tested (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID
10945 11370]). Increases in luminal epithelium anomalies and gland cell anomalies at PND360 were reported
10946 in the other Tier 1 study (Vigezzi et al., 2015 [RefID 7472]) with the two doses tested (i.e. 0.5 and 50
10947 µg/kg bw per day) and with the higher dose, respectively. In one Tier 1 study (NTP Clarity Report,
10948 2018/Camacho et al., 2019 [RefID 11370]), cystic endometrial hyperplasia was increased with the
10949 highest dose after 1 year and at the highest two doses after 2 years. In the same study, no changes
10950 were seen for endometrial hyperplasia, whereas a slight increase in squamous metaplasia was observed
10951 at the highest dose (25000 µg/kg bw per day) after 1 year (NTP Clarity Report, 2018/Camacho et al.,
10952 2019 [RefID 11370]). In the other Tier 1 study no squamous metaplasia was detected in animals treated
10953 with lower doses (0.5 µg/kg and 50 µg/kg bw per day) (Vigezzi et al., 2015 [RefID 7472]). Based on
10954 these results, the CEP Panel judged the non-neoplastic changes of the uterus in the developmental
10955 exposure period as Likely.

10956 One **neoplastic** lesion was reported, i.e. stromal polyps. For this endpoint, a decreased incidence was
10957 seen in a Tier 1 study at the highest dose after 2 years (NTP Clarity Report, 2018/Camacho et al., 2019
10958 [RefID 11370]). Given that a decreased incidence of stromal polyps is not adverse, the CEP Panel
10959 judged uterine neoplastic changes in the developmental exposure period as Not Likely.

10960 No statistically significant effect on **apoptosis** was reported in one study (NTP Clarity Report, 10961 2018/Camacho et al., 2019 [RefID 11370]). Therefore, the CEP Panel judged the uterine 10962 proliferative/apoptotic changes in the developmental exposure period as Not Likely.

10963 The CEP Panel assigned a likelihood level of Likely to the uterine histological effects of BPA based on 10964 non-neoplastic changes (gland cellular anomalies, squamous metaplasia and cystic endometrial 10965 hyperplasia) observed in two rat Tier 1 studies (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 10966 11370]; Vigezzi et al., 2015 [RefID 7472]). Therefore, these endpoints were taken forward for BMD 10967 analysis (see Chapter 3.2.1) and for uncertainty analysis (see Appendix D).

10968 Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

10969 For effects on uterus histology following developmental and adult exposure to BPA, two Tier 1 studies 10970 in rats were identified: NTP Clarity Report (2018)/Camacho et al. (2019) [RefID 11370] and the Clarity- 10971 BPA Consortium grantee study of Leung et al. (2020) [RefID 13789].

10972 The following **non-neoplastic** changes were evaluated: uterine dilatation, cystic endometrial 10973 hyperplasia, endometrial hyperplasia and squamous metaplasia. Regarding uterine dilatation, a 10974 significant dose positive 'trend' at 2 years was reported in one study (NTP Clarity Report, 2018/Camacho 10975 et al., 2019 [RefID 11370]). In the Camacho et al. study, cystic endometrial hyperplasia was decreased 10976 in animals treated with the lowest dose (2.5 µg/kg bw/day; not adverse) at 1 year, while an increase 10977 of endometrial hyperplasia was seen in animals treated with 2.5 and 250 µg/kg bw per day at 1 year 10978 but not at 2 years. Additionally, a significant positive dose trend was reported for the incidence of 10979 squamous metaplasia at 1 year (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]) but no 10980 statistically significant effect on this endpoint (% of animals with squamous metaplasia) was reported 10981 in the other Tier 1 study, neither at PND90 nor at 1 year (Leung et al., 2020 [RefID 13789]). Based on 10982 the results from one Tier 1 study with no effect and another Tier 1 study with a statistical trend and no 10983 evident MDR or NMDR, the CEP Panel judged the non-neoplastic changes of the uterus in the 10984 developmental and adult exposure period as ALAN.

10985 One **neoplastic** lesion was evaluated, i.e. stromal polyps. For this endpoint, a significant dose trend 10986 towards increased incidence at the higher doses at 1 year was observed, while a negative trend (no 10987 adverse effect) was observed at 2 years in a Tier 1 study (NTP Clarity Report, 2018/Camacho et al., 10988 2019 [RefID 11370]). Based on this implausible biological result, the CEP Panel judged the uterine 10989 neoplastic changes in the developmental and adult exposure period as Not Likely.

10990 **Apoptosis** was evaluated with standard histology in one study (NTP Clarity Report, 2018/Camacho et 10991 al., 2019 [RefID 11370]) that reported an increase (trend) at the highest dose (25,000 µg/kg bw per 10992 day) only at 1 year. However, no effect was demonstrated in the other study (Leung et al., 2020 [RefID 10993 13789]) in which apoptosis at PND90 and at 1 year was evaluated with a specific and sensitive method 10994 (TUNEL). Based on these divergent results the CEP Panel judged the changes in uterine 10995 proliferation/apoptosis in the developmental until adult exposure period as ALAN.

10996 Based on non-neoplastic changes and apoptosis, the CEP Panel assigned a likelihood level of ALAN to 10997 the uterine histological effects of BPA during the developmental and adult exposure period, hence, none 10998 of the endpoints was taken forward for BMD analysis. However, they were considered in the uncertainty 10999 analysis (see Appendix D).

11000 Growth phase/young age exposure

11001 No studies were available for this exposure period.

11002 Adult exposure (after puberty)

11003 For effects on uterus histology following adult exposure to BPA, one study was considered (Kendriorski 11004 and Belcher, 2015 [RefID 3453]). In this study, two strains of mice were used in different experiments. 11005 Due to the low number of animals for one strain (C57Bl/6J) part of the study was considered Tier 3, 11006 while the other part performed on CD-1 mice was considered Tier 1.

11007 The following **non-neoplastic** changes were evaluated: gland nests and gland nest density. An 11008 increase in gland nests and gland nest density was reported in the Tier 1 experiment on CD-1 mice 11009 treated with the second highest dose (4000 µg/kg bw per day). Effects on gland density (with a high

11010 incidence in controls) was not confirmed by the Tier 3 part (C57Bl/6J) of the experiment, whereas an
 11011 increase of gland nest density was seen with a U-shaped NMDR (in the lowest and the two highest
 11012 doses). Based on the results without clear dose-responses from one Tier 1 part of the experiment and
 11013 from the Tier 3 part, the CEP Panel judged the non-neoplastic changes of the uterus in the adult
 11014 exposure period as ALAN.

11015 Based on non-neoplastic changes, the CEP Panel assigned a likelihood level of ALAN to the uterine
 11016 histological effects of BPA during the adult exposure period. This endpoint was considered for
 11017 uncertainty analysis (see Appendix D).

11018 Indirect (germline) exposure

11019 No studies were available for this exposure period.

11020 Overall cluster selection of the endpoints/studies for BMD analysis for effects on uterus histology

11021 Overall, the CEP Panel assigned a likelihood level of Likely to the uterine histological effects of BPA in
 11022 the developmental exposure period and ALAN in the developmental and adult and in the adult exposure
 11023 periods. The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster
 11024 uterus, was Likely.

11025 The CEP Panel considered that the evidence from the studies available showed a Likely effect for gland
 11026 cell anomalies, squamous metaplasia and endometrial cystic hyperplasia. Therefore, these endpoints
 11027 were taken forward for BMD analysis (see Chapter 3.2.1).

11028 **3.1.8.3. Integration of likelihoods from human and animal studies**

11029 There was no human evidence available for this category. Thus, the overall likelihood of effects of BPA
 11030 was based on the animal evidence.

11031 Table 14 presents the likelihoods of effect for each cluster and the overall likelihood in the animal
 11032 stream in Carcinogenicity and mammary gland proliferative effects.

11033 **Table 14:** Overall likelihood from the animal studies for Carcinogenicity and mammary gland
 11034 proliferative effects.

Human stream	Animal stream	Integrated likelihood
Cluster: Effects on Mammary gland weight:	Cluster: Effects on Mammary gland weight:	
Not applicable	Developmental exposure (pre-natal and/or post-natal until weaning) Not Likely	
	<i>Overall likelihood:</i>	<i>Not likely</i>
Cluster: Effects on Mammary gland histology	Cluster: Effects on Mammary gland histology	
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) ALAN	
	<i>Overall likelihood:</i> ALAN	ALAN
Cluster: Effects on Prostate histology	Cluster: Effects on Prostate histology	
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	
	Adult exposure (after puberty)	Inadequate evidence	
	<i>Overall likelihood:</i>	<i>ALAN</i>	<i>ALAN</i>
Cluster: Effects on Uterus weight	Cluster: Effects on Uterus weight		
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	
	<i>Overall likelihood</i>	<i>ALAN</i>	<i>ALAN</i>
Cluster: Effects on Uterus histology	Cluster: Effects on Uterus histology		
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)	ALAN	
	Adult exposure (after puberty)	ALAN	
	<i>Overall likelihood</i>	<i>Likely</i>	<i>Likely</i>

11035 3.1.8.4. *In vitro* and Mechanistic studies

11036 Regarding scoring of likelihood of effects in the WoE for the HOC Carcinogenicity, no relevant clusters
 11037 were available from the human studies. A few subcluster/endpoints were scored Likely and several
 11038 were scored ALAN. In the following, MoA studies for these subclusters are considered.

11039 Effects on Mammary gland

11040 Among the HOC Carcinogenicity, the cluster mammary gland histology was considered with the
 11041 following subclusters: non-neoplastic changes, pre-neoplastic lesions, neoplastic lesions, proliferation
 11042 and apoptosis.

11043 Mammary gland histology

11044 Based on the integration of the evidence from human and animal studies, the overall likelihood of
 11045 effects of BPA for mammary gland histology was scored ALAN. This scoring was mainly based on
 11046 evidence from the subclusters neoplastic changes, proliferation and apoptosis while the subclusters
 11047 pre-neoplastic lesions (only for the developmental exposure period) and neoplastic lesions (only for the
 11048 developmental until adulthood exposure period) were considered Not Likely and Likely, respectively.

11049 Several *in vivo* studies addressed the role of proliferation and ER α in the action of BPA in different
 11050 compartments of the mammary gland. Oral treatment of adult female rats with BPA (5000 $\mu\text{g}/\text{kg}$ bw
 11051 per day for 8 weeks) resulted in significant increases in proliferation and apoptosis indices in epithelial
 11052 cells but not in ER α expression (Ibrahim et al., 2016 [RefID 2972]). An increase in proliferation and in
 11053 the proliferation-to-apoptosis ratio was also observed in female rats at PND50 exposed to BPA (250
 11054 $\mu\text{g}/\text{kg}$ bw per day) via lactating dams (Wang J et al., 2014 [RefID 7640]). BPA exposure did not alter
 11055 ER α expression but reduced the expression of ER β which was shown to reduce breast cancer growth.
 11056 Hindman et al. (2017) [RefID 2711] studied the role of ER α activation in fetal stroma of *in utero* treated
 11057 female mice (i.p. 25 $\mu\text{g}/\text{kg}$ bw per day equivalent to the oral dose 2790 $\mu\text{g}/\text{kg}$ bw per day) on
 11058 histological changes in the mammary gland. In mammary gland sections of 4.5-week-old mice epithelial
 11059 elongation was directly correlated with proliferation and inversely correlated with ER α expression in the
 11060 stroma indicating a critical role of the stroma in BPA effects on mammary gland morphology. *In utero*

11061 exposure to BPA (250 ng/kg bw per day, s.c. equivalent to the oral dose 55.5 µg/kg bw per day) of
11062 wild-type and ER α knockout mice (Wadia et al., 2013 [RefID 7531]) indicated that BPA-induced changes
11063 in the expression of genes involved in the mammary stromal–epithelial interactions were ER α
11064 dependent. Gomez et al. (2017) [RefID 2308] studied the effects of perinatal BPA treatment (GD9 to
11065 PND21 with 0.5 µg or 50 µg/kg bw per day) on 17 β -E2 treated ovariectomised rats. While the
11066 histological findings showed a BPA (0.5 µg)-induced increase in ductal and atypical lobular hyperplasia,
11067 the immunohistochemistry data did not demonstrate increases in the proliferation index or significant
11068 changes in the expression levels of ER α or the progesterone receptor except for a decrease in ER α in
11069 ducts from the low BPA dose group.

11070 Data on BPA-induced epigenetic changes in mammary glands were reported in several *in vivo* studies.
11071 Camacho et al. (2015) [RefID 802] addressed the BPA effects on gene expression and global genomic
11072 DNA methylation in mammary glands of female rats exposed to a wide range of BPA doses (0.5 to
11073 300000 mg/kg bw per day from GD6 to PND90). While only limited BPA effects were observed on DNA
11074 methyltransferase coding genes and ER and ERR genes at PND4 and PND90, genome-wide gene
11075 expression data analysed by DNA microarray technology (PND4) showed effects of the highest BPA
11076 dose (300000 mg/kg bw per day) similar to those in tissues of oestrogen-treated rats. In addition, the
11077 expression levels of several genes were modulated in the low-dose range of BPA (0.5 to 2700 µg/kg
11078 bw per day) but mostly without dose–response. In another study BPA effects on the methylation pattern
11079 were reported in female rats exposed *in utero* to BPA (250 µg/kg bw per day from GD9 to PND1,
11080 subcutaneously equivalent to the oral dose 8925 µg/kg bw per day) at different time points, i.e. PND4,
11081 PND21 and PND50 (Dhimolea et al., 2014 [RefID 1576]). According to the authors the time-dependent
11082 changes in the methylation pattern of genomic DNA may reflect the developmental morphological
11083 changes observed in BPA-treated animals rather than causal events resulting in carcinogenesis later in
11084 life. Epigenetic effects of BPA were also studied in female rat offspring exposed via lactating dams (250
11085 µg/kg bw per day, PND2 to PND1) at PND100 (Jadhav et al., 2017 [RefID 3045]). The authors found a
11086 large number of genes with hypermethylated loci following pre-pubertal BPA exposure. Using network
11087 and pathway analysis enriched networks related to cancer, cell death and proliferation were identified.
11088 Bhan et al. (2014a) [RefID 557] studied altered epigenetic programming by BPA in mammary glands
11089 of ovariectomised adult female rats. Their findings indicate that BPA (two subcutaneous injections with
11090 25 µg/kg bw equivalent to the oral dose 892.5 µg/kg bw per day) induced long non-coding RNA
11091 HOTAIR, a regulatory RNA associated with breast cancer. In an additional study with the same study
11092 design the authors Bhan et al. (2014b) [RefID 558] provided evidence of BPA-induced expression of
11093 the methyltransferase EZH2 which is overexpressed in breast cancer and associated with proliferation,
11094 tumour invasiveness and metastasis. In the same laboratory the expression levels of HOXC6 (Hussain
11095 et al., 2015 [RefID 2960]) and HOXB9 (Deb et al., 2016 [RefID 1483]), oestrogen-regulated homeobox-
11096 containing genes (HOX genes) which are overexpressed in breast cancer, were studied with the same
11097 design. The results indicate that the expression levels of HOXC6 and HOXB9 were increased by BPA
11098 treatment.

11099 Expression of proteins was studied by Lee et al. (2017) [RefID 3907] in the human MCF-7 breast cancer
11100 cell line and in a xenograft mouse model of breast cancer (mice injected s.c. with MCF-7 cells). It was
11101 shown that treatment of xenografted mice with BPA (50 mg/kg bw, injected s.c. for 10 weeks) induced
11102 breast cancer growth and proteins related to epithelial–mesenchymal transition and metastasis. In a
11103 rodent cancer model with DMBA (at PND50) Leung et al. (2017) [RefID 3990] observed that exposure
11104 of rats to a low pre-natal dose of BPA (25 µg/kg bw per day) together with a HBF diet fed to the dams
11105 increased the tumour incidence (at PND140) and shortened the tumour-free survival time. The increase
11106 in tumour incidence was not observed at higher BPA doses, i.e. 250 and 2500 µg/kg bw per day, and
11107 in BPA (25 µg) exposed rats from dams fed a control diet. The authors also studied the methylation
11108 pattern (PND21) in offspring exposed to BPA and HBF and concluded that some of the differentially
11109 expressed genes may be related to breast cancer. Using quantitative proteomic techniques Betancourt
11110 et al. (2014) [RefID 548] reported on the expression of proteins in sera of rats exposed via lactating
11111 dams (250 µg/kg bw per day). The authors discussed the altered protein expression in serum (at PND21
11112 and PND35) in the context with their potential roles in proliferation/apoptosis and carcinogenesis in the
11113 mammary gland.

11114 Wang DH et al. (2014) [RefID 7598] reported increases in lateral branching and hyperplasia in
11115 mammary glands of 4-month-old female mice exposed to BPA (25 µg/kg bw per day from PND21 for 3

11116 weeks). The authors suggest that BPA affects mammary stem cells by altering the expression of genes
11117 associated with early neoplastic lesions.

11118 A role of increased production of ROS in female rat following long-term treatment with BPA (10, 50 or
11119 100 µg/kg bw per day for 12 weeks) was addressed in a study with female rats (Thilagavathi et al.,
11120 2017 [RefID 9247]). Dose-related decreases in SOD and CAT activities and GSH levels along with
11121 increased TBARS in the mammary glands were observed. At the lowest BPA dose there was an increase
11122 of Enos synthase associated with decreased levels of steroidogenic enzymes and decreased levels of
11123 serum E2. Histological analysis revealed hyperplasia in mammary epithelial cells in all dose groups.

11124 Only some changes in nuclear receptor expression (decreased ER α and AR) were observed in a study
11125 on female mice at 8 months after treatment with BPA from GD10.5 to GD17 (Tucker et al., 2018 [RefID
11126 13275]). The histological changes in this study are described in 3.1.8.2.

11127 In male rat offspring a delayed mammary gland development was reported by Kass et al. (2015) [RefID
11128 3402] who observed at PND30 a reduced ductal growth along with a reduced expression of the AR
11129 following pre-natal exposure to BPA (64 µg/kg bw per day orally or 250 µg/kg bw per day s.c. equivalent
11130 to an oral dose 8925 µg/kg bw per day; see 3.1.8.2).

11131 In an *ex vivo* experiment morphological changes were studied in embryonic mouse mammary buds
11132 dissected at embryonic day 14 and cultured for 5 days (Speroni et al., 2017 [RefID 6866]). Ductal
11133 growth and branching were increased following treatment with 1 nM BPA but inhibited by a high
11134 concentration of BPA (1,000 nM). The increase in ductal growth was inhibited by a nuclear ER antagonist
11135 (fulvestrant, ICI 182,780). The results suggest a direct oestrogenic action of BPA on the gland
11136 development in a concentration dependent manner. In another study using primary organotypic 3D
11137 cultures of mammary glands from 6-week-old to 8-week-old mice (Williams et al., 2016 [RefID 7958])
11138 an altered branching pattern was observed along with proteomic changes after treatment with 20 nM
11139 BPA for 6 days. Such culture models may be useful to elucidate further the mechanisms of action of
11140 BPA involved in morphological changes in the mammary gland.

11141 Potential MoAs for BPA-induced effects in the mammary gland were addressed inter alia by studies with
11142 breast cancer or normal breast epithelial cell lines on signalling pathways, ROS, epigenetic processes,
11143 gene and protein expression. Several of the studied mechanisms were associated with proliferation or
11144 apoptosis, cell migration and cell invasion.

11145 Recent *in vitro* studies on signalling pathways confirmed previous findings that BPA acts via ER α /ER β
11146 (Lee et al., 2014 [RefID 3922]; Potratz et al., 2017 [RefID 5906]; Li Y et al., 2018b [RefID 12494]) and
11147 non-classical ERs or ER-independent mechanisms. Chronic exposure to BPA (50 nM) may decrease the
11148 expression of ER target genes and initiate transcriptional reprogramming of breast cancer cells
11149 (Patterson et al., 2015 [RefID 5725]). An increased proliferation and anchorage-independent growth in
11150 ER-negative breast cancer cells by BPA (40 nM) was reported to be mediated via eGFR activation (Sauer
11151 et al., 2017 [RefID 6493]). The induction of rapid signalling via the G protein-coupled oestrogen
11152 receptor (GPER) was reported to be involved in breast cancer cell adhesion (Magruder et al., 2014
11153 [RefID 4777]), migration (Sanchez et al., 2016 [RefID 6427]) and proliferation under hypoxia (Xu FY
11154 et al., 2017 [RefID 8183]). Other BPA effects are dependent on the ERR γ , e.g. proliferation of ER-
11155 positive (MCF-7) and ER-negative (SkBr3) cells (Song et al., 2015 [RefID 6818]) or migration and
11156 invasion of the triple-negative breast cancer cells (Zhang et al., 2016 [RefID 8865]) induced by
11157 nanomolar concentrations of BPA (10 nM). In summary, the data confirm that different signalling
11158 pathways can contribute to BPA-stimulated effects at nanomolar concentrations.

11159 A variety of *in vitro* studies with breast cancer cells showed alterations of gene and protein expression
11160 by BPA. The expression of HOX genes which are of particular interest in the regulation of perinatal
11161 development and known to be overexpressed in breast cancer were reported to be transcriptionally
11162 regulated by nanomolar concentrations of BPA (Bhan et al., 2014a [RefID 557]; Bhan et al., 2014b
11163 [RefID 558]; Deb et al., 2016 [RefID 1483]; Hussain et al., 2015 [RefID 2960]). BPA (10 nM) enhanced
11164 cancer stem cell activity via induction of the transcription factor SOX2 (Lillo et al., 2017 [RefID 4277])
11165 and upregulated the octamer-binding transcription factor 4 (Oct4) in human embryonic stem cells
11166 indicating the disturbance of expression of markers for stem cells and mammary epithelial cells (Yang
11167 LQ et al., 2013 [RefID 8370]). Results from proteomic analyses of mouse mammary tissue cultured in
11168 a 3D model (Williams et al., 2016 [RefID 7958]) showed that 20 nM BPA altered proteins involved in

11169 pathways related to different cellular processes such as proliferation, focal adhesion assembly,
11170 substrate adhesion-dependent cell spreading/cell migration and epithelium morphogenesis. Using a
11171 reporter gene assay (VM7Luc4E2) in MCF-7 cells the EC50 value of 260 nM was derived for BPA-induced
11172 RNA expression (Peng et al., 2018 [RefID 12887]). The induction of the expression of cell cycle genes
11173 (Kim et al., 2017 [RefID 3525]; Lee et al., 2014 [RefID 3922]; Li XT et al., 2014 [RefID 4187]) and ER-
11174 regulated key proteins (Potratz et al., 2017 [RefID 5906]; Wang T et al., 2018 [RefID 13374]) were
11175 tested only at micromolar BPA concentrations (e.g. 10 µM). At a high concentration (100 µM) BPA
11176 induced the expression of COX-2 via the transcription factor NF-κB (Song et al., 2017 [RefID 6815]).
11177 Overall, the expression of regulatory proteins critical for cell differentiation may be affected by low and
11178 high concentrations of BPA.

11179 Two *in vitro* studies investigated the formation of ROS by BPA. Lei et al. (2017) [RefID 3960] observed
11180 in MCF-7 cells a slight increase of the viability up to 1 µM BPA while ROS levels were enhanced at 50–
11181 100 µM where cell viability was markedly decreased and LDH release was increased. Similarly, in a
11182 study using a normal mammary epithelial cell line (MCF10A) no increase of ROS production was
11183 detected with 1 and 10 nM BPA (Kang et al., 2013 [RefID 3348]), i.e. in a concentration in which
11184 enhanced proliferation was observed in another study (Pfeifer et al., 2015 [RefID 5815]). Based on
11185 these *in vitro* findings intracellular ROS formation may be linked rather to cytotoxicity than to
11186 physiological processes, e.g. proliferation.

11187 Mammary gland proliferation and apoptosis

11188 Proliferation was studied in numerous *in vitro* studies using ER-positive breast cancer cells (e.g. MCF-7
11189 and T47D cells) which usually require ≥100 nM BPA for growth stimulation (Katchy et al., 2014 [RefID
11190 3410]; Kim et al., 2017 [RefID 3525]; La Rosa et al., 2014 [RefID 3794]; Lee et al., 2014 [RefID 3922];
11191 Li XT et al., 2014 [RefID 4187]; Mesnage et al., 2017 [RefID 5057]; Potratz et al., 2017 [RefID 5906];
11192 Rotroff et al., 2013 [RefID 6302]; Wang T et al., 2018 [RefID 13374]). Related oestrogenic effects such
11193 as the expression of cell cycle genes (Kim et al., 2017 [RefID 3525]; Lee et al., 2014 [RefID 3922]; Li
11194 XT et al., 2014 [RefID 4187]) and ER-regulated key proteins (Potratz et al., 2017 [RefID 5906]; Wang
11195 T et al., 2018 [RefID 13374]) were tested at micromolar concentrations of BPA. Induction of
11196 proliferation and apoptosis at 1 µM BPA was observed (Sengupta et al., 2013 [RefID 6567]), while a
11197 reduction of apoptosis and ROS production was reported in ER-positive VM7Luc4E2 breast cancer cells
11198 (Lee GA et al., 2018 [RefID 12427]). BPA-induced proliferation was mainly induced by an ER-dependent
11199 mechanism and was not observed in ER-negative cells, i.e. MDA-MB-231 cells (Li XT et al., 2014 [RefID
11200 4187]; Mesnage et al., 2017 [RefID 5057]). However, Pfeifer et al. (2015) [RefID 5815] using ERα-
11201 negative normal breast epithelial cell lines (MCF10A, 184A1) showed that 10 nM BPA induced
11202 proliferation via upregulation of the c-Myc protein. Using the same low BPA concentration (10 nM) Song
11203 et al. (2015) [RefID 6818] also reported an increased proliferation in ER-positive (MCF-7) and ER-
11204 negative (SkBr3) cells mediated by the receptor ERRγ. Additionally, it was reported that BPA (≥100 nM)
11205 stimulated proliferation and migration of ER-negative breast cancer cells (SkBr3, MDA-MB-231) via the
11206 G-protein-coupled oestrogen receptor (GPER) pathway and under hypoxic culture (Xu FY et al., 2017
11207 [RefID 8183]). In summary, the results from *in vitro* studies suggest that ERα-dependent as well as
11208 ER-independent mechanisms lead to BPA-induced proliferation in breast cancer cells, also depending
11209 on the study design, cell lines and culture conditions used.

11210 Effects related to Carcinogenicity

11211 Two *in vitro* studies reported DNA damage only at high concentrations of BPA (10 µM) which also
11212 induced cytotoxicity (Aghajanjpour-Mir et al., 2016 [RefID 57]; Lei et al., 2017 [RefID 3960]). Therefore,
11213 these genotoxic effects were considered by the CEP Panel in the Genotoxicity chapter (Chapter 3.1.9).

11214 In several other studies BPA promoted altered cell adhesion, migration, epithelial–mesenchymal
11215 transition and invasion mediated via various signalling pathways at micromolar concentrations (Kim et
11216 al., 2017 [RefID 3525]; Lee et al., 2017 [RefID 3907]; Magruder et al., 2014 [RefID 4777]; Sanchez et
11217 al., 2016 [RefID 6427]; Zhang et al., 2016 [RefID 8865]), except for two studies which showed an
11218 enhanced migration potential at 10 nM BPA (Zhang et al., 2015 [RefID 8866]) or 100 nM (Cao et al.,
11219 2017 [RefID 837]) in ER-negative breast cancer cells. No effect on migration and cell invasion was
11220 observed following long-term exposure (at least 4 weeks) to 250 nM BPA in MCF-7 cells (Norberto et
11221 al., 2017 [RefID 5440]). The reported findings on cellular cancer-related endpoints indicate that BPA
11222 may contribute to tumorigenesis in breast cancer cells.

11223 In conclusion, the CEP Panel considered that the *in vivo* studies show evidence that stromal–epithelial
11224 interactions may play a crucial role in the BPA-induced developmental changes in the mammary gland.
11225 Epigenetic effects of BPA including changes in methylation patterns are frequently reported along with
11226 altered expression of genes involved in proliferation/apoptosis and other cellular functions. Results from
11227 two breast cancer models (i.e. xenograft mouse model and chemically (DMBA) induced cancer) suggest
11228 that BPA may enhance the susceptibility to carcinogenic stimuli. In addition, there is some evidence
11229 from *in vitro* studies with breast cancer cells which supports the hypothesis that BPA promotes changes
11230 in proliferation and pathological processes which ultimately could contribute to carcinogenesis in the
11231 mammary gland.

11232 **Effects on prostate**

11233 Among the HOC Carcinogenicity, the cluster ‘prostate histology’ was considered and the following
11234 subclusters were considered if available: non-neoplastic changes, pre-neoplastic lesions, neoplastic
11235 lesions, proliferation and apoptosis.

11236 Eighteen MoA studies specifically addressing the carcinogenic effects on the prostate were considered,
11237 five within the mammalian stream, one human and 12 in the *in vitro* stream. MoA studies from the *in*
11238 *vitro* stream often used prostate cancer cell lines (in seven studies) and in particular LNCaP, PC3 and
11239 C4–2 cell lines, in some cases stem cells (in four studies) and normal cells (in two studies). Other
11240 studies related to non-neoplastic changes have also taken in consideration.

11241 Prostate histology (*in vivo* studies)

11242 *In vivo* studies on mechanisms for rodent developmental BPA-induced prostate inflammation and
11243 reactive hyperplasia have explored a number of endpoints, including alterations in ER-related and AR-
11244 related gene and protein expression in the prostate. Proposed mechanisms include increased expression
11245 of AR, decreased expression of ER α , increased prostate aromatase expression leading to increased
11246 intraprostatic E2, and altered expression of other androgen-related genes (e.g. Scgb2a1, Ptgds).

11247 In conclusion, despite the relatively large amount of data available, there is no consensus on
11248 mechanisms underlying developmental BPA-induced prostate effects, in particular whether effects are
11249 upstream or downstream. More details are reported in Chapter 3.1.6.4, *In Vitro* and Mechanistic studies
11250 on Reproductive and developmental Toxicity.

11251 Prostate histology (*in vitro* studies)

11252 • Prostate histology (non-neoplastic changes)

11253 Two histological non-neoplastic findings (inflammation and hyperplasia) were evaluated in animal
11254 studies. Based on the inconsistencies in the outcomes and the diversity of the effects, the CEP Panel
11255 judged the prostatic non-neoplastic changes as ALAN. MoA *in vitro* studies indicate that BPA may
11256 increase the proliferative rate of different types of prostate cells while no data are available for
11257 inflammation.

11258 • Prostate histology (pre-neoplastic lesions)

11259 Two histological pre-neoplastic findings (atypical hyperplasia and dysplasia) were evaluated in animal
11260 studies in the developmental exposure period and were judged as ALAN.

11261 No MoA *in vitro* study specifically addressed these outcomes.

11262 • Prostate histology (neoplastic lesions)

11263 In animal studies, no neoplastic lesions of prostate induced by BPA without additional hormonal
11264 treatment (testosterone and oestrogen) were identified by the CEP Panel.

11265 Data obtained from MoA studies indicate that BPA can enhance prostate cancer susceptibility while its
11266 direct carcinogenicity is still debated. However, the results of studies carried out in laboratory rodents
11267 do not demonstrate a direct evidence of tumorigenic activity of BPA alone. More details can be found
11268 in the Chapter 3.1.6.4 in the HOC Reproductive and developmental toxicity.

11269 • Prostate histology (proliferation and apoptosis)

11270 In animal studies carried out in rats, the CEP Panel judged proliferation and apoptosis in the
11271 developmental exposure period as ALAN.

11272 Data obtained from a few *in vitro* MoA studies indicate that BPA can enhance proliferation of the
11273 prostate cells supporting the weak evidence from studies in animals. Data from *in vitro* MoA studies
11274 regarding apoptosis do not shed light on this aspect. More details can be found in the Chapter 3.1.6.4
11275 in the HOC Reproductive and developmental toxicity.

11276 **Effects on uterus**

11277 Among the HOC Carcinogenicity, the clusters 'uterus weight' and 'uterus histology' were considered.
11278 The CEP Panel judged the likelihood for an increase in uterus weight ALAN during developmental
11279 exposure.

11280 For histology, the following subclusters were considered if available: non-neoplastic changes, pre-
11281 neoplastic lesions, neoplastic lesions, proliferation and apoptosis. The CEP Panel considered the overall
11282 likelihood of this cluster Likely during the developmental exposure based on effects on non-neoplastic
11283 changes and ALAN during the developmental and adult exposure period.

11284 Uterus weight

11285 The CEP Panel considered the likelihood for an increase in uterus weight ALAN during developmental
11286 exposure. The possible MoA may be mediated by ERs. No MoA study specially addressed this outcome.

11287 Uterus histology (non-neoplastic changes)

11288 Most MoA studies indicate that BPA may increase the proliferative rate of different types of uterine cells.
11289 More details can be found in the Chapter 3.1.6.4 in the HOC Reproductive and developmental toxicity.

11290 Uterus histology (pre-neoplastic lesions)

11291 No pre-neoplastic lesions were evaluated in human or animal studies and no MoA study specifically
11292 addressed this outcome.

11293 Uterus histology (neoplastic lesions)

11294 No neoplastic lesions were evaluated in human studies. Based on evidence from animal (rats and mice)
11295 studies, the overall likelihood of effects of BPA for uterine neoplastic lesions was considered Not Likely.

11296 Uterus histology (proliferation and apoptosis)

11297 MoA studies do not support/explain the increase of apoptosis observed in BPA-treated animals in one
11298 *in vivo* study. More details can be found in the Chapter 3.1.6.4 in the HOC Reproductive and
11299 developmental toxicity.

11300 **3.1.8.5. Conclusion on hazard identification for Carcinogenicity and mammary gland** 11301 **proliferative effects of BPA**

11302 In the 2015 EFSA opinion on BPA (EFSA CEF Panel, 2015), BPA effects on mammary gland proliferation
11303 were considered Likely based on results from a subchronic rat study with pre-natal exposure to BPA as
11304 well as proliferative and related morphological changes in the mammary gland reported in other studies.
11305 The data on mammary gland proliferation were taken forward for a BMD–response modelling but these
11306 data were not suitable to provide a RP (BMDL) due to considerable uncertainty in the outcome of
11307 modelling. However based on the WoE evaluation, effects related to proliferation in the mammary gland
11308 were taken into account in the evaluation of uncertainty for hazard characterisation and included in the
11309 risk assessment. The evidence for proliferative changes in prostate and other organs (testis, liver) was
11310 evaluated as too weak to reach a definite conclusion. Overall, the findings in mammary gland, prostate
11311 and other organs were considered 'insufficient to conclude that there is a link to cancer development
11312 in later life' and the likelihood level of 'unlikely to ALAN' was assigned to carcinogenic effects of BPA
11313 (EFSA CEF Panel, 2015).

11314 A new pre-natal and chronic toxicity study of BPA in rats and additional studies in rodents were
11315 conducted to further clarify whether changes in proliferation and differentiation in mammary gland,
11316 prostate, uterus and other organs eventually result in an increased incidence of tumours. Having
11317 assessed these data in the current evaluation the CEP Panel considered that the new findings support

11318 the earlier observations. While no effects on mammary gland weight of female rats treated with BPA at
11319 doses from 2.5 to 25,000 µg/kg bw per day were observed up to 2 years of age, several histological
11320 changes, e.g. in longitudinal growth, TEBs, branching, gland density and mammary gland scores were
11321 reported in studies with female and/or male rats and mice after BPA exposure during the developmental
11322 until weaning or adulthood period. Due to the diversity of these outcomes assessed at different
11323 timepoints and doses the CEP Panel considered the induction of these non-neoplastic effects ALAN.
11324 Regarding pre-neoplastic lesions the CEP Panel noted an increase of atypical foci at the lowest dose
11325 (2.5 µg/kg bw per day) in female rats following treatment during developmental until adult exposure
11326 (Likely) but no effect after only developmental exposure until weaning (Not Likely) in female rats in the
11327 chronic study. On the other hand, regarding neoplastic lesions in the same study there was an increase
11328 of adenomas and/or adenocarcinomas at the lowest dose in female rats following treatment during
11329 developmental until weaning period (Likely) but no statistically significant effect was found after long-
11330 term exposure from *in utero* until adulthood (Not Likely) in female rats.

11331 Overall, the CEP Panel considered the sum of the histopathological findings ALAN and therefore, the
11332 effects were taken into account in the evaluation of uncertainty for hazard characterisation and included
11333 in the risk assessment.

11334 MoA studies addressing epigenetic effects, changes in gene expression and hormone receptor levels *in*
11335 *vivo* and *in vitro* suggest various mechanisms of action of BPA possibly involved in the induction of
11336 proliferative/morphological changes in the mammary gland. Some evidence from *in vitro* studies with
11337 breast cancer cells supports the hypothesis that BPA promotes changes in proliferation and pathological
11338 processes which ultimately contribute to a higher susceptibility to mammary gland carcinogenesis.

11339 In the current evaluation also data on histological effects in prostate from the new pre-natal and chronic
11340 toxicity study of BPA in rats and additional studies in rodents were considered in the WoE approach.
11341 Non-neoplastic changes related to inflammation and hyperplasia following developmental exposure until
11342 weaning were graded as ALAN due to inconsistencies in the outcome among studies. In the
11343 developmental and adult exposure period these effects were considered as Not Likely and in the adult
11344 exposure period the non-neoplastic changes were Inadequate due to the limitations in the study
11345 database. Pre-neoplastic lesions, i.e. atypical hyperplasia and dysplasia were considered as ALAN after
11346 developmental exposure and Unlikely after developmental until adult exposure in rat studies. Moreover,
11347 proliferation and apoptosis were considered as ALAN following developmental exposure in two rat
11348 studies. No evidence of induction of neoplastic lesions were seen in any of the exposure periods.

11349 MoA studies confirmed the role of sex hormones in prostate cancer development. In a rat model using
11350 testosterone and two-fold elevated oestrogen levels, developmental BPA treatment increased the
11351 severity score of prostatic intraepithelial neoplasia (PIN) and adenocarcinoma multiplicity. BPA
11352 treatment alone was reported to increase prostate stem cell proliferation and disrupt prostate stem cell
11353 homeostasis. Via epigenetic or additional mechanisms BPA can modulate gene and protein expression,
11354 signalling pathways, stem cell homeostasis or centrosome activity. In conclusion, MoA studies indicate
11355 that BPA can enhance the susceptibility to prostate cancer while the results of studies in rodents do not
11356 demonstrate a direct tumorigenic effect of developmental and chronic exposure to BPA.

11357 Regarding uterus, several non-neoplastic changes were considered as Likely after developmental
11358 exposure to BPA in rats: gland cell anomalies, endometrial cystic hyperplasia and squamous metaplasia.
11359 Therefore, these endpoints were taken forward for BMD analysis (see Chapter 3.2.1). Uterine dilatation,
11360 endometrial hyperplasia and squamous metaplasia were considered ALAN after developmental and
11361 adult exposure. Gland nests and gland nest density were non-neoplastic changes observed in the adult
11362 exposure period. Apoptosis was considered ALAN after developmental and adult exposure period and
11363 Not Likely after developmental exposure. No evidence of induction of neoplastic lesions were seen in
11364 any of the exposure periods, since a negative dose trend (no adverse effect) was observed at two years
11365 with a significant decrease at the highest dose (developmental exposure), while a positive trend without
11366 statistical significance at the higher doses was observed at one year (developmental and adult
11367 exposure). Therefore, neoplastic lesions in the uterus were considered as Not Likely.

11368 Most MoA studies indicate that BPA increases the proliferative rate of different types of uterine cells.
11369 Data obtained from *in vitro* MoA studies indicate that BPA can modulate many mechanisms underlying
11370 the onset of growth and invasion of uterine tumours. However, the results of rodent studies do not
11371 provide evidence of a carcinogenic activity of BPA.

11372 3.1.9. Genotoxicity

11373 In the present assessment, the CEP Panel examined whether new data from the published literature
 11374 could provide new evidence on the potential genotoxicity of BPA. To this aim, a literature search was
 11375 performed as reported in Annex A. Also the references from the previous CEF Panel opinion (EFSA CEF
 11376 Panel, 2015) have been included in the current assessment using the same appraisal criteria applied to
 11377 the newly published data and considering the EFSA Scientific Committee guidance documents on
 11378 genotoxicity published after 2015 (EFSA Scientific Committee, 2017c, 2021a).

11379
 11380 Genotoxicity studies considered for this assessment are:

- 11381 • *in vitro* and *in vivo* studies (88 publications) retrieved from the literature search (Annex A and
 11382 Annex L),
- 11383 • *in vitro* and *in vivo* studies (15 publications) considered in the Scientific opinion on the risks
 11384 to public health related to the presence of BPA in foodstuffs (EFSA CEF Panel, 2015) (Appendix
 11385 E and Annex L).

11386
 11387
 11388 *In vitro* and *in vivo* studies were grouped based on the genotoxicity endpoint investigated:

- 11389 • gene mutations (e.g. bacterial reverse mutation assay);
- 11390 • chromosomal damage (CA and micronucleus assays);
- 11391 • DNA damage (comet assay).

11392 These studies were summarized in synoptic tables (Annex L), evaluated for reliability and relevance
 11393 and grouped into lines of evidence in a WoE approach (see Chapter 2.3.5).

11394 The genotoxicity studies have been assessed using a scoring system for reliability based on criteria
 11395 published by Klimisch et al. (1997) as explained in Chapter 2.3.5. In a second step, the relevance (high,
 11396 limited, low) of study results was assessed based on reliability of the study and some general aspects
 11397 e.g. genetic endpoint, purity of the test substance, route of administration and status of validation of
 11398 the assay (see Chapter 2.3.5).

11399 Genotoxicity studies evaluated as of low relevance have not been further considered in the assessment.
 11400 Studies not investigating classical genotoxicity endpoints (e.g. γ H2AX, oxidative DNA damage, DNA
 11401 binding, ROS generation) and studies in humans are considered in the MoA and as supportive evidence.
 11402 All the studies evaluated were summarised in a narrative form (Appendix E).

11403 3.1.9.1 WoE

11404 1. Gene mutations *in vitro* and *in vivo*

11405 1a. *In vitro* gene mutation

11406 Of the six available studies of the mutagenicity of BPA in bacteria, only one describes the application
 11407 of the Ames test in a comprehensive battery of *Salmonella* Typhimurium strains (TA1535, TA97, TA98,
 11408 TA100 and TA102) at a range of concentrations up to 5000 μ g/plate. It reports negative results both
 11409 in the presence and absence of metabolic activation (Xin et al., 2015 [RefID 8150]). Three studies
 11410 reported negative results in TA98 and TA100 (Masuda et al., 2005; Fic et al., 2013; Zemheri and Uguz,
 11411 2016 [RefID 9535]). A study shows negative results in TA98, TA100 and TA102 strains (Tiwari et al.,
 11412 2012). The sixth used the bacterial SOS/umuC assay with a range of concentrations from 1 to 1000
 11413 μ g/L in presence and absence of S9 mix. It also reported negative results (Balabanić et al., 2021 [RefID
 11414 224-G]).

11415 The CEP Panel concluded that BPA does not induce gene mutations in bacteria.

11416 No studies on gene mutation assays in mammalian cells following the OECD guidelines were available.

11417 1b. *In vivo* gene mutation

11418 No studies on gene mutation assays *in vivo* were available.

11419

11420 **2. Induction of chromosomal aberrations/micronuclei *in vitro* and *in vivo***

 11421 **2a. *In vitro* chromosomal aberrations/micronuclei**

11422 Fifteen *in vitro* studies of micronuclei (MN) and structural CA induction in different cell lines were
 11423 available for evaluation. Of these, nine were further considered in the assessment (Figure 2), classified
 11424 as having high (1 study) or limited relevance (8 studies).

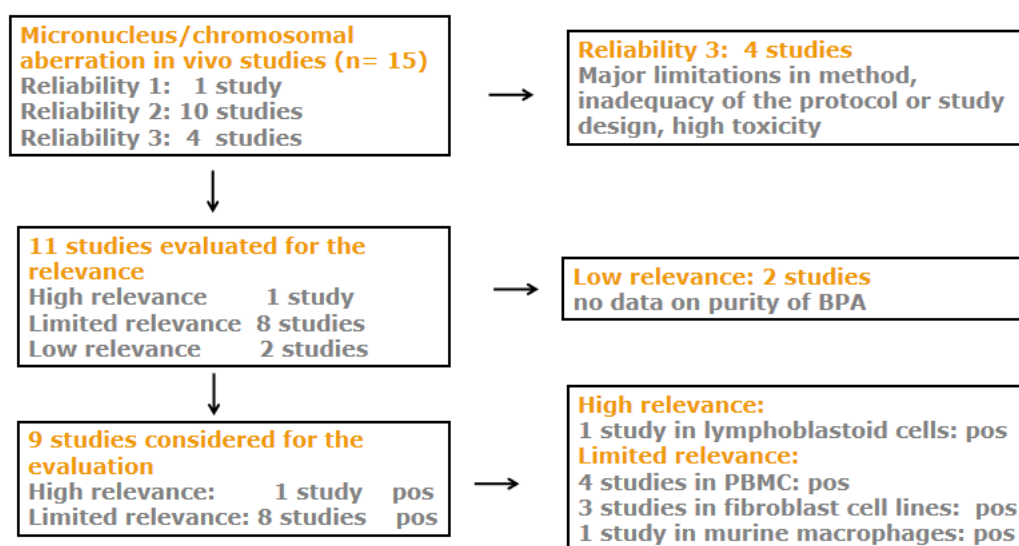
11425 All showed positive results in both blood cells and established cell lines. In the single study classified
 11426 as of high relevance, a concentration-dependent increase of MN frequency over a wide range of
 11427 concentrations (1.5 to 37 µg/ml corresponding to 6.6 µM and 162 µM) was observed in the AHH-
 11428 1 human lymphoblastoid cell line (Johnson and Parry, 2008). Positive CA results were also reported
 11429 from cultures of human peripheral lymphocytes in two studies with limited relevance (Santovito et al.,
 11430 2018 [RefID 11220]; Di Pietro et al., 2020 [RefID 258-G]). In one of these (Santovito et al., 2018
 11431 [RefID 11220]), MN frequency was also measured. A study of MN in bovine peripheral blood
 11432 lymphocytes also reported positive findings (Šutiaková et al., 2014 [RefID 7026]).

11433 In murine macrophage RAW264.7 cells, positive MN results were associated with an increase in reactive
 11434 oxygen species (ROS), and a decreased level of antioxidant enzymes (GPx, SOD and CAT. Concomitant
 11435 phosphorylation of P53 and release of cytochrome C from mitochondria were detected along with
 11436 increased apoptosis. Pretreatment with N-acetylcysteine (NAC) reduced BPA-induced cytotoxicity,
 11437 apoptosis and genotoxicity (MN frequency was reduced by 30%). These results indicate that the toxic
 11438 effect of BPA in macrophages was mainly through the oxidative stress-associated mitochondrial
 11439 apoptotic pathway (Huang FM et al., 2018 [RefID 296-G]).

11440 Finally, two studies in the Chinese hamster ovary (CHO) and V79 cell lines reported positive results (Xin
 11441 et al., 2015 [RefID 8150]; Yu et al., 2020 [RefID 475-G]). Xin and co-workers reported a concentration
 11442 dependent increase of both MN and CAs in CHO cells in the absence of metabolic activation. In
 11443 contrast, the BPA-induced increase in MN frequency in V79, reported by Yu and colleagues, apparently
 11444 required CYP1A1 and CYP1B1 expression.

11445 Overall, the significant increases of chromatid and chromosome breaks observed in several studies *in*
 11446 *vitro* indicated that BPA has clastogenic activity also at non-cytotoxic concentrations. Two reports
 11447 indicated that oxidative stress is implicated in the observed induction of chromosomal damage. In
 11448 addition, Johnson and Parry (2008) reported the formation of aberrant mitotic spindles, with multiple
 11449 poles, in cells treated with BPA.

11450 In conclusion, the *in vitro* studies on CA and MN induced by BPA indicated that both clastogenic and
 11451 aneugenic mechanisms may operate.



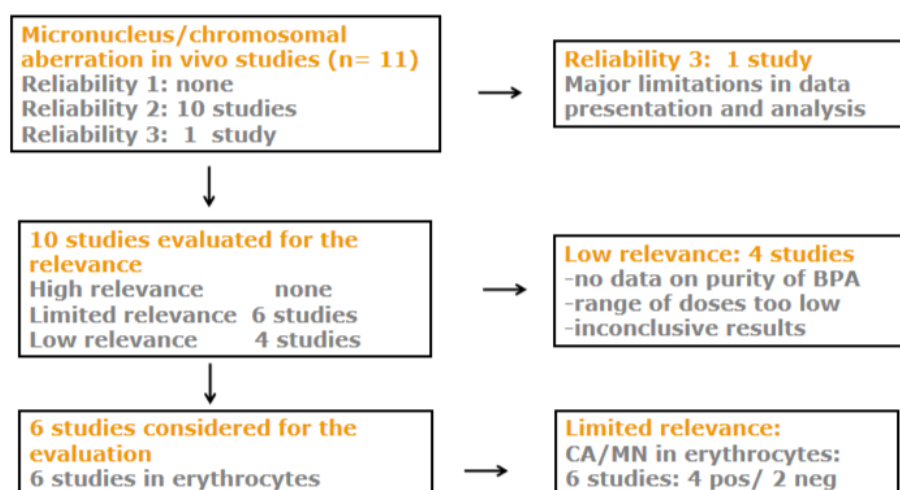
11452

11453 **Figure 2:** *In vitro* chromosomal aberration and micronucleus studies: evaluation and summary of test
 11454 results from 15 studies.

11455 **2b. *In vivo* chromosomal aberrations/micronuclei**

11456 Eleven *in vivo* studies addressing BPA-induced MN and structural CA after oral exposure were evaluated.
 11457 After a screening for the reliability and relevance of the results, six studies from four publications, all
 11458 ranked as of limited relevance, were selected for further consideration (Figure 3 and Table 15). Of
 11459 these, three studies were considered positive for the induction of MN and CA in the same publication
 11460 (Tiwari et al., 2012) or of MN (Panpatil et al., 2020 [RefID 379-G]) in rats following daily oral BPA
 11461 administrations for 6 and 28 days, respectively. Tiwari et al. (2012) applied a range of doses from 2.4
 11462 µg up to 50 mg/kg bw per day. In a separate publication, the same authors (Tiwari and Vanage, 2017)
 11463 reported that these experimental conditions were associated with the induction of lipid peroxidation
 11464 (malonaldehyde, MDA) and oxidative stress (decreased SOD, CAT, GSH) in rat bone marrow and
 11465 peripheral blood lymphocytes. In Panpatil et al. (2020) [RefID 379-G] the dose range was much lower
 11466 (50 and 100 µg/kg bw per day). A fourth study tested positive in the mouse bone marrow MN test after
 11467 the administration of a daily dose of 50 mg/kg bw for 28 days in presence of high level of cytotoxicity
 11468 (Fawzy et al., 2018 [RefID 270-G]). A study by Naik and Vijayalaxmi (2009) reported negative findings
 11469 in the mouse bone marrow MN test and CAs following a single dose in the range 10 to 100 mg/kg bw.

11470
 11471 Overall, the available data provided evidence of chromosomal damage after multiple oral
 11472 administrations but not after single oral administration of BPA.



11473
 11474 **Figure 3:** *In vivo* chromosomal aberration and micronucleus studies: evaluation and summary of
 11475 test results from 11 studies.

11476 **Table 15:** Summary table of test results of MN and CAs *in vivo* studies.

Test system	Dose	Results	Reference
MN and CA in bone marrow Swiss albino mice 6 animals/group	10, 50 and 100 mg/kg bw, single dose by gavage; 10 mg/kg bw for 5 days (50 mg) by gavage	NEGATIVE No significant decrease of PCE/NCE ratio, but significant increase of gaps and C-mitoses	Naik and Vijayalaxmi, 2009

MN and CA in bone marrow Holtzman rats 10 animals/group	2.4 µg, 10 µg, 5 mg and 50 mg/kg bw per day orally for 6 days	POSITIVE Dose-related increase of CA and MN-PCEs starting from 10 µg	Tiwari et al., 2012
MN in bone marrow Male Swiss albino mice 10 animals/group	50 mg/kg bw per day orally for 28 days	POSITIVE Significant reduction in the ratio of PCE/NCE	Fawzy et al., 2018 [RefID 270-G]
MN in bone marrow Male Wistar rats 6 animals/group	50 and 100 µg/kg bw per day orally for 28 days	POSITIVE Dose-related increase of MDA in blood and of urinary 8-OHdG	Panpatil et al., 2020 [RefID 379-G]

11477

11478 **3. Comet assay**11479 **3a. *In vitro* comet assay**

11480 Twenty-two *in vitro* studies using a comet assay in different cell lines were available for evaluation.
 11481 Twelve were classified as of limited relevance and further considered in the assessment. Most cell lines
 11482 used in these studies were of human origin from blood, mammary gland and prostate. Rodent cell lines
 11483 from rat, mouse and hamster and one cell line from monkey were also considered.

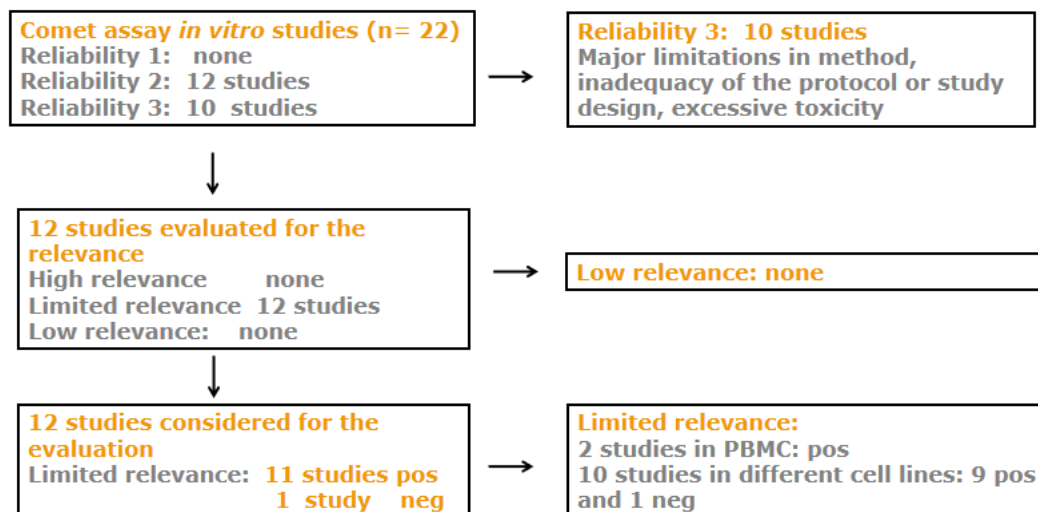
11484 Eleven of the 12 studies reported positive results. Three studies on HepG2 cell line yielded both positive
 11485 (Li XH et al., 2017 [RefID 4176]; Balabanič et al., 2021 [RefID 224-G]) and negative (Fic et al., 2013)
 11486 results. In a non-tumorigenic human prostatic cell line, BPA induced a significant increase in DNA strand
 11487 breaks paralleled by a decrease in total GSH, antioxidant capacity, glutathione peroxidase 1 (GPx1) and
 11488 SOD activity and an increase in glutathione reductase (Kose et al., 2020 [RefID 325-G]). Positive results
 11489 were also reported in CHO cells (Xin et al., 2015 [RefID 8150]). Positive results were reported from two
 11490 studies in which human PBMC were analysed by both alkaline and neutral comet assays (Mokra et al.,
 11491 2017 [RefID 5170]). Evidence of oxidative damage to DNA bases was provided by the addition
 11492 of endonuclease III (Nth) and 8-oxoguanine DNA glycosylase (hOGG1) DNA repair enzymes (Mokra et
 11493 al., 2018 [RefID 364-G]). DNA strand breaks induction by BPA was associated with increased ROS, MDA
 11494 and reduced SOD activity in HepG2 (Li XH et al., 2017 [RefID 4176]). In murine macrophage RAW264.7
 11495 cells, positive DNA strand breaks were associated with an increase in ROS and decreased level
 11496 of antioxidant enzymes (Huang FM et al., 2018 [RefID 296-G]). In Marc-145 rhesus monkey embryo
 11497 renal epithelial cells, DNA strand breaks induction was associated with increased ROS and TBARS and
 11498 decrease in GSH and SOD activity (Yuan et al., 2019 [RefID 478-G]).

11499 DNA strand breaks induction in mouse embryonic fibroblast cell line (NIH3T3) is associated with
 11500 elevated ROS and a modest increase in DNA 8-hydroxy-2'-deoxyguanosine (8-OHdG) at the highest
 11501 concentration tested (Chen et al., 2016 [RefID 1130]). In rat INS-1 insulinoma cells DNA strand breaks
 11502 and ROS level increased in parallel along with the induction of DNA damage-associated proteins (p53
 11503 and p-Chk2). At the highest concentration of 100 µM, pre-treatment with NAC reduced the number of
 11504 induced DNA strand breaks by two-fold (Xin et al., 2014 [RefID 8147]). Finally, ER-positive MCF-7 cells
 11505 were more sensitive than ER-negative MDA-MB-231 cells to BPA-induced DNA damage, as measured
 11506 by comet assay (Iso et al., 2006).

11507 The available *in vitro* studies provided evidence that BPA induces DNA strand breaks most likely related
 11508 to the induction of oxidative stress.

11509

11510



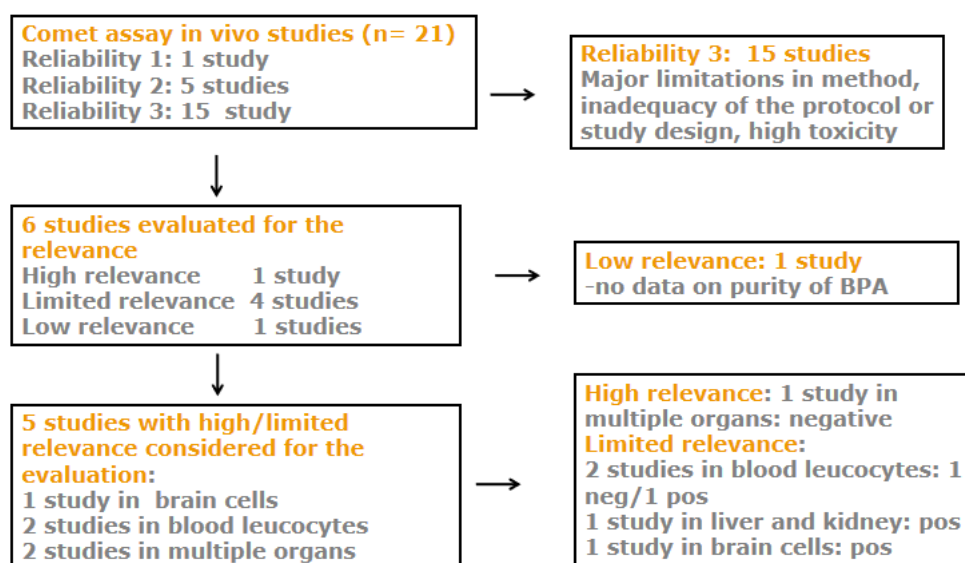
11511

11512 **Figure 4:** *In vitro* comet assay: evaluation and summary of test results from 22 studies.

11513 3b. *In vivo* comet assay

11514 In the current assessment only five of 21 *in vivo* comet assay studies of DNA strand breaks induction
 11515 by BPA were classified as of high (one study) or limited relevance and have been considered for
 11516 evaluation. Among the five oral studies selected, three were positive and two were negative. A single
 11517 study of high relevance reported negative results in multiple mouse organs (liver, kidney, testes, urinary
 11518 bladder, colon and lungs) after single treatment at three doses up to the MTD of 500 mg/kg bw (Sharma
 11519 et al., 2018 [RefID 662-G]). Negative results were also reported in rats exposed to 200 mg/kg bw per
 11520 day orally for 10 days (De Flora et al., 2011). In contrast, dose-related increases in DNA strand breaks
 11521 were reported at doses greater than 10 µg/kg bw in rats treated for 6 days with a range of doses
 11522 between 2.4 µg and 50 mg/kg bw per day (Tiwari et al., 2012). A weak and dose-dependent increase
 11523 in liver DNA strand breaks was observed at 50 and 100 mg/kg bw per day, whereas the increase in
 11524 kidney was limited to 50 µg/kg bw (Panpatil et al., 2020 [RefID 379-G]). Finally, in a study on BPA
 11525 neurotoxicity, a significant increase of strand breaks in brain cells was observed after treatment in a
 11526 range of doses from 0.5 to 5000 µg/kg bw per day for 8 weeks (Zhou YX et al., 2017 [RefID 9083]).

11527 Overall, the comet assays provided only limited evidence of DNA damage following multiple
 11528 administrations of BPA, but not following single dose administrations.



11529

11530 **Figure 5:** *In vivo* comet assay: evaluation and summary of test results from 21 studies.

11531 **Table 16:** Summary table of test results of Comet *in vivo* studies.

Test system	Dose	Results	Reference
<i>Comet assay in liver, kidney, testes, urinary bladder, colon and lungs</i>			
CD-1 male mice 5 animals/group	125, 250 and 500 (MTD) mg/kg bw Single dose by gavage	NEGATIVE	Sharma et al., 2018 [RefID 662-G]
<i>Comet assay in blood leucocytes</i>			
Sprague-Dawley rats 8 animals/group	200 mg/kg bw per day orally for 10 days	NEGATIVE	De Flora et al., 2011
Holtzman rats 10 animals/group	2.4 µg, 10 µg, 5 mg and 50 mg/kg bw per day orally for 6 days	POSITIVE Dose-related increase starting from 10 µg/kg	Tiwari et al., 2012
<i>Comet assay in liver and kidney</i>			
Male Wistar rats (WNIN) 6 animals/group	50 and 100 µg/kg orally for 4 weeks	POSITIVE Weak dose-related in liver; only at 50 µg/kg in kidney	Panpatil et al., 2020 [RefID 379-G]
<i>Comet assay in brain cells</i>			
KM male mice 11 animals/group	0.5, 50 and 5000 µg/kg bw per day orally for 8 weeks	POSITIVE	Zhou YX et al., 2017 [RefID 9083]

11532

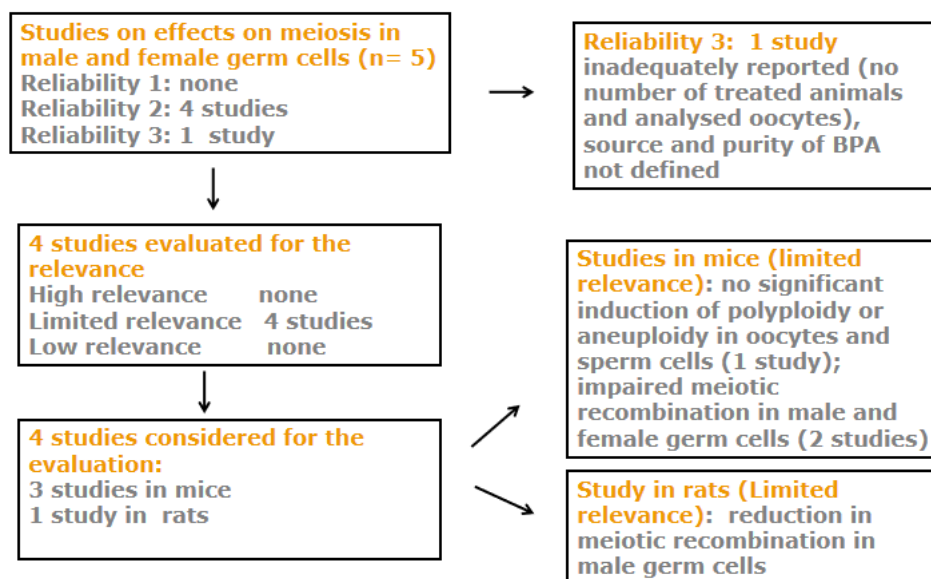
11533 **4. Effects in germ cells**

11534 A dominant mutation test carried out in Holtzman rats examined the mutagenic activity of BPA in male
 11535 germ cells (Tiwari and Vanage, 2013). In this study a decrease in total implants/female and live
 11536 implants/female, with a concurrent significant increase in the number of resorbed embryos per female,
 11537 was observed during the fourth week and sixth week in females mated with males treated with 5.0 mg
 11538 BPA/kg bw per day for 6 days. The timing of response may indicate the induction of post-implantation
 11539 loss due to dominant lethal mutations in mid-spermatids and spermatocytes. However, the CEP Panel
 11540 noted that the limited protocol of the study, with less analysable total implants and resorptions than

11541 recommended, as well as the lack of positive and historical controls, prevents to reach a firm conclusion
11542 on the dominant lethal effect of BPA on male germ cells.
11543 A comprehensive evaluation of the aneugenic potential of BPA in rodent germ cells was carried out in
11544 the framework of a European collaborative project on aneugenic chemicals (Pacchierotti et al., 2008).
11545 Hyperploidy and polyploidy were evaluated in metaphase II (MII) oocytes and zygotes following acute
11546 (0.2 and 20 mg/kg bw), subacute (0.04 mg/kg bw for 7 days) and subchronic (0.5 mg/L equivalent to
11547 0.09 mg/kg¹⁹ for 7 weeks) administration of BPA to young adult (4 or 9 weeks old) females, and in
11548 epididymal sperm after subacute treatment of adult males (six daily administrations of 0.002, 0.02 and
11549 0.2 mg/kg bw). The results of the project did not provide any evidence of increased frequency of
11550 aneuploidy in mouse oocytes and zygotes and in sperm cells following low dose BPA exposure.
11551 Other studies on male and female germ cells investigated the effect of BPA exposure on meiotic
11552 progression and recombination.
11553 In a study on adult (8 weeks old) male rats (Liu C et al., 2014 [RefID 4378]), the administration of BPA
11554 (20 µg/kg bw per day) by gavage for 60 consecutive days (one spermatogenesis cycle) inhibited
11555 spermiation and delayed meiosis initiation. An accumulation of unresolved DSBs and chromosomal
11556 abnormalities (asynapsis, end-to-end associations and altered synaptonemal complex staining) was
11557 observed in the late pachytene stage. These data indicate that the inhibition of meiotic DSBs repair
11558 following activation of checkpoints may halt meiotic progression and inhibit meiotic entry.
11559 In mice, oral exposure to BPA in neonatal life (20 or 500 µg/kg bw per day on days 1-12 post-partum)
11560 resulted in a reduction of meiotic recombination in pachytene cells, which persisted in the adult males
11561 (Vrooman et al., 2015 [RefID 7521]). A reduction in meiotic recombination (measured by the number
11562 of MLH1 foci in pachytene stage meiocytes) was also observed in another study in young (6 weeks old)
11563 male adult mice following oral administration of BPA (20 µg/kg bw per day) on days 1-8 post-partum
11564 (Horan et al., 2018 [RefID 291-G]). In the same study, administration of BPA (20 µg/kg bw per day)
11565 to pregnant females on days 14-15 post-coitum, to coincide with the time of meiotic entry in the fetal
11566 ovary, resulted in an increase of meiotic recombination in developing oocytes.
11567 It was suggested that a decreased level of recombination in spermatocytes may be lethal in these cells
11568 due to the actions of the spindle assembly checkpoints (SAC). In contrast, increased meiotic
11569 recombination, which is compatible with continued oocyte survival, may increase the frequency of
11570 aneuploid eggs and embryos (Horan et al., 2018 [RefID 291-G]). However, the CEP Panel noted that
11571 adequately conducted studies in male and female mouse germ cells failed to demonstrate an increase
11572 in aneuploid gametes following oral exposure to low doses of BPA under several exposure regimens
11573 (Pacchierotti et al., 2008).
11574
11575 Overall, the CEP Panel concluded that there is no evidence for aneugenic activity in germ cells in studies
11576 in adult animals using low doses of BPA.

11577

¹⁹ According to EFSA Scientific Committee (2012)



11578

11579 **Figure 6:** Effects in germ cells: summary of evaluation and results from five studies.

11580

11581 **5. Other studies**

11582

11583 **5a. DNA adducts**

11584 In early publications, ³²P post-labelling indicated the formation of several DNA adducts of BPA *in vitro*
 11585 in the presence of a microsomal activation system and *in vivo* in mouse and rat liver and in mouse
 11586 mammary tissue (Atkinson and Roy, 1995a,b; Izzotti et al., 2009). These reactions were proposed to
 11587 occur *via* oxidation of BPA to bisphenol-*o*-quinone. Formation of DNA adducts was confirmed in human
 11588 prostate cell lines (De Flora et al., 2011). No further characterisation of BPA-induced DNA adducts has
 11589 been reported since the last EFSA opinion.

11590 The possible biological significance of these DNA adducts is undermined by the limitation of the
 11591 postlabelling technique together with multiple studies that indicate that BPA induces mutagenic and
 11592 clastogenic DNA damage in the absence of metabolic activation.

11593 **5b. Induction of γH2AX foci**

11594 Several studies have investigated the induction of γH2AX foci (generally regarded as a marker of DNA
 11595 DSBs) following BPA treatment (Iso et al., 2006; Pfeifer et al., 2015 [RefID 5815]; George and
 11596 Rupasinghe, 2018 [RefID 277-G]; Kim et al., 2018b [RefID 11137]; Mahemuti et al., 2018 [RefID
 11597 11171]; Hercog et al., 2019 [RefID 278-G]; Hercog et al., 2020 [RefID 288-G]; Nair et al., 2020 [RefID
 11598 367-G]; Yin et al., 2020 [RefID 474-G]; Escarda-Castro et al., 2021 [RefID 266-G]; Yuan et al., 2021
 11599 [RefID 477-G]).

11600 Iso et al. (2006) reported increased levels of γH2AX foci after treatment with 17β-E2 or BPA in ER-
 11601 positive MCF-7 cells (1000x higher concentrations of BPA were needed to induce the same levels of
 11602 effects as E2). Induction was less severe in ER-negative MDA-MB-231 cells and the ER antagonist
 11603 ICI182780 blocked BPA-induced γH2AX focus formation in MCF-7 cells. Taken together, these findings
 11604 indicate that BPA-induced genotoxicity is ER-dependent.

11605 The effects of low-dose BPA were studied in the ER α -negative MCF10A and in 184A1 normal breast
11606 epithelial cell lines and the ER α -positive MCF7 and MDA-MB-231 human breast epithelial
11607 adenocarcinomas. Low doses (10 and 100 nM) induced DSBs as measured by γ H2AX foci in all cell lines
11608 and increased the level of c-Myc and of the cell-cycle regulatory proteins cyclins D1 and E and E2F1.
11609 Silencing c-Myc reduced BPA-induced γ -H2AX foci and abolished BPA-mediated mitochondrial ROS
11610 production. BPA also induced proliferation in ER α -negative 184A1 mammary cells. The authors conclude
11611 that low-dose BPA exerts a c-Myc-dependent genotoxicity and mitogenicity in ER α -negative mammary
11612 cells (Pfeifer et al., 2015 [RefID 5815]).

11613 A concentration-dependent increase in γ -H2AX foci and ROS levels was reported following exposure to
11614 BPA in the sub-micromolar range in HepG2 and NKNT human hepatocyte cell lines. Similar effects were
11615 also observed in liver tissue of juvenile rats exposed to a relatively low dose of BPA (0.5 mg/kg bw for
11616 90 days). These effects were correlated to the BPA-associated promotion of cell proliferation (Kim et
11617 al., 2018b [RefID 11137]).

11618 A 2-month exposure of HME1 mammary epithelial cells and MCF7 breast cancer cell line to low, non-
11619 toxic BPA concentration (0.0043 nM) increased levels of DNA damage as shown by upregulation of DNA
11620 damage markers (γ H2AX, pCHK1, pCHK2, p-P53) and disruption of the cell cycle progression. In
11621 addition, hypomethylation was observed in a panel of 24 tumour suppressor genes consistent with an
11622 epigenetic mechanism (Nair et al., 2020 [RefID 367-G]).

11623 Together these data suggest that the BPA-mediated increase in γ H2AX foci is associated with an
11624 increased rate of cell proliferation induced by exposure to low concentrations of this compound. In
11625 contrast, only the highest tested concentration (20 μ g/ml for 72 h) of BPA induced a significant increase
11626 in γ H2AX foci in HepG2 cells (Hercog et al., 2019 [RefID 287-G], 2020 [RefID 288-G]). This increase
11627 was accompanied by changes in the expression of some genes involved in xenobiotic metabolism and
11628 response to oxidative stress (but no changes in any of the genes involved in the DNA damage response
11629 DDR), although the relevance of these changes is uncertain.

11630 Mahemuti et al. (2018) [RefID 11171] examined γ H2AX foci and changes in global gene expression
11631 induced by BPA in cultured human foetal lung fibroblasts. BPA (100 μ M) increased DNA DSBs as shown
11632 by induction of γ H2AX foci. Activated ATM signalling (increased phosphorylation of p53) resulted in
11633 increased cell cycle arrest at G1 phase accompanied by senescence, autophagy and decreased cell
11634 proliferation. Finally, BPA increases cellular ROS and activates the Nrf2-regulated stress response and
11635 xenobiotic detoxification pathways. The unclear levels of toxicity in this study does not allow any clear
11636 conclusion to be reached (Mahemuti et al., 2018 [RefID 11171]).

11637 The quality of some of the studies (including undefined levels of toxicity) precludes any evaluation of
11638 the relevance of the findings reported by George and Rupasinghe (2018) [RefID 277-G], Yin et al.
11639 (2020) [RefID 474-G], Escarda-Castro et al. (2021) [RefID 266-G] and Yuan et al. (2021) [RefID 477-
11640 G].

11641 In conclusion, γ H2AX foci are induced by BPA exposure, both at low and high concentrations. These
11642 foci may however arise from different mechanisms (increased cell proliferation with concurrent ROS
11643 production as well as DSB associated with toxic events).

11644 **5c. Oxidized DNA bases**

11645 A significant increase in DNA 8-OHdG was observed in sperm exposed to high concentrations of BPA
11646 (Barbonetti et al., 2016 [RefID 419]). The significance of changes in oxidative DNA damage in the
11647 extreme experimental conditions of sperm with no motility and/or viability is, however, questionable.

11648 In a short report published in conference proceedings, 8-OHdG was produced *in vitro* by reacting dG
11649 with BPA in the presence of the Fenton reagent. The authors conclude that BPA may act as a prooxidant

11650 (Budiawan et al., 2018 [RefID 757-G]) although the very low levels of 8-OHdG formation (42.26 ppb,
11651 analyzed by HPLC/EC) raise questions about the relevance of this short report.

11652 No convincing evidence of BPA induction of oxidized DNA base(s) is available.

11653 **5d. Analysis of mutations by whole genome sequencing (WGS)**

11654 In comparison with untreated controls, increased levels of single and double base substitutions, small
11655 insertion/deletions and structural variants (mainly translocations) were identified by WGS in human
11656 embryonic HEK 293T kidney cells following exposure to 100 μ M BPA (Hu et al., 2021 [RefID 295-G]).
11657 In these experimental conditions, a large increase of γ H2AX foci was also reported. Increased levels of
11658 GC>TA transversions and mutations at A:T base pairs were reported in three clonally amplified BPA-
11659 treated cell populations in comparison with one clonally amplified control cell line, which could suggest
11660 a mutagenic potential

11661 This study represents a novel approach to study mutations at genome level. Although this is a very
11662 powerful method able to identify also very weak mutagens, the novelty of the techniques, the lack of
11663 information on the reliability of the methodology and the relatively small number of clonal cell lines
11664 analysed do not allow firm conclusions to be reached on the mutagenic potential of BPA.

11665 **5e. Changes in gene expression and DNA methylation**

11666 Changes in DNA methylation have been investigated in several studies (De Felice et al, 2015 [RefID
11667 1462]; Porreca et al., 2016 [RefID 5892]; Karmakar et al., 2017 [RefID 3388]; Karaman et al., 2019
11668 [RefID 269-G]). No specific discussion on DNA repair or DDR genes is reported in these publications.

11669 None of the information present in these studies is relevant for the clarification of the genotoxic
11670 potential of BPA.

11671 **5f. Studies in humans**

11672 A few studies have investigated the association between environmental or occupational BPA exposure,
11673 measured by urinary BPA levels, and makers of oxidative DNA damage and/or oxidative stress in urine
11674 (Huang YF et al., 2017b [RefID 2962]; Lv YS et al., 2017 [RefID 4702]; Rocha et al., 2018 [RefID 402-
11675 G]), sperm DNA damage (Omran et al., 2018 [RefID 373-G]; Radwan et al., 2018 [RefID 390-G]; Kiwitt-
11676 Cárdenas et al., 2021 [RefID 321-G]) and sperm aneuploidy (Radwan et al., 2018 [RefID 390-G]) in
11677 humans. Mixed results were reported for BPA and urinary 8-OHdG (weak association in Lv YS et al.,
11678 2017 [RefID 4702], no or doubtful association in Huang YF et al., 2017b [RefID 2962] and Rocha et
11679 al., 2018 [RefID 402-G]) or sperm DNA damage (positive in Omran et al., 2018 [RefID 373-G], equivocal
11680 in Kiwitt-Cárdenas et al., 2021 [RefID 321-G] and negative in Radwan et al., 2018 [RefID 390-G]).
11681 However, all studies were considered Inadequate to establish a causal association between the markers
11682 analyzed and BPA exposure, because of their observational nature and/or the low number of subjects
11683 recruited and/or the Inadequate control of confounders.

11684 Overall, human studies are not considered to provide additional relevant information for the evaluation
11685 of BPA genotoxicity.

11686 **6. Mode of action**

11687 BPA did not induce gene mutations in bacteria. All the available *in vitro* studies on chromosomal
11688 damage, classified as of high or limited relevance, reported positive results such as increase of CA or
11689 MN frequency, in different cellular systems. The increases of BPA-induced chromatid and chromosome
11690 breaks observed in some studies (Xin et al., 2015 [RefID 8150]; Santovito et al., 2018 [RefID 11220];
11691 Di Pietro et al., 2020 [RefID 258-G]) in association with the induction of DNA strand breaks, detected
11692 by comet assay (Xin et al., 2015 [RefID 8150]) are consistent with a clastogenic activity. Moreover, the

11693 potential of BPA to affect the spindle integrity and interfere with the chromosome segregation
11694 machinery was demonstrated in some reliable studies. Johnson and Parry (2008) reported the formation
11695 of aberrant mitotic spindles, with multiple poles, in V79 cells treated with BPA. Altered cytoskeleton
11696 organization, with multipolar spindles, failure of microtubule attachment to the kinetochore with the
11697 concomitant activation of SAC and chromosome misalignment, were also observed in HeLa cells (Kim
11698 et al., 2019 [RefID 319-G]). Studies on spindle morphology of mouse (Yang et al., 2020 [RefID 469-
11699 G]) and bovine (Campen et al., 2018 [RefID 240-G]) oocytes during *in vitro* maturation reported a
11700 pattern of alterations similar to that observed in permanent cell lines, namely shorter and multipolar
11701 spindles, with altered kinetochore-microtubule attachment and chromosome misalignment at M II.

11702 The conclusion, based on these *in vitro* studies, is that BPA may act by both clastogenic and aneugenic
11703 mechanisms.

11704 The large majority (11 out of 12) of the *in vitro* studies on comet assay, classified as of limited relevance,
11705 reported BPA-induced increases of DNA strand breaks. In some studies, the increase of DNA damage
11706 was associated with a parallel increase of ROS and MDA and decrease in antioxidant capacity and in
11707 total GSH (Xin et al., 2014 [RefID 8147]; Li XH et al., 2017 [RefID 4176]; Huang FM et al., 2018 [RefID
11708 296-G]; Yuan et al., 2019 [RefID 478-G]; Kose et al., 2020 [RefID 325-G]). A study in macrophages
11709 reported also a release of cytochrome c from mitochondria along with increased apoptosis with the
11710 indication that the DNA strand breaks could be mainly through the oxidative stress-associated
11711 mitochondrial apoptotic pathway (Huang FM et al., 2018 [RefID 296-G]). This mechanism was reported
11712 also in studies on liver effects (see Chapter 3.1.2.4). In a study on human PBMC, the application of
11713 comet assay with the addition of endonuclease III (Nth) and 8-oxoguanine DNA glycosylase (hOGG1)
11714 DNA repair enzymes allowed the detection of oxidative damage to DNA bases (Mokra et al., 2018 [RefID
11715 364-G]). Further indication of the role of oxidative damage in induction of DNA strand breaks was
11716 provided by the protective effects on DNA damage induced by the pre-treatment with NAC (Xin et al.,
11717 2014 [RefID 8147]; Huang FM et al., 2018 [RefID 296-G]).

11718 In conclusion, the evidence of DNA strand breaks *in vitro* is in agreement with the ability of BPA to
11719 induce clastogenic damage. In addition, the studies on comet assay provide consistent evidence that
11720 BPA induces DNA strand breaks most probably related to the induction of oxidative stress.

11721 The available *in vivo* studies for BPA-induced chromosomal damage in somatic cells reported mixed
11722 results. No increase of CA and MN frequency was reported after a single administration of BPA to mice
11723 in a range of doses inducing toxicity at the bone marrow level (Naik and Vijayalaxmi, 2009). In contrast,
11724 in another study in mice, increased MN frequency was detected in the presence of high bone marrow
11725 toxicity (Fawzy et al., 2018 [RefID 270-G]). Positive results were observed in two rat studies (Tiwari et
11726 al., 2012; Panpatil et al., 2020 [RefID 379-G]) after repeated dose administration, possibly associated
11727 with lipid peroxidation and oxidative stress in the first study. No induction of hyperploidy or polyploidy
11728 was observed in these studies.

11729 These results indicate that the *in vivo* induction of chromosomal damage requires specific conditions
11730 such as repeated exposure to BPA.

11731 Induction of DNA strand breaks, detected by comet assay *in vivo*, was observed only after repeated
11732 exposure for extensive periods of time up to 8 weeks (Tiwari et al., 2012; Zhou YX et al., 2017 [RefID
11733 9083]; Panpatil et al., 2020 [RefID 379-G]). Only one study of high relevance was available on single
11734 administration of BPA reporting negative results in multiple mouse organs in a range of doses up to the
11735 MTD of 500 mg/kg bw (Sharma et al., 2018 [RefID 662-G]). An indication of a possible role of the
11736 oxidative stress in inducing DNA strand breaks by BPA was provided by the results of several studies
11737 (Abdel-Rahman et al., 2018 [RefID 199-G]; Fawzy et al., 2018 [RefID 270-G]; Kazmi et al., 2018 [RefID
11738 315-G]; Majid et al., 2019 [RefID 354-G]; Mohammed et al., 2020 [RefID 363-G]) showing the

11739 protective effects of natural extracts with antioxidant properties. However, these studies were evaluated
11740 as low relevance.

11741 Finally, studies on germ cells, carried out by four laboratories in the framework of a collaborative project
11742 on aneugenic chemicals, did not provide any evidence of increased frequency of aneuploidy in mouse
11743 oocytes and zygotes and in sperm cells following exposure to low BPA doses (Pacchierotti et al., 2008).

11744 BPA is genotoxic *in vitro* inducing chromosomal damage and DNA breaks. However, *in vivo* the evidence
11745 of genotoxic properties of BPA is contradictory. This might depend on multiple mechanisms of action
11746 described or proposed for BPA. A major difficulty in the interpretation of these contradictory results is
11747 the lack of knowledge on the role of BPA metabolism that could be operational in genotoxic activity.
11748 Indeed, the role of the proposed DNA adducts has not been clarified. Other uncertainties include the
11749 role of ER receptors in the oxidative stress induced by BPA.

11750 **3.1.9.2. Conclusion on hazard identification for Genotoxicity effects of BPA**

11751 In 2015, the CEF Panel concluded that:

11752 The available data support that BPA is not mutagenic (in bacteria or mammalian cells), or
11753 clastogenic (MN and CAs). The potential of BPA to produce aneuploidy *in vitro* was not
11754 expressed *in vivo*. The positive finding in the postlabelling assays *in vitro* and *in vivo* is unlikely
11755 to be of concern, given the lack of mutagenicity and clastogenicity of BPA *in vitro* and *in vivo*.

11756 Based on the scientific literature considered in the previous EFSA opinions and published thereafter
11757 until 21 July 2021, the CEP Panel concluded that:

- 11758
- 11759 • BPA does not induce gene mutations in bacteria;
 - 11760 • BPA induces DNA strand breaks, clastogenic and aneugenic effects in mammalian cells *in vitro*;
 - 11761 • oxidative stress related mechanism(s) are likely to be involved in the DNA damaging and
11762 clastogenic activity elicited by BPA *in vitro*;
 - 11763 • there is some evidence for DNA and chromosomal damaging activities of BPA *in vivo* following
11764 repeated administrations, but not following single administrations;
 - 11765 • the available studies do not provide evidence of aneugenicity of BPA in germ cells *in vivo*.

11765 In contrast with consistent positive *in vitro* findings, the *in vivo* findings in several studies with
11766 high/limited reliability were inconsistent. The CEP Panel concluded that the evidence does not support
11767 an *in vivo* genotoxic hazard posed by BPA through direct interaction with DNA.

11768

11769 **3.1.9.3 Uncertainty analysis for genotoxicity**

11770 The purpose of the uncertainty analysis for genotoxicity was to assess the degree of certainty for the
11771 conclusion on whether BPA presents a genotoxic hazard by a direct mechanism (direct interaction with
11772 DNA), taking into account the available evidence and also the associated uncertainties. This overall
11773 question was divided into two sub-questions, which were assessed by three WG members with specialist
11774 expertise in genotoxicity assessment:

11775 Sub-question 1: What is your probability (%) that there is a genotoxic hazard in humans from BPA?

11776 Sub-question 2: If there would be a genotoxic hazard in humans from BPA, what is your probability
11777 that its causes include a direct mechanism?

11778 The experts' judgements were elicited by the structured procedure described in Chapter 2.3.4.2. When
11779 assessing the two sub-questions, the experts considered all the data they had reviewed for the
11780 genotoxicity assessment, including results from *in vitro* studies and animal models, taking into account

11781 their relevance to humans; the available data from human studies were considered not relevant (see
 11782 Chapter 3.1.9.1, Paragraph 5f).

11783 Table 17 shows the revised judgements provided by the three experts together after sharing and
 11784 discussing their initial judgements and reasoning. The third row of Table 17 shows their probabilities
 11785 for the overall question, which were obtained by multiplying each expert’s probabilities for the two sub-
 11786 questions, as explained in Chapter 2.3.4.2. These are their probabilities that BPA does present a
 11787 genotoxic hazard and that there is a direct mechanism. The bottom row of Table 17 shows the
 11788 complement of the probabilities in the third row, obtained by subtracting each probability from 100%.
 11789 These are the experts’ probabilities for the opposite outcome: that BPA does *not* present a genotoxic
 11790 hazard by a direct mechanism. The fifth column of Table 17 shows the ‘envelope’ of the probabilities
 11791 for the three experts, obtained by taking the lowest and highest probabilities in each row. These express
 11792 the range of opinion across the three experts.

11793 **Table 17:** Results of the uncertainty analysis for the genotoxicity assessment.

	Expert A	Expert B	Expert C	Envelope of the three experts	Assessment (rounded values) *
Experts’ probabilities that BPA presents a genotoxic hazard in humans (sub-question 1)	70 – 90%	66 – 90%	70 – 90%	66 – 90%	66 – 90%
Experts’ probabilities that, if BPA is genotoxic, there is a direct mechanism (sub-question 2)	10 – 33%	10 – 33%	20 – 30%	10 – 33%	10 – 33%
Calculated probabilities that BPA is genotoxic by a direct mechanism (sub-question 1 x sub-question 2)	7 – 29.7%	6.6 – 29.7%	14 – 27%	6.6 – 29.7%	5 – 30%
Calculated probabilities that BPA is not genotoxic by a direct mechanism (100% minus row above)	70.3 – 93%	70.3 – 93.4%	73 – 86%	70.3 – 93.4%	70 – 95%

11794 *The calculated probabilities were rounded to the nearest 5%. The experts’ probabilities of 33% and 66% were not changed
 11795 because they correspond approximately to a 1 in 3 chance and a 2 in 3 chance, respectively.

11797 The results in Table 17 and the reasoning of the three experts were presented and discussed in detail
 11798 at a facilitated meeting with the full WG. It was agreed to take the envelope of the 3 experts’ results
 11799 as the consensus of the WG, taking account of the available evidence and associated uncertainties. The
 11800 WG also agreed that their consensus probability that BPA is genotoxic by a direct mechanism should
 11801 be rounded to 5 – 30%, as shown in the right-hand column of Table 17, to take account that it is based
 11802 on expert judgement and avoid the implied precision of the calculated values. Similarly, the WG rounded
 11803 their consensus probability that BPA is not genotoxic by a direct mechanism to 70 – 95%.

11804 The width of the consensus probability range for BPA not being genotoxic by a direct mechanism,
 11805 reflects the uncertainty of the three experts and the other WG members about the judgements on sub-
 11806 questions 1 and 2. The WG discussed in more detail which lines of evidence tended to support
 11807 probabilities in the lower end of this range, and which tended to support the upper end of the range
 11808 (Table 18).

11809 **Table 18:** Summary of lines of evidence supporting either lower or higher probabilities that BPA
 11810 does not present a genotoxic hazard by a direct mechanism, within the range assessed by the WG
 11811 (70-95%).

Evidence supporting probabilities closer to 95%	<ul style="list-style-type: none"> Consistent negative Ames tests
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	<ul style="list-style-type: none"> • Indications of carcinogenic effects of BPA do not indicate direct genotoxic mechanism because only at very low doses and not higher doses (non-monotonic), only after developmental exposure (up to weaning) and only in one target tissue • Reactive non-conjugated metabolites of BPA are observed in animals but not in humans • Effects only from repeated exposures, so might be secondary • Evidence for several indirect mechanisms
Evidence supporting probabilities closer to 70%	<ul style="list-style-type: none"> • Presence of uncharacterised DNA adducts • Mutational spectrum from whole genome assessment

11812

11813 It was concluded that it is Unlikely to Very Unlikely (5 – 30% probability) that BPA presents a genotoxic hazard, the causes of which include a direct mechanism (combining subquestion 1 and 2, see third row
11814 of Table 17). Accordingly, it was concluded that it is Likely to Very Likely (70 - 95% probability) that
11815 BPA either presents a genotoxic hazard only through indirect mechanism(s) or is not genotoxic. The
11816 likelihood terms used in these conclusions are taken from the approximate probability scale, which is
11817 recommended by EFSA (Table 2 in EFSA Scientific Committee, 2018a) for harmonised use in EFSA
11818 assessments.
11819

11820 EFSA Scientific Committee (2017c) has advised that, where the overall evaluation of genotoxicity for a
11821 substance leaves no concerns for genotoxicity, HBGVs may be established. However, if concerns for
11822 genotoxicity remain, establishing a HBGV is not considered appropriate and a Margin of Exposure (MoE)
11823 approach should be followed.

11824 Considering the WoE for probabilities closer to either 70% or 95% that BPA does not present a
11825 genotoxic hazard by a direct mechanism (Table 18), the CEP Panel concluded that probabilities close
11826 to 95% are more strongly supported by the evidence than probabilities close to 70% and, therefore,
11827 the balance of evidence allows a HBGV to be established.

11828

11829 **3.2. Hazard characterisation**

11830 **3.2.1 Dose–response modelling**

11831 The CEP Panel used BMD analysis for dose-response modelling and performed it in accordance with the
11832 updated guidance of the Scientific Committee on BMD modelling (EFSA Scientific Committee, 2017a),
11833 using the R-package PROAST as made available in the EFSA BMD online web-tool. The detailed reports
11834 of the BMD analyses are presented in Annex I, including the rationale for the selection of the BMRs. A
11835 summary of the outcome of the analyses is shown in Table 19.

11836 The approach described in the guidance on BMD modelling includes criteria to evaluate whether a dose-
11837 response relationship is present. However, when the data are relatively poor or uninformative, the
11838 resulting BMD confidence interval (CI) will tend to be wide, and the BMDL might be much lower than
11839 the true BMD. It might happen that the data are so poor that using the associated BMDL as a potential
11840 RP appears unwarranted. This might be concluded when the BMD CI is wide or when different models
11841 result in widely different BMDL values (EFSA Scientific Committee, 2017a). The current EFSA guidance
11842 does not provide guidance on how to deal with poor data sets.

11843 It was noted that a cut-off value of 10 for the ratio of the lowest non-zero dose and the BMDL is used
11844 in the BMDS software to discard datasets for BMD analysis (US EPA, 2020). In the absence of EFSA
11845 guidance, the CEP Panel decided to apply this cut-off value to bring a study forward for selection of the
11846 RP. If this criterion is not fulfilled, the BMDL is extrapolated too far outside the dose range and the
11847 study design is considered not adequate to evaluate the relevant effect sizes. However, the study will
11848 be considered in the uncertainty analysis.

11849 BMD analysis was performed using the BPA administered doses without conversion to HED. HED
11850 converted values will be used subsequently to compare the different modelling outcomes.

11851 **3.2.1.1 Immunotoxicity**

11852 Using a WoE approach, the CEP Panel assigned a likelihood level of Likely to BPA induced effects on
 11853 the Th17 cells (Luo et al., 2016 [RefID 4679]), on the neutrophils in epididymis (Ogo et al., 2018 [RefID
 11854 11201]) and on eosinophils in the BAL (Tajiki-Nishino et al., 2018 [RefID 13221]) and of Very Likely to
 11855 BPA-induced effects on serum OVA-specific IgE (O'Brien et al., 2014a [RefID 5462]). Therefore, these
 11856 endpoints were brought forward for BMD analysis; the CEP Panel noted that the datasets on neutrophils
 11857 in epididymis (Ogo et al., 2018 [RefID 11201]) and eosinophils in the BAL (Tajiki-Nishino et al., 2018
 11858 [RefID 13221]) included only two dose groups and a control group.

11859 Due to a litter effect in the dataset on the effect of BPA on serum OVA-specific IgE (O'Brien et al.,
 11860 2014a [RefID 5462]) and the lack of information to take the litter effect into account in the BMD
 11861 analysis, no BMD analysis was performed on this endpoint.

11862 For the effect of BPA on Th17 cells in mice, the lowest BMDL₂₀–BMDU₂₀ CI (0.06–0.74 µg/kg bw per
 11863 day) was observed in females at PND21; at PND42 the BMDL₂₀–BMDU₂₀ CI in females was 0.17–1.79
 11864 µg/kg bw per day. In males, similar BMDL₂₀–BMDU₂₀ CI were observed for both time points of
 11865 measurement (0.30–3.39 µg/kg bw per day for PND21 and 0.35–3.38 µg/kg bw per day for PND42).

11866 For the effect of BPA on the neutrophils in the epididymis, a BMDL₂₀–BMDU₂₀ CI of 6.8–90.4 µg/kg bw
 11867 per day was calculated for the caput/corpus while for the cauda, the BMDL₂₀ (0.5 µg/kg bw per day)
 11868 was too far outside the tested dose-range to be taken forward for identifying the RP. Also the BMDL₂₀
 11869 (0.00046 µg/kg bw per day) calculated for the effect on eosinophils in the BAL was too far outside the
 11870 tested dose range to be taken forward for identifying the RP.

11871 **3.2.1.2 Metabolic effects**

11872 The CEP Panel considered that the available evidence showed a Likely effect of BPA for the endpoint
 11873 uric acid. Therefore, the endpoints hepatic and serum uric acid concentrations reported by Ma et al.
 11874 (2018) [RefID 12637] were taken forward for BMD analysis (see Table 19 and Annex I for further
 11875 details).

11876 For the effect of BPA on serum uric acid in C57BL6 mice, none of the fitted models was better than the
 11877 null model, indicating that there is no observable trend. For the effect of BPA on serum uric acid in CD1
 11878 mice, the BMDL₂₀ (0.39 µg/kg bw per day) was too far outside the tested dose-range to be used for
 11879 identifying the RP. For the effect on hepatic uric acid a BMDL₂₀–BMDU₂₀ CI of 1.59–399 µg/kg bw per
 11880 day was calculated; the Panel noted the wide CI. However, as explained above, the EFSA guidance
 11881 does not provide guidance on how to deal with poor data sets.

11882 **3.2.1.3 Neurotoxicity and developmental neurotoxicity**

11883 The CEP Panel considered that the available evidence showed a Likely effect of BPA for the following
 11884 endpoints:

- 11885 • anxiety/emotionality (Xu XH et al., 2015 [RefID 8232]; Chen Z et al., 2018 [RefID 11734];
 11886 Liang et al., 2018 [RefID 12508]; Xin et al., 2018 [RefID 13482]);
- 11887 • learning and memory (Wang C et al., 2014 [RefID 7579]; Johnson et al., 2016 [RefID 3241];
 11888 Wang C et al., 2016 [RefID 7576]; Zhou YX et al., 2017 [RefID 9083]; Chen Z et al., 2018
 11889 [RefID 11734]);
- 11890 • male sexual behaviour (Picot et al., [RefID 5830]);
- 11891 • salt preference (Nuñez et al., 2018 [RefID 11199]);
- 11892 • dendritic spine density (Liu ZH et al., 2014 [RefID 10411]; Kimura et al., 2016 [RefID 3566];
 11893 Chen Z et al., 2018 [RefID 11734]);
- 11894 • number of neurons in hippocampus (CA1 and CA3 areas) (Zhou YX et al., 2017 [RefID 9083]);
- 11895 • AChE activity (Khadrawy et al., 2016 [RefID 3462]).

11896 Therefore, these endpoints were taken forward for BMD analysis; the CEP Panel noted that the datasets
 11897 on anxiety/emotionality (Xu XH et al., 2015 [RefID 8232]), dendritic spine density (Kimura et al., 2016
 11898 [RefID 3566]) and AChE activity (Khadrawy et al., 2016 [RefID 3462]) included only two dose groups
 11899 and a control group.

11900 Due to lack of information on litter effects in the learning and memory studies (Wang C et al., 2014
11901 [RefID 7579] and 2016 [RefID 7576]), no BMD analyses were performed.

11902 No observable trend was noted for the endpoints number of neurons in hippocampus (CA1 and CA3
11903 areas) (Zhou YX et al., 2017 [RefID 9083]), learning and memory (Zhou YX et al., 2017 [RefID 9083]),
11904 salt preference (Nuñez et al., 2018 [RefID 11199]) and anxiety/emotionality (immobility) in females
11905 (Xu XH et al., 2015 [RefID 8232]) (see Table 19 and Annex I for further details).

11906 For anxiety/emotionality, the BMDL₅₀ calculated from the dataset reported by Xin et al. (2018) [RefID
11907 13482] was not further considered due to the large uncertainty as illustrated by the large BMDL₅₀-
11908 BMDU₅₀ CI interval (6.1 to 1.06e+14 µg/kg bw per day). The BMDL₅₀ calculated from the data reported
11909 by Chen Z et al. (2018) [RefID 11734] was not further considered based on the ratio of the lowest non-
11910 zero dose and the BMDL. Xu XH et al. (2015) [RefID 8232] studied immobility and the time in open
11911 arms. A BMDL₅₀-BMDU₅₀ CI of 497-80400 µg/kg bw per day was calculated for males; the Panel noted
11912 the wide CI. The other BMDL₅₀ values, calculated from the data reported by Xu XH et al. (2015) [RefID
11913 8232], were too far outside the tested dose-range to be taken forward for identifying the RP.

11914 For the effect of BPA on learning and memory as studied by Johnson et al. (2016) [RefID 3241], a
11915 BMDL₅₀-BMDU₅₀ CI of 10.10-2160 µg/kg bw per day was calculated for females and 1.47-1520 µg/kg
11916 bw per day for males; the Panel noted the wide CIs. Chen Z et al. (2018) [RefID 11734] studied several
11917 endpoints in relation to this cluster. For the relative expression of NR2 in V1, a BMDL₂₀-BMDU₂₀ CI of
11918 7.96-842 µg/kg bw per day was calculated and for platform duration a BMDL₅₀-BMDU₅₀ of 10700-
11919 2.4e+7 µg/kg bw per day; the Panel noted the wide CIs. The BMDL values for the relative expression
11920 of NR2 and GluR1 in the hippocampus were too far outside the tested dose-range to be taken forward
11921 for identifying the RP.

11922 For dendritic spine density a BMDL₂₀-BMDU₂₀ CI of 4.24-2350 µg/kg bw per day was estimated from
11923 the dataset reported by Chen Z et al. (2018) [RefID 11734]; the Panel noted the wide CI. For the same
11924 endpoint, a BMDL₂₀-BMDU₂₀ CI of 16,800-70,100 µg/kg bw per day was calculated from the dataset
11925 reported by Liu ZH et al. (2014) [RefID 10411]; the Panel noted that the latter CI was completely
11926 outside the dose range. The BMDL₂₀ value calculated from the data reported by Kimura et al. (2016)
11927 [RefID 3566] was not considered further based on the ratio of the lowest non-zero dose and the BMDL.
11928 The same applied to the BMDL values calculated for AChE activity.

11929 **3.2.1.4 Reproductive and developmental toxicity**

11930 The CEP Panel considered that the available evidence showed a Likely effect of BPA for the following
11931 endpoints:

- 11932 • ovary weight (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]);
- 11933 • uterus histology (Vigezzi et al., 2015 [RefID 7472]; NTP Clarity Report, 2018/Camacho et al.,
11934 2019 [RefID 11370]);
- 11935 • ovary histology (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370] during the
11936 developmental exposure period; NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]
11937 during developmental and adult exposure; Hu et al., 2018 [RefID 11119] during adult
11938 exposure);
- 11939 • decreased implantation incidence (Li et al., 2016 [RefID 4128]);
- 11940 • epididymis histology (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]);
- 11941 • testis histology (Gurmeet et al., 2014 [RefID 2502]);
- 11942 • effects on sperm (Wang HF et al., 2016 [RefID 7618]).

11943 Therefore, these endpoints were taken forward for BMD analysis; the CEP Panel noted that the dataset
11944 on uterus histology (Vigezzi et al., 2015 [RefID 7472]) included only two dose groups and a control
11945 group.

11946 The dataset on decreased implantation incidence (Li et al., 2016 [RefID 4128]) could not be used for
11947 BMD analysis as the actual number of animals per dose group was not reported.

11948 Within the cluster female reproductive toxicity, no observable trend was noted for the endpoints uterus
11949 histology during developmental exposure and ovary histology during developmental and adult exposure
11950 reported by NTP Clarity Report (2018)/Camacho et al. (2019) [RefID 11370].

11951 A BMDL₀₅–BMDU₀₅ CI of 0.63–25000 µg/kg bw per day was calculated for ovary weight (NTP Clarity
11952 Report, 2018/Camacho et al., 2019 [RefID 11370]) and a BMDL₁₀–BMDU₁₀ CI of 5.53–3680 µg/kg bw
11953 per day for ovary histology during developmental exposure period reported by NTP Clarity Report
11954 (2018)/Camacho et al. (2019) [RefID 11370]. A BMDL₀₅–BMDU₀₅ CI of 0.96–349 µg/kg bw per day was
11955 calculated for the ratio of primordial and total follicles as reported by Hu et al. (2018) [RefID 11119].
11956 The Panel noted the wide CIs for these data sets. For the other datasets, the BMDL values were too
11957 far outside the tested dose-range to be taken forward for identifying the RP.

11958 Within the cluster male reproductive toxicity, no observable trend was noted for the incidence of
11959 inflammation in the epididymis as reported by NTP Clarity Report (2018)/Camacho et al. (2019) [RefID
11960 11370] and for testis histology as reported by Gurmeet et al. (2014) [RefID 2502]. For the incidence
11961 of exfoliated germ cells as reported by NTP Clarity Report (2018)/Camacho et al. (2019) [RefID 11370],
11962 a BMDL₁₀–BMDU₁₀ CI of 2,260–27,500 µg/kg bw per day was estimated. BMDL₂₀–BMDU₂₀ CIs of 26.1–
11963 2460 and 3.41–74.8 µg/kg bw per day were calculated for sperm viability and motility, respectively
11964 (Wang HF et al., 2016 [RefID 7618]). For the effect of BPA on AR, the BMDL value was too far outside
11965 the tested dose-range to be taken forward for identifying the RP.

11966 **3.2.1.5 Carcinogenicity and mammary gland proliferative effects**

11967 Overall, the CEP Panel did not assign a likelihood level of Likely to the pre-neoplastic and neoplastic
11968 histological changes. However, the Panel assigned a likelihood level of Likely to the following non-
11969 neoplastic uterine histological effects of BPA: gland cell anomalies (Vigazzi et al., 2015 [RefID 7472])
11970 and endometrial cystic hyperplasia (NTP Clarity Report, 2018/Camacho et al., 2019 [RefID 11370]).
11971 Therefore, these endpoints were taken forward for BMD analysis. The outcome of the BMD analysis on
11972 these endpoints is reported in Chapter 3.2.1.4.

11973 **3.2.2 Identification of the reference point**

11974 All BMDL values identified for the selection of the RP were converted to HED (Table 20). The CEP Panel
11975 selected the lowest BMDL value (expressed as HED) of 0.93 ng/kg bw per day for the effect of BPA on
11976 Th17 cells in mice to be used as RP for the risk assessment of BPA.

11977 The BMD analysis from which the RP was selected is repeated in Appendix F.

11978

11979 **Table 19:** Overview of BMD analyses.

Reference	Endpoint	Species	Dose range ^(b) (µg/kg bw per day)	BMR	Group	BMDL (µg/kg bw per day)	BMDU (µg/kg bw per day)	Ratio dose /BMDL ^(d)
Immunotoxicity								
Luo et al. (2016) [RefID 4679]	Th17 cells	Mouse	0.475–47.5	20%	F PND21	0.06	0.74	7.9
		Mouse	0.475–47.5	20%	F PND42	0.17	1.79	2.8
		Mouse	0.475–47.5	20%	M PND21	0.30	3.39	1.6
		Mouse	0.475–47.5	20%	M PND42	0.35	3.38	1.4
Ogo et al. (2018) [RefID 11201]	Neutrophils in epididymis: Caput/corpus	Rat	20–200	20%		6.8	90.4	2.9
	Neutrophils in epididymis: cauda	Rat	20–200	20%		0.5	4.62	40
Tajiki-Nishino et al. (2018) [RefID 13221]	Eosinophil infiltration in BAL fluid	Mouse	60–200	20%		0.00046	34.5	130434.8
Metabolic effects								
Ma et al. (2018) [RefID 12637]	Hepatic uric acid concentration	Mouse	5–500	20%		1.59	399	3.1
	Serum uric acid concentration	Mouse	5–500	20%	CD1 mice	0.39	91.5	12.8
		Mouse	5–500	20%	C57BL6 mice	None of the fitted models is better than the null model ^(a)		
Neurotoxicity- and developmental neurotoxicity								
Xin et al. (2018) [RefID 13482]	Time spent immobile in the forced swim test (Anxiety/emotionality)	Mouse	10–10000	50%		6.1	1.06e+14	1.6
Johnson et al. (2016) [RefID 3241]	Sniffing incorrect holes on day 7 (learning and memory)	Rat	2.5–2500	50%	F	10.10	2160	0.2
		Rat	2.5–2500	50%	M	1.47	1520	1.7
Chen Z et al. (2018) [RefID 11734]	First entry time in the open field test (Anxiety/emotionality).	Rat	40–4000	50%		0.03	517	1333.3
	Platform duration (learning and memory)	Rat	40–4000	50%		10700	2.4e+7	0.004

	Relative expression NR2 in the hippocampus (learning and memory)	Rat	40-4000	20%		2.18	439	18.3
	Relative expression GluR1 in the hippocampus (learning and memory)	Rat	40-4000	20%		0.31	2410	129.0
	Relative expression NR2 in V1 (learning and memory)	Rat	40-4000	20%		7.96	842	5.0
Zhou YX et al. (2017) [RefID 9083]	Quantity of hippocampal CA1 neurons	Mouse	0.5–500	20%		None of the fitted models is better than the null model(a)		
	Quantity of hippocampal CA3 neurons	Mouse	0.5–500	20%		None of the fitted models is better than the null model(a)		
	Trials to qualify for the standard (learning and memory)	Mouse	0.5–500	50%		None of the fitted models is better than the null model(a)		
Xu XH et al. (2015) [RefID 8232]	Time in open arms (Anxiety/emotionality)	Mouse	40–40,000	50%	F	1.71	1260	23.4
	Immobility (Anxiety/emotionality)	Mouse	40–40000	50%	M	497	80,400	0.1
		Mouse	40–40000	50%	F	None of the fitted models is better than the null model(a)		
		Mouse	40–40000	50%	M	0.03	89.4	1333.3
Núñez et al. (2018) [RefID 11199]	Salt preference	Rat	10–500	10%		None of the fitted models is better than the null model(a)		
Kimura et al. (2016) [RefID 3566]	Dendritic spine density	Mouse	40–400	20%		2.43	58.5	16.5
Liu ZH et al. (2014) [RefID 10411]	Dendritic spine density	Rats	918–9175	20%		16800	70100	0.1
Chen Z et al. (2018) [RefID 11734]	Dendritic spine density	Rat	40–4000	20%		4.24	2350	9.4
Khadrawy et al. (2016) [RefID 3462]	AChE activity in the cortex	Rat	10000–25000	20%		570	20700	17.5
	AChE activity in the hippocampus	Rat	10000–25000	20%		2.7	6330	3703.7

**Reproductive and developmental toxicity
Carcinogenicity and mammary gland proliferative effects ^(c)**

Camacho et al., 2019 [RefID 11370] ^(e)	Ovary weight	Rat	2.5–25000	5%	0.63	25000	4.0
Camacho et al., 2019 [RefID 11370] ^(c) ^(e)	Incidence of hyperplasia, cystic, endometrium (uterus histology)	Rat	2.5–25000	10%	None of the fitted models is better than the null model ^(a)		
	Incidence of squamous metaplasia (uterus histology)	Rat	2.5–25000	10%	None of the fitted models is better than the null model ^(a)		
Vigizzi et al. (2015) [RefID 7472] ^(c)	Incidence of glands with cellular anomalies (uterus histology)	Rat	0.5–50	10%	2.8e–05	8.34	17857.1
Camacho et al. (2019) [RefID 11370] ^(e)	Incidence of follicle cysts (ovary histology)	Rat	2.5–25000	10%	5.53	3680	0.5
Camacho et al. (2019) [RefID 11370] ^(e)	Incidence of interstitial cell hypertrophy (ovary histology)	Rat	2.5–25000	10%	None of the fitted models is better than the null model ^(a)		
Hu et al. (2018) [RefID 11119]	Ratio of primordial and primary follicles	Mouse	1–10000	5%	0.08	2.34	12.5
	Ratio of primordial and total follicles	Mouse	1–10000	5%	0.96	349	1.0
Camacho et al. (2019) [RefID 11370] ^(e)	Incidence of exfoliated germ cells (epididymis histology)	Rat	2.5–25000	10%	2260	27500	0.001
	Incidence of inflammation (epididymis histology)	Rat	2.5–25000	10%	None of the fitted models is better than the null model ^(a)		
Gurmeet et al. (2014) [RefID 2502]	Seminiferous tubule diameter (testis histology)	Rat	1000–100000	5%	None of the fitted models is better than the null model ^(a)		
Wang HF et al. (2016) [RefID 7618]	Viability (effects on sperm)	Mouse	10–250	20%	26.1	2460	0.4
	Motility (effects on sperm)	Mouse	10–250	20%	3.41	74.8	2.9
	AR (effects on sperm)	Mouse	10–250	20%	0.31	1230	32.3

11980 AR: acrosome reaction; AChE: acetylcholinesterase; BMDL: lower confidence limit of the benchmark dose; BMDU: upper confidence limit of the benchmark dose; BMR: benchmark response; F: female; M:
11981 male; PND: post-natal day.

11982 (a): All fitted models' Akaike information criterion (AIC) values are larger than null model's AIC – 2.

11983 (b): Dose range of BPA treated animals; the dose of 0 µg/kg bw per day was included in all studies but not included in the presented dose range. All doses are expressed as oral.

11984 (c): Studies with footnote (c) were identified for both HOCs; the other studies for Reproductive and developmental toxicity only.

11985 (d): Ratio of the lowest non-zero dose and the BMDL; values below 10 are shown in bold.

11986 (e): Full reference: NTP Clarity Report (2018)/Camacho et al. (2019).

11987 **Table 20:** Overview of BMD confidence intervals used for the identification of the Reference Point^(c) to derive a HBGV.

Reference	Endpoint	Species	BMR	Group	Administered doses		Administered doses converted to HED ^(b)	
					BMDL (µg/kg bw per day)	BMDU (µg/kg bw per day)	BMDL (ng/kg bw per day)	BMDU (ng/kg bw per day)
Immunotoxicity								
Luo et al. (2016) [RefID 4679]	Th17 cells	Mouse	20%	F PND21	0.06	0.74	0.93	11.5
		Mouse	20%	F PND42	0.17	1.79	2.64	27.7
		Mouse	20%	M PND21	0.30	3.39	4.65	52.5
		Mouse	20%	M PND42	0.35	3.38	5.43	52.4
Ogo et al. (2018) [RefID 11201]	Neutrophils in epididymis: Caput/corpus	Rat	20%		6.8	90.4	1126	14970
Metabolic effects								
Ma et al. (2018) [RefID 12637]	Hepatic uric acid	Mouse	20%		1.59	399	24.6	6185
Neurotoxicity and developmental neurotoxicity								
Johnson et al. (2016) [RefID 3241]	Sniffing incorrect holes on day 7 (learning and memory)	Rat	50%	F	10.1	2160	1673	357696
		Rat	50%	M	1.47	1520	243	251712
Chen Z et al. (2018) [RefID 11734]	Platform duration (learning and memory)	Rat	50%		10700	2.4e+7	1.77e+6	4.02e+9
	Relative expression NR2 in V1 (learning and memory)	Rat	20%		7.96	842	1318	139435
Xu XH et al. (2015) [RefID 8232]	Time in open arms (Anxiety/emotionality)	Mouse	50%	M	497	80400	7704	1.25e+06
Liu ZH et al. (2014) [RefID 10411]	Dendritic spine density	Rat	20%		16800	70100	2.78e+06	1.16e+07
Chen Z et al. (2018) [RefID 11734]	Dendritic spine density	Rat	20%		4.24	2350	702	389160
Reproductive and developmental toxicity								
Camacho et al. (2019) [RefID 11370] ^(e)	Ovary weight	Rat	5%		0.63	25,000	104	4.14e+06
Camacho et al. (2019) [RefID 11370] ^(e)	Incidence of follicle cysts (ovary histology)	Rat	10%		5.53	3680	916	6.09e+05
Hu et al. (2018) [RefID 11119]	Ratio of primordial and total follicles	Mouse	5%		0.96	349	14.9	5410
Camacho et al. (2019) [RefID 11370] ^(e)	Incidence of exfoliated germ cells (epididymis histology)	Rat	10%		2260	27500	3.74e+05	4.55e+06
Wang HF et al. (2016) [RefID 7618]	Viability (effects on sperm)	Mouse	20%		26.1	2460	405	38130
	Motility (effects on sperm)	Mouse	20%		3.41	74.8	53	1159

11988 BMDL: lower confidence limit of the benchmark dose; BMDU: upper confidence limit of the benchmark dose; BMR: benchmark response; F: female; M: male; PND: post-natal day.

11989 (a): All fitted models' Akaike information criterion (AIC) values are larger than null model's AIC - 2.

11990 (b) For the calculation of the BMDL/BMDU values expressed as HED, a HEDF of 0.0155 was used for studies in mice and a HEDF of 0.1656 for studies in rats. The lowest BMDL value is shown in bold.

11991 (c) The confidence intervals are shown as administered and as the corresponding human equivalent dose (HED).

11992 (e): Full reference: NTP Clarity Report (2018)/Camacho et al. (2019).

11993 3.2.3 Uncertainty in the hazard characterisation

11994 The purpose of the uncertainty analysis was to assess whether other effects of BPA may potentially occur
11995 after exposure to lower doses than the endpoint on which the RP is based and, if so, inform a decision on
11996 what size of additional UF would be suitable to take those effects into account. This was carried out in the
11997 series of steps described in Chapter 2.3.4, where an overview of the approach is provided in Figure 1. The
11998 final outcome of the uncertainty analysis is reported in this section. Detailed results of the steps leading to
11999 this outcome are presented in Appendix D.

12000 Five HOCs were considered at this phase of the uncertainty analysis: Immunotoxicity, Metabolic effects,
12001 Neurotoxicity and developmental neurotoxicity, Reproductive and developmental toxicity, and
12002 Carcinogenicity and mammary gland proliferative effects. Cardiotoxicity was not considered in the
12003 uncertainty analysis because the evidence was either judged as Not Likely or as Inadequate. The WG
12004 experts noted that no Likely or Very Likely clusters were identified for the HOC General toxicity, and the
12005 lowest effect level reported was 8 ng/kg bw per day (HED) for relative liver weight (Ke et al., 2016 [RefID
12006 3447]; single dose study) with an effect size of about 4%, which was considered not adverse. Therefore
12007 this HOC was not considered further in the uncertainty analysis.

12008 Within the other five HOCs, the uncertainty analysis focused on 21 clusters of endpoints that were rated
12009 ALAN, Likely or Very Likely in the WoE assessment. Uncertainty was assessed by quantitative expert
12010 judgements for each cluster (see Chapter 2.3.4), elicited from two or three experts per cluster. The expert's
12011 judgements were combined to produce a distribution quantifying the experts' uncertainty about the
12012 estimated lowest BMD in each cluster. The distributions for the 21 clusters were then combined to produce
12013 a distribution quantifying uncertainty about the estimated lowest BMD across all clusters, which is the
12014 distribution required to inform consideration of the need for an additional UF for deriving the TDI (illustrated
12015 by the pink distribution shown at the top of Figure 1 in Chapter 2.3.4).

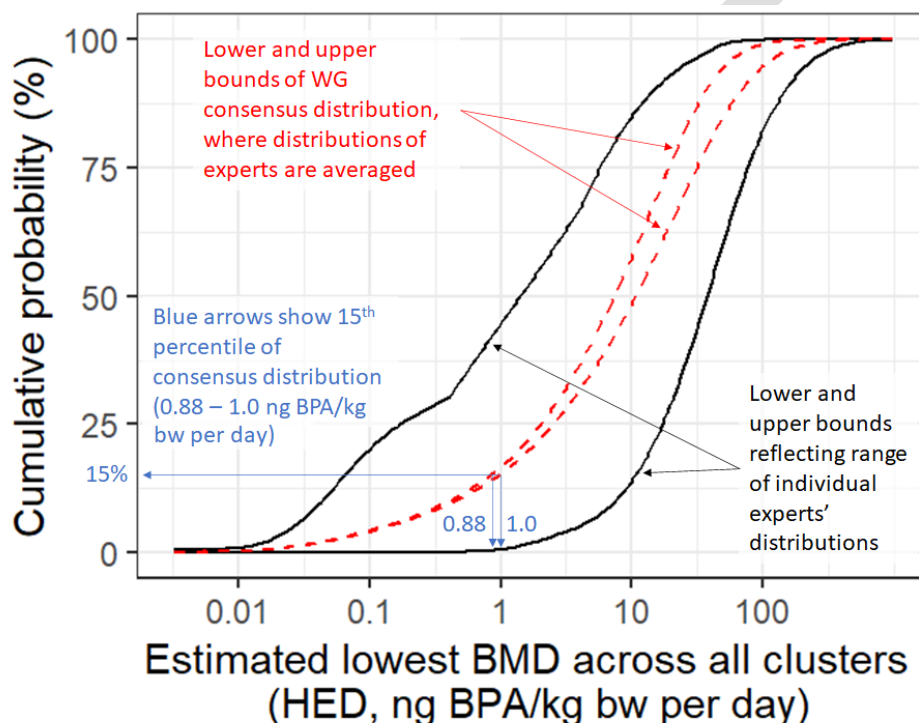
12016 Sensitivity analysis showed that the combined distribution was determined mainly by just one of the 21
12017 clusters: allergic lung inflammation. Uncertainties affecting this cluster were therefore subjected to further
12018 assessment by the whole WG. After reviewing the judgements and reasoning of the two experts with
12019 specialist knowledge of this cluster and considering the opinions of other WG members, the WG agreed on
12020 a consensus probability of 66% that at least one of the endpoints in this cluster that occurs in animals, is
12021 both relevant and adverse for humans.

12022 Distributions were elicited separately from 14 WG experts for the lowest BMD for an endpoint in the cluster
12023 allergic lung inflammation that occurs in animals and is both relevant and adverse for humans. These were
12024 then used to produce lower and upper bounds for a revised distribution quantifying uncertainty about the
12025 estimated lowest BMD across all clusters, which is shown by the black solid curves in Figure 7. The distance
12026 between the lower and upper bounds is mostly due to differences between experts in their assessment of
12027 the lowest BMD in each cluster, primarily for the cluster allergic lung inflammation where the experts'
12028 individual distributions ranged over five orders of magnitude.

12029 The WG experts identified and discussed a list of additional uncertainties potentially affecting the hazard
12030 assessment (see Appendix D). The WG had already considered many of these when making their
12031 judgements earlier in the uncertainty analysis and judged that the additional uncertainties would not alter
12032 the assessment provided by the wide bounds of the distribution resulting from the judgements of different
12033 experts (black curves in Figure 7).

12034 The distance between the lower and upper bounds was reduced when the distributions of different experts
 12035 were combined by averaging for each cluster, as shown by the red dashed curves in Figure 7. The remaining
 12036 distance between the red curves is due to the range of responses given by the experts when assessing, for
 12037 each cluster other than allergic lung inflammation, their probability that at least one endpoint occurs in
 12038 animals and is both relevant and adverse for humans. The WG experts agreed on the red dashed curves
 12039 in Figure 7 as their consensus assessment of overall uncertainty for the estimated lowest BMD across all
 12040 clusters that were rated ALAN, Likely or Very Likely in the WoE assessment (including General toxicity
 12041 clusters, for the reasons given above).

12042



12043

12044 The solid black curves show the lower and upper bounds of the distribution resulting from the range of judgements between WG
 12045 experts. The red dashed curves show the lower and upper bounds of the consensus distribution of the WG, where judgements of
 12046 different experts for each cluster were aggregated by averaging. Selected percentiles of the consensus distribution are shown in
 12047 Table 21.

12048 **Figure 7:** Lower and upper bounds for cumulative distributions quantifying uncertainty about the
 12049 estimated lowest BMD across all 21 clusters considered in the uncertainty analysis.

12050 As the final step in the uncertainty analysis the WG experts considered whether an additional UF is needed
 12051 when deriving the TDI. To inform this, lower and upper bounds for different percentiles of the consensus
 12052 distribution for the estimated lowest BMD across all clusters were considered (the red dashed curves in
 12053 Figure 7), together with the ratios of the RP (BMDL) to each of those percentiles. These results are shown
 12054 in Table 21.

12055 The WG experts noted from Table 21 that the RP of 0.93 ng BPA/kg bw per day is close to the 15th
 12056 percentile of both the lower and upper bounds of the consensus cpf. This implies about 15% probability
 12057 that the estimated lowest BMD for all clusters is lower than the RP and therefore 85% probability that the
 12058 estimated lowest BMD for all clusters is above the RP.

12059 Accordingly, in the right-hand columns of Table 21, the ratio of the RP to the 15th percentile of both bounds
 12060 of the cpf is close to 1. This implies about 85% probability that an additional UF of 1 (i.e. no additional UF)
 12061 would be sufficient to cover all clusters of endpoints that were rated ALAN, Likely or Very Likely in the WoE
 12062 assessment. When the calculations were repeated with the histograms provided by the experts, rather than
 12063 fitted parametric distributions, the probability that the estimated lowest BMD for all clusters is below the
 12064 RP increased slightly, to 87%.

12065 The WG experts also noted that the large range of endpoints tested for BPA makes the hazard assessment
 12066 for BPA more conservative than for most other chemicals, where only the standard endpoints are tested.

12067 Taking all these considerations together, the CEP Panel concluded that no additional UF is needed.

12068 **Table 21:** Percentiles of the lower and upper bounds of the consensus distribution for the estimated
 12069 lowest BMD across all clusters (columns 2 and 3) and ratios of the RP of 0.93 ng BPA/kg bw per day
 12070 to each percentile (columns 4 and 5). The row shown in bold is the basis for the CEP Panel's
 12071 conclusion that an additional UF is not needed. See text for details and Figure 7 for explanation of
 12072 how percentiles were derived from the consensus distribution.

Percentile of consensus cpf	Lower bound for percentile (ng BPA/kg bw per day)	Upper bound for percentile (ng BPA/kg bw per day)	Ratio of reference point to lower bound	Ratio of reference point to upper bound
1%	0.028	0.028	33.8	32.7
2.5%	0.059	0.061	15.7	15.2
5%	0.135	0.141	6.9	6.6
10%	0.412	0.447	2.3	2.1
15%	0.881	1.002	1.1	0.9
20%	1.485	1.754	0.6	0.5
25%	2.182	2.673	0.4	0.3
30%	2.989	3.800	0.3	0.2
35%	3.931	5.171	0.2	0.2
40%	5.028	6.797	0.2	0.1
45%	6.293	8.705	0.1	0.1
50%	7.742	10.955	0.1	0.1
55%	9.406	13.591	0.1	0.1
60%	11.323	16.671	0.1	0.1
65%	13.501	20.438	0.1	0.0
70%	15.991	25.137	0.1	0.0

75%	19.054	31.067	0.0	0.0
80%	23.057	38.870	0.0	0.0
85%	28.472	49.854	0.0	0.0
90%	36.534	67.143	0.0	0.0
95%	51.747	101.729	0.0	0.0

12073

12074

3.2.4 Derivation of a health-based guidance value (HBGV)

12075 Of all endpoints considered for the identification of a RP (Table 20), the CEP Panel noted that the effect of
 12076 BPA on Th17 cells in mice (Luo et al., 2016 [RefID 4679]) was the most sensitive. A BMDL₂₀ corresponding
 12077 to a HED of 0.93 ng/kg bw per day was derived from that study and used to establish a TDI. The CEP Panel
 12078 did not apply the UF for inter-species variability in toxicokinetics because this was already taken into account
 12079 by the conversion into HED. The remaining UF of 25 was applied to derive the HBGV, thus accounting for
 12080 inter-species toxicodynamic difference (2.5) and intra-human variability in toxicokinetics and
 12081 toxicodynamics (10).

12082 However, as dose-response analyses could not be performed for several endpoints (see Chapter 3.2.1), an
 12083 uncertainty analysis was performed, using EKE, to identify if an additional UF would be needed. This was
 12084 done by taking into considerations all other endpoints in clusters judged to be ALAN, Likely or Very Likely.
 12085 After screening of all such endpoints, the study by O'Brien et al. (2014a) [RefID 5462], reporting effects
 12086 on mast cell-mediated production of pro-inflammatory mediators and specific IgE in mice exposed pre-
 12087 natally to BPA, was considered the most critical. All other endpoints in the other clusters were much less
 12088 sensitive and judged to be of no influence for the uncertainty analysis.

12089 In the O'Brien study, effects on specific IgE were observed at the lowest dose tested (LOAEL) corresponding
 12090 to 7.5 ng/kg bw per day, corresponding to a HED of 0.116 ng/kg bw per day. As such it could not be
 12091 excluded, *a priori*, that if the O'Brien study had been conducted in such a way that a BMD analysis could
 12092 have been performed, a lower RP might have been derived. The uncertainty analysis tried to address this,
 12093 first by considering the probability that the findings in the O'Brien study would be adverse in humans and
 12094 what the true BMD from the O'Brien study might have been if the study had been without any limitations
 12095 or weaknesses. The relevance of the effect observed in the O'Brien study for humans was estimated to be
 12096 66%. In addition, individual experts' judgments (EKE) on where the true BMD of that study would have
 12097 been were scattered over two orders of magnitude. Based on that assessment, the WG's overall probability
 12098 that no additional UF was needed was in the range 85 –87%.

12099 The CEP Panel concluded that no additional UF was needed and that a HBGV based on the identified RP is
 12100 justified. Therefore, an UF of 25 was applied to the RP of 0.93 ng/kg bw per day for the effect of BPA on
 12101 Th17 cells in mice (Luo et al., 2016 [RefID 4679]), resulting in a TDI of 0.04 ng/kg bw per day.

12102 3.3. Risk characterisation

12103 The CEP Panel had to carry out an assessment of 'the risk to public health related to the presence of BPA
 12104 in foodstuffs', in accordance with the Terms of Reference as provided by the requestor, without performing
 12105 an updated exposure assessment. Therefore, the CEP Panel compared the newly derived TDI of 0.04 ng/kg
 12106 bw per day with the dietary exposure estimates for BPA (see Table 22), taken from Table 20 in the 2015

12107 EFSA opinion (EFSA CEF Panel, 2015), in which the data are provided for different age groups and
 12108 subpopulations.

12109 **Table 22:** Summary table on average (=mean) (A) and high (H) (=95th percentile) dietary exposure
 12110 ($\mu\text{g}/\text{kg}$ bw per day) to BPA in the different age groups of the general population (taken from EFSA
 12111 CEF Panel, 2015).

Age group	Exposure level	Dietary exposure ($\mu\text{g}/\text{kg}$ bw per day)	Dietary exposure/TDI (0.04 ng/kg bw)
		Oral (food and beverages)	
Infants 1–5 days (breastfed)	A	0.225	5625
	H	0.435	10875
Infants 6 days – 3 months (breastfed)	A	0.165	4125
	H	0.6	15000
Infants 4–6 months (breastfed)	A	0.145	13625
	H	0.528	13200
Infants 0–6 months (formula fed)	A	0.03	750
	H	0.08	2000
Infants 6–12 months	A	0.375	9375
	H	0.857	21425
Toddlers 1–3 years	A	0.375	9375
	H	0.857	21425
Children 3–10 years	A	0.290	7250
	H	0.813	20325
Adolescents 10–18 years	A	0.159	3975
	H	0.381	9525
Women 18–45 years	A	0.132	3300
	H	0.388	9700
Men 18–45 years	A	0.126	3150
	H	0.335	8375
Other adults 45–65 years	A	0.126	3150
	H	0.341	8525
Elderly and very elderly, 65 years and over	A	0.116	2900
	H	0.375	9375

12112
 12113 The comparison of the dietary exposure estimates with the TDI showed that both the mean and the 95th
 12114 percentile dietary exposures in all age groups (including all infants and toddler groups) exceeded the TDI
 12115 by two to four orders of magnitude.

12116 The CEP Panel noted that a TDI based on the second most sensitive endpoint (ovarian follicle counts, Hu
 12117 et al., 2018 [RefID 11119]) would also be exceeded by the dietary exposure by two to three orders of
 12118 magnitude.

12119 The CEP Panel considered the uncertainties associated with the characterisation of risks posed by BPA in
 12120 foodstuffs to different age groups. These uncertainties come from two sources: (1) uncertainties in
 12121 exposure (as discussed in the earlier EFSA opinion, EFSA CEF Panel 2015, in Section 4.7.2 in Part I-Exposure

12122 assessment), and (2) uncertainties in the identification and characterisation of hazard (Chapter 3.2.3 of
12123 this scientific opinion). The CEP Panel noted that the use of exposure data presented in the 2015
12124 assessment adds to the uncertainty. Following the 2015 opinion, the SML for the authorisation of BPA in
12125 plastic FCMs was revised at the EU level (from 0.6 mg/kg to 0.05 mg/kg), and the same SML of 0.05 mg/kg
12126 was introduced for varnishes and coatings. The ban on the use of BPA in the manufacture of PC baby
12127 bottles was extended to its use to manufacture sippy cups for infants and young children (= toddlers) and
12128 to the migration of BPA from varnishes and coatings applied to FCMs specifically intended to come into
12129 contact with foods for babies, infants and toddlers. The CEP Panel is aware that the exposure assessment
12130 presented in the 2015 opinion may therefore not fully represent the current dietary exposure.

12131 Even considering this uncertainty, as the TDI was exceeded by two to four orders of magnitude for both
12132 mean and 95th percentile estimates of dietary exposures, the CEP Panel concluded that there is a health
12133 concern from dietary BPA exposure for all age groups of the general population.

12134 4. Conclusions

12135 4.1 Hazard identification

12136 4.1.1 Toxicokinetics

- 12137 • The studies in mice and rats did not contribute to a better understanding of toxicokinetic aspects
12138 of BPA. The studies in ewes showed that the absolute bioavailability was lower when BPA was
12139 given by nasogastric tubing compared with BPA administration via pellets. This finding is most
12140 probably explained by the buccal absorption of BPA.
- 12141 • The human data showed that BPA is absorbed to nearly 100% and pre-systemically metabolised
12142 to a great extent to glucuronide and sulfate conjugates. The concentration in the systemic
12143 circulation is low (mean C_{max} = 0.43 nM, following 30 µg/kg bw; mean C_{max} = 6.5 nM, following 100
12144 µg/kg bw). The dose-corrected AUCs were clearly different in the two studies. The most probable
12145 explanation would be that in the study with dose-corrected higher C_{max} and higher AUC, the contact
12146 time with the buccal mucosa could be longer compared with the other study because of the mode
12147 of administration (BPA in cookies versus BPA in soup).
- 12148 • The CEP Panel decided to use the median value of the AUCs from both studies for the calculation
12149 of the HEDF, because both modes of administration are realistic for humans. The median value
12150 was 15.7 nM × h, which is 4-fold higher than the modelled AUC value used for calculating the HEDF
12151 in the 2015 EFSA opinion (EFSA CEF Panel, 2015).
- 12152 • To calculate the HEDF, the AUC data were used from the 2015 EFSA opinion (EFSA CEF Panel,
12153 2015) opinion for mice, rats, monkeys and dogs. For ewes, the data reported in the current opinion
12154 were used.
- 12155 • The following HEDFs were obtained: 0.0115 for mice, 0.165 for rats, 0.095 for monkeys, 0.1395
12156 for dogs, 0.1197 for ewes (gavage) and 0.4357 for ewes (diet).
- 12157 • Specific factors were applied to convert the doses from studies in which BPA was given by other
12158 routes than the oral one to allow to compare the doses.

12159 **4.1.2 General toxicity**

- 12160 • The newly available literature data indicate that in the HOC General toxicity several organs are
12161 potential targets of toxicity for BPA and that haematological parameters can be affected by this
12162 compound.
- 12163 • Within the HOC General toxicity, no human studies were available, while 10 clusters with relevant
12164 endpoints were identified in animal studies: body weight, liver effects, kidney effects, lung effects,
12165 thyroid effects, parathyroid effects, pituitary gland effects, adrenal gland effects, bone marrow
12166 effects and effects on haematological parameters.
- 12167 • Overall, none of the evaluated clusters' effects in the HOC General toxicity was considered Very
12168 Likely or Likely. In each of the evaluated clusters, effects were noted at least in one exposure
12169 period, but there were less consistent results among the available studies and, therefore, these
12170 effects were judged as ALAN in all the clusters.
- 12171 • MoA studies suggested oxidative stress as a potential pathogenetic mechanism for kidney damage.
12172 Similarly, oxidative stress in liver cells may be related to impaired mitochondrial function and liver
12173 toxicity. MoA studies also proposed that epigenetic changes via DNA methylation may have an
12174 impact on different signalling pathways related to lipid and carbohydrate metabolism. MoA studies
12175 in lungs suggested that BPA can delay fetal lung maturation evaluated through reduced alveolar
12176 airspace and thickened septa. Both these findings may be related to an increase in the lung weight.
12177 MoA studies on thyroid cells suggested mechanisms responsible for an increase in proliferation,
12178 supporting the limited evidence of hyperplastic changes observed in the animal studies. Moreover,
12179 it was suggested that BPA could enhance the susceptibility to thyroid carcinoma in combination
12180 with other endogenous or external factors.

12181 **4.1.3 Immunotoxicity**

- 12182 • The newly available data from literature indicate that the immune system is a target of toxicity for
12183 BPA.
- 12184 • Within the HOC Immunotoxicity, one relevant cluster of endpoints was identified in the human
12185 studies: asthma/allergy, including data from the exposure periods pregnancy and childhood.
- 12186 • In the animal studies, five clusters of relevant endpoints were identified: innate immunity, cellular
12187 immunity, humoral immunity, inflammation and allergic lung inflammation.
- 12188 • Based on the human data, a positive association between BPA exposure and asthma/allergy was
12189 judged as ALAN.
- 12190 • Based on the animal data, the clusters cellular immunity and allergic lung inflammation showed
12191 effects that were judged as Likely. In the other clusters, effects were also noted, but there were
12192 less consistent results, and these effects were judged as ALAN. In the cluster allergic lung
12193 inflammation, the effect noted was on the production of specific IgE in response to an allergen,
12194 which is deemed as adverse as it is a crucial parameter in inducing allergic reactions in the
12195 respiratory tract. Other effects in that cluster supported the likelihood of this effect. The likely effect

12196 in the cluster cellular immunity was supported by the consistency of the different endpoints within
12197 that cluster. The most sensitive parameter affected by BPA was the increased number of Th17
12198 cells. Although Th17 cells are T cells, and therefore were put in the cluster cellular immunity, they
12199 play a role in allergic responses, and therefore the effect on Th17 cells is consistent with the effect
12200 on specific IgE. *In vivo* evidence was supported by MoA studies. *In vitro* studies indicated the ability
12201 of BPA to induce immune deregulation, increasing susceptibility to develop inflammatory diseases.
12202 Th17 cells are a specific subset of CD4+ T helper cells, which participate in various immune
12203 diseases, including asthma and autoimmune diseases. Potential mechanisms by which BPA may
12204 contribute to immune-mediated disorders included modulation of ERK1/2 phosphorylation, NF- κ B
12205 activation, modulation of ERs, GR and AR as well as cytokine/chemokine secretion, and oxidative
12206 stress. Effects may be on non-specific cells belonging to the immune system or influencing the
12207 immune system (such as APCs and epithelial cells). This will, through presentation of antigens to
12208 T-lymphocytes or release of mediators, influence the regulatory homeostasis of the immune
12209 system, suppressing T regulatory cells and stimulating Th-17 cells. Thus, BPA appeared to promote
12210 multiple interwoven pathways involved in immune deregulation that may play a role in immune-
12211 related disorders.

12212 4.1.4 Metabolic effects

- 12213 • The newly available literature data indicate that BPA may induce adverse metabolic effects.
- 12214 • Within the HOC Metabolic effects, five clusters of endpoints were identified in the human studies:
12215 obesity, cardiometabolic effects, thyroid effects, T2DM and gestational diabetes mellitus, including
12216 data from one or more of the exposure periods pregnancy, childhood and adulthood.
- 12217 • In the animal studies, eight clusters of relevant endpoints were identified: obesity, fat deposition
12218 in the liver, glucose regulation, blood lipids, uric acid, T1DM, other metabolic hormones and thyroid
12219 hormones. The clusters included data from one or more of the exposure periods developmental
12220 until weaning, developmental until adulthood, growth phase, adult exposure and indirect (germline)
12221 exposure.
- 12222 • Based on the human data, none of the metabolic clusters showed effects that were considered
12223 Likely or Very Likely. A positive association between BPA exposure and obesity and T2DM was
12224 judged as ALAN, while a positive association between BPA exposure and cardiometabolic effects,
12225 thyroid effects and gestational diabetes mellitus was judged as Not Likely.
- 12226 • Based on the animal data, no metabolic clusters were considered Very Likely. The cluster uric acid
12227 was considered Likely (in the adult exposure period), as increased levels were observed in the liver
12228 of mice and in the serum of mice and rats after BPA exposure. The other metabolic endpoints were
12229 considered either ALAN (obesity, fat deposition in the liver, glucose regulation, blood lipids and
12230 T1DM) or Not Likely (other metabolic hormones and thyroid hormones), in one or more exposure
12231 periods.
- 12232 • Substantial amounts of supporting evidence for plausible MoAs of BPA were available on obesity,
12233 fat deposition in the liver and glucose regulation, mostly from animal and *in vitro* studies. The MoA
12234 data in animals showed that BPA could increase the formation of hepatic uric acid by increasing

12235 the activity of the enzyme xanthinoxidase, which catalyses the conversion of purines hypoxanthine
12236 and xanthine into uric acid. The MoA data on T1DM were very limited and the results depended on
12237 the animal model used.
12238

12239 **4.1.5 Neurotoxicity and developmental neurotoxicity**

12240 • The newly available literature data indicate that the central nervous system is a target of toxicity
12241 for BPA.

12242 • Within the HOC Neurotoxicity and developmental neurotoxicity, the evaluation of the human data
12243 considered endpoints from the cluster neurodevelopment. In the animal studies, three clusters of
12244 endpoints were identified: neuromorphology, nervous system functionality and behaviour.

12245 • Based on the human data, it was concluded that the evidence for an association between BPA
12246 exposure and impaired neurodevelopment was Not Likely.

12247 • Based on the animal data, all three neurotoxicity clusters showed effects that were judged as
12248 Likely:

12249 - In the neuromorphology cluster, Likely effects were found for the endpoints dendritic spine
12250 density of pyramidal cells in hippocampus (CA1 and dentate gyrus areas) after developmental
12251 exposure and for the endpoints number of neurons in hippocampus (CA1 and CA3 areas), and
12252 dendritic spine density in pyramidal cells in the medial part of the PFC after exposure during
12253 the growth phase/young age.

12254 - In the nervous system functionality cluster, a Likely effect on the endpoint AChE activity during
12255 the adult exposure period was identified.

12256 - In the behaviour cluster, Likely effects were noted for the endpoint anxiety/emotionality during
12257 all exposure periods (developmental, growth phase/young age, adult and exposure through
12258 the male germline). Furthermore, the endpoint learning/memory showed a Likely influence of
12259 BPA from developmental and growth phase/young age exposure, and effects on sensory-motor
12260 coordination and salt preference were considered Likely in adults.

12261 • The mechanisms of action that link the identified effects of BPA on various endpoints of brain
12262 structure, function and development have not been sufficiently explored in the literature to draw
12263 conclusions. There is evidence for an involvement of steroid-hormone-dependent pathways
12264 (oestrogen, androgens, corticosterone); oxidative stress, mitochondrial function and calcium
12265 regulation; gene expression changes through DNA methylation and other signaling pathways
12266 (canonical and non-canonical Wnt pathways, kinases).

12267 **4.1.6 Reproductive and developmental toxicity**

12268 • The newly available literature data indicate that the reproductive system is a target of toxicity for
12269 BPA.

12270 • Within the HOC Reproductive and developmental toxicity, five relevant clusters of endpoints were
12271 identified in the human studies: fetal and post-natal growth, prematurity, pre-eclampsia, male

- 12272 fertility and female fertility, including data from one or more of the exposure periods pregnancy,
12273 childhood and adulthood.
- 12274 • In the animal studies, three clusters of relevant endpoints were identified: developmental toxicity,
12275 female reproductive toxicity and male reproductive toxicity. The clusters included data from one or
12276 more of the exposure periods developmental until weaning, developmental until adulthood, growth
12277 phase, adult exposure and indirect (germline) exposure.
- 12278 • Based on the human data, none of the clusters showed effects that were judged as Likely or Very
12279 Likely. An association between maternal BPA exposure and impaired pre- and post-natal growth,
12280 shorter duration of gestation or preterm delivery, reduced male fertility and pubertal development
12281 when exposed during childhood, was judged as Not Likely. An association between BPA exposure
12282 and reduced female fertility and pre-eclampsia during adulthood and pubertal development when
12283 exposed during pregnancy was judged as ALAN
- 12284 • Based on the animal data, both female and male reproductive toxicity clusters showed effects that
12285 were judged as Likely:
- 12286 - In the female reproductive toxicity cluster, there were Likely effects on ovary weight and
12287 histology and uterus histology after developmental exposure, on ovary histology after
12288 developmental and adult exposure, on implantation rate after growth phase/young age
12289 exposure and on ovary histology (follicle counts) after adult exposure.
- 12290 - In the male reproductive toxicity cluster, there were Likely effects on epididymis (exfoliated
12291 germ cells and inflammation) after developmental exposure (pre-natal and/or post-natal until
12292 adult), on testis histology (decreased seminiferous tubule diameter) after growth phase/young
12293 age exposure and on sperm (motility, viability and acrosome reaction) after adult exposure.
- 12294 • In the developmental toxicity cluster, effects were also noted, but the results were less consistent
12295 results, and these effects were judged as ALAN for the endpoints bone development, mammary
12296 gland histology, body weight (in the developmental exposure), mammary gland weight and
12297 mammary gland histology (in the developmental and adult exposure) as well as body weight and
12298 age at first oestrus (in the growth phase/young age exposure).
- 12299 • Supporting evidence for plausible MoAs of BPA on reproductive toxicity effects was available. They
12300 include ER and AR interactions and associated downstream and cross-stream effects, including
12301 epigenetic changes. Other possible mechanisms, including BPA-induced generation of oxidative
12302 stress, have been less explored.

12303 4.1.7 Cardiotoxicity

- 12304 • The newly available literature data investigated the cardiovascular system as a target of toxicity
12305 for BPA.
- 12306 • Within the human HOC Cardiotoxicity, no case-control or cohort studies were available. Therefore,
12307 the evidence for a positive association between BPA exposure and cardiotoxicity in human was
12308 considered Inadequate.

12309 • In the animal studies, five clusters of relevant endpoints were identified: absolute and relative
12310 heart weight, incidence of cardiac lesions, cardiac structural changes (as measured by
12311 echocardiography), effects on cardiac function (as measured by echocardiography), blood pressure
12312 and atherosclerotic lesions.

12313 • Based on the animal studies, the evidence of BPA effects was judged as Not Likely in the majority
12314 of the cardiotoxicity clusters, and in few clusters as Inadequate, in one or more exposure periods.
12315 Given the functional relationship between the endpoints, the outcome of the WoE was considered
12316 biologically plausible.

12317 • Considering the Not Likely evidence for BPA on cardiotoxic effects, the data from *in vitro* and
12318 mechanistic studies were not included for this HOC.

12319 **4.1.8 Carcinogenicity and mammary gland proliferative effects**

12320 • The newly available literature data indicate that, in the HOC Carcinogenicity and mammary gland
12321 proliferative effects, the following organs are targets of BPA-induced toxicity: mammary gland,
12322 prostate and uterus.

12323 • Within the HOC Carcinogenicity and mammary gland proliferative effects, no human studies were
12324 available, while five clusters with relevant endpoints were identified in animal studies: mammary
12325 gland weight, mammary gland histology, prostate histology, uterus weight and uterus histology.
12326 For histology, four subclusters were considered, if available: non-neoplastic changes, pre-
12327 neoplastic lesions, neoplastic lesions, proliferation and apoptosis as evaluated by quantitative
12328 immunohistochemistry.

12329 • The cluster mammary gland weight was judged Not Likely. The clusters mammary gland histology,
12330 prostate histology and uterus weight showed effects that were not consistently reported in the
12331 available studies and, therefore, these effects were judged as ALAN.

12332 • Also, regarding the subclusters linked to lesions in the mammary gland, inconsistencies were noted:
12333 in the developmental until weaning exposure period no increase in pre-neoplastic lesions (Not
12334 Likely), but a higher incidence in neoplastic lesions (Likely) was observed. In the developmental to
12335 adult exposure period an increase in pre-neoplastic lesions (ALAN) was reported but no increase
12336 in neoplastic lesions was detected (Not Likely). Therefore, these effects contributed to the overall
12337 judgement ALAN in the cluster mammary gland histology.

12338 • In the cluster uterus histology, the non-neoplastic changes gland cellular anomalies, squamous
12339 metaplasia and cystic endometrial hyperplasia were considered adverse and judged as Likely based
12340 on studies with developmental exposure (pre-natal and/or post-natal until weaning) to BPA.

12341 • MoA studies in mammary gland addressing epigenetic effects, changes in gene expression and
12342 changes in hormone receptor levels suggested various MoAs of BPA possibly involved in the
12343 induction of proliferative/morphological changes. Some *in vivo* studies indicated that stromal-
12344 epithelial interactions may play a crucial role in the BPA-induced developmental changes in the
12345

12350 mammary gland. *In vitro* studies provided some support for the hypothesis that BPA contributes
12351 to a higher susceptibility to mammary gland carcinogenesis. MoA studies on prostate cancer
12352 indicated that BPA can enhance the susceptibility to tumorigenesis in rodents co-treated with very
12353 high levels of E2 and testosterone, while developmental and chronic exposure to BPA without
12354 additional sex hormones did not demonstrate a direct tumorigenic effect. *In vitro* MoA studies on
12355 uterine cells indicated that BPA increases the proliferative rate. Data from other *in vitro* studies
12356 suggested that BPA modulates various mechanisms underlying the onset, growth and invasion of
12357 uterine tumours. However, the results of rodent studies did not demonstrate a tumorigenic activity
12358 of BPA.

12359 4.1.9 Genotoxicity

- 12360 • The analysis of the available literature data indicate that BPA does not induce gene mutations in
12361 bacteria. BPA induces DNA strand breaks, clastogenic and aneugenic effects in mammalian cells *in*
12362 *vitro*. Oxidative stress-related mechanism(s) are likely to be involved in this DNA damaging and
12363 clastogenic activity.
- 12364 • In contrast with consistent positive *in vitro* findings, the *in vivo* findings in several studies with
12365 high/limited reliability were inconsistent. The CEP Panel concluded that the evidence does not
12366 support an *in vivo* genotoxic hazard posed by BPA through direct interaction with DNA.
- 12367 • The CEP Panel concluded that it is unlikely to very unlikely that BPA presents a genotoxic hazard,
12368 the causes of which include a direct mechanism, and that the balance of evidence allows a HBGV
12369 to be established.

12370 4.2 Hazard characterisation

- 12371 • The CEP Panel used BMD analysis for dose-response modelling of the endpoints that were assigned
12372 a likelihood level of Likely or Very Likely.
- 12373 • After conversion of the doses to HED, the CEP Panel selected the lowest BMDL value of 0.93 ng/kg
12374 bw per day for the effect of BPA on Th17 cells in mice to be used as RP for the risk assessment of
12375 BPA.
- 12376 • Based on an assessment of other endpoints that could require an additional UF, the WG's overall
12377 probability that no additional UF was needed was in the range 85-87%. The CEP Panel concluded
12378 that no additional UF was needed and that a HBGV based on the identified RP is justified.
- 12379 • The CEP Panel applied the UFs for inter-species toxicodynamic difference (2.5) and intra-human
12380 variability in toxicokinetics and toxicodynamics (10) and established a TDI of 0.04 ng/kg bw per
12381 day.

12382 4.3 Risk characterisation

- 12383 • The comparison of the dietary exposure estimates from the 2015 EFSA opinion with the new TDI
12384 showed that both the mean and the 95th percentile dietary exposures in all age groups (including
12385 all infants and toddler groups) exceeded the TDI by two to four orders of magnitude.
- 12386 • The CEP Panel is aware that the exposure assessment presented in the 2015 opinion may not fully
12387 represent the current dietary exposure. Even considering this uncertainty, since the exceedance

12388 was so large, the CEP Panel concluded that there is a health concern from dietary BPA exposure
12389 for all age groups of the general population.

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DRAFT

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Abbreviations

8-isoPF2 α	Isoprostaglandin F2 α
8-NO ₂ Gua	8-Nitroguanine
8-OHdG	8-Hydroxy-2'-deoxyguanosine
ACC	Acetyl-CoA carboxylase
AChE	Acetylcholine esterase
ACTH	Adrenocorticotrophic hormone
ADHD	Attention deficit hyperactivity disorder
AGD	Anogenital distance
AGDac	Anogenital distance anus–clitoris
AGDaf	Anogenital distance anus–fourchette
AGDap	Anogenital distance anus–penis
AGDas	Anogenital distance anus–scrotum
AIF	Apoptosis-inducing factor
AIR	Acute insulin response
Akt	Protein kinase B
ALAN	As Likely As Not
ALB/GLO	Albumin/globulin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMH	Anti-Müllerian hormone
AMPK	Adenosine monophosphate-activated protein kinase
ANSES	French Agency for Food, Environmental and Occupational Health and Safety
APCs	Antigen-presenting cells
AR	Androgen receptor
ASC	Adipose stromal/stem cells
ASP	Aspartate
AST	Aspartate aminotransferase
ATGL	Adipose triglyceride lipase
ATM	Ataxia-telangiectasia mutated
AU	Arbitrary units
AUC	Area under the curve

AVPV	Anteroventral periventricular nucleus
BAL	Bronchoalveolar lavage
BASC-2	Behaviour Assessment System for Children-2
BAT	Brown adipose tissue
BDNF	Brain-derived neurotrophic factor
BER	Base excision repair
BL	Body length
BMD	Benchmark dose
BMDL	Benchmark dose lower confidence interval
BMDU	Benchmark dose upper confidence interval
BMI	Body mass index
BMR	Benchmark response
BNST _{dl}	Dorsolateral bed nucleus of stria terminalis
BNST _p	Posterior bed nucleus of stria terminalis
BP-1	Sulphonylbis(benzene-4,1-diyloxy)]diethanol
BP-2	4,4'-Sulphanediylidiphenol
BPA	Bisphenol A
BPAF	Bisphenol AF
BPA-G	Bisphenol A-glucuronide
BPA-S	Bisphenol A-sulfate
BPF	Bisphenol F
BPS	Bisphenol S
BPZ	Bisphenol Z
BrdU	5-bromo-2'-deoxyuridine
BRIEF-P	Behaviour Rating Inventory of Executive Function–Preschool
BSID	Bayley Scales of Infant Development
BSID-II	Bayley Scales of Infant Development-II
BTB	Blood-testis barrier
BUN	Blood urea nitrogen
BW	Birth weight
CA	Chromosomal aberrations
CAT	Catalase

CBCL	Child Behaviour Checklist
CBMA	Cytokinesis blocked micronucleus assay
CCCEH	Columbia Center for Children's Environmental Health
Cd	Cadmium
CDIIT	Comprehensive Developmental Inventory for Infants and Toddlers
CDRS	Children's Depression Rating Scale-Revised
CEBS	Chemical Effects in Biological Systems
CEF	Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO cells	Chinese hamster ovary cells
CI	Confidence interval
cPEN	Caudal periventricular nucleus
Cpf	Cumulative probability function
CPI	C-peptide index
CPT	Camptothecin
CRH	Corticotropin releasing hormone
CRP	C-reactive protein
CT	Computed tomography
DAMP	Damage-associated molecular pattern
DBP	Dibutyl phthalate
DBS	Double base substitutions
DCFH-DA	Dichlorofluorescein diacetate assay
DDR	DNA damage response
DEXA	Dual energy X-ray absorptiometry
DFI	DNA fragmentation index
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone sulfate
DHPN	<i>N</i> -bis(2-hydroxypropyl)nitrosamine
DLPFC	Dorsolateral prefrontal cortex
DLT	Dark Light Test
DMBPA	bis(4-hydroxy-3-methylphenyl)propane
DMBPS	4,4'-Sulfonylbis(2-methylphenol
DOR	Decreased ovarian reserve

DSBs	DNA double-strand breaks
E2	Oestradiol
ECHA	European Chemicals Agency
ECoMRI	Echo Magnetic Resonance Imaging System
EDC	Endocrine-disrupting chemical
EDSP	Endocrine disruptor screening program
EE	Ethinyl oestradiol
EF	Ejection fraction
eGFR	Epidermal growth factor receptor
EKE	Expert knowledge elicitation
ELISA	Enzyme-linked immunosorbent assay
EMS	Ethyl methanesulfonate
eNOS	Endothelial nitric oxide
EPM	Elevated plus maze
ER	Oestrogen receptor
ERK	Extracellular-signal-regulated kinase
ERR	Oestrogen related receptor
ERR α	Oestrogen related receptor- α
ERR γ	Oestrogen related receptor gamma
ER2	Oestrogen receptor 2
ESR	Erythrocyte sedimentation rate
ESC	Endometrial stromal cells
ESI	Electrospray ionization
eWAT	Epididymal white adipose tissue
EZM	Elevated zero maze
FATP1	Fatty acid transport protein 1
FDA	Food and Drug Administration
FENO	Fraction of exhaled nitric oxide
FEV ₁	Forced respiratory volume in 1 s
FFA	Free fatty acids
FGV	Mammary fibroglandular volume
FMI	Fat mass index

Fpg	Formamidopyrimidine-DNA glycosylase
FS	Fractional shortening
FSH	Follicle stimulating hormone
FST	Forced swimming test
FT ₃	Free triiodothyronine
FT ₄	Free thyroxine
GABA	Gamma amino butyric acid
GD	Gestation day
GDM	Gestational diabetes mellitus
GE	Ginger extract
GGT	Gamma-glutamyl transferase
GLN	Glutamine
GLP	Good laboratory practice
GLU	Glutamate
GLY	Glycine
GPx	Glutathione peroxidase
GR	Glucocorticoid receptor
GSH	Reduced glutathione
γ-GTP	Gamma-glutamyl transpeptidase
GTT	Glucose tolerance test
gWAT	Gonadal white adipose tissue
HBF	High butter fat
HBGV	Health-based guidance value
HDL	High-density lipoprotein
HED	Human equivalent dose
HEDF	Human equivalent dose factor
HFD	High-fat diet
HFLF	Human fetal lung fibroblasts
HNE-MA	4-Hydroxy-2-nonenal-mercapturic acid
HoC	Health outcome category
hOGG1	8-Oxoguanine DNA glycosylase
HOMA	Homeostatic Model Assessment

HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HOME	Health Outcomes and Measures of the Environment
HOX	Homeobox-containing genes
HPA	Hypothalamic–pituitary–adrenal axis
HPG	Hypothalamic-pituitary-gonadal axis
HPLC	High-performance liquid chromatography
HPT	Hypothalamic–pituitary–thyroid axis
hUM	Human uterine myoma tissue
HUVEC	Human umbilical vascular endothelial cells
HVA	Homovanillic acid
ICD	International Classification of Diseases
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IGF-1	Insulin-like growth factor 1
IgG	Immunoglobulin G
IgM	Immunoglobulin M
InDel	Insertions and deletions
INMA	INfancia y Medio Ambiente (Environment and Childhood) Project
INSL3	Insulin-like peptide 3
IpGTT	Intraperitoneal glucose tolerance test
IpITT	Intraperitoneal insulin tolerance test
ITT	Insulin tolerance test
IvGTT	Intravenous glucose tolerance test
IVS	Interventricular septum
iWAT	Inguinal white adipocytes
JNK	c-Jun N-terminal kinase
KC	Kupffer cells
LBW	Low birth weight
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LH	Luteinizing hormone

LIFE	Longitudinal Investigation of Fertility and the Environment
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOQ	Limit of quantification
LPL	Lipoprotein lipase
LPS	Lipopolysaccharides
LVIDd	Left ventricular internal dimension, at diastole
LVIDs	Left ventricular internal dimension, at systole
LVPWd	Left ventricle posterior wall thickness, at diastole
LVWT	Left ventricle wall thickness
MAO	Monoamine oxidase
MAPK	Mitogen-activated protein kinase
MCH	Mean corpuscular haemoglobin
MDA	Malondialdehyde
MDR	Monotonic dose–response
MEFs	Mouse embryonic fibroblasts
MEST	Mesoderm specific transcript
MII	Metaphase II
MMP	Mitochondrial transmembrane potential
MMTV	Mouse mammary tumour virus
MN	Micronuclei
MoA	Mode of action
mPFC	Medial prefrontal cortex
MSC	Mesenchymal stem cell
MTOCs	Modifications of the microtubule organizing centres
mTOR	Mammalian target of rapamycin
MTP	Microsomal triglyceride transfer protein
MUFA	Monounsaturated fatty acids
MWM	Morris water maze
NA	Noradrenaline
n.a.	Not available
NAC	<i>N</i> -acetylcysteine

NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NCTR	National Centre for Toxicological Research
NDI	Nuclear division index
NHANES	National Health and Nutrition Examination Survey
NHSII	Nurses' Health Study II
NIEHS	National Institute of Environmental Health Science
NMDR	Non monotonic dose–response
NMR	Nuclear magnetic resonance
NOAEL	No observed adverse effect level
NOD	Non-obese diabetic
NPC	Neuroprogenitor cell
NSC	Neural stem cells
NTP	National Toxicology Program
OECD	Organization for European Economic Cooperation and Development
OFT	Open field test
oGTT	Oral glucose tolerance test
OR	Odds Ratio
OTR	Oxytocin receptor
OTM	Olive tail moment
OV	Ovarian volume
OVA	Ovalbumin
OVX	Ovariectomy/ ovariectomised
PA	Premature anaphase
PBG	Phenylbiguanide
PBMC	Peripheral blood mononuclear cells
PBPK	Physiologically-based pharmacokinetic (model)
PC	Polycarbonate
PCBs	Polychlorinated biphenyls
PCE	Polychromatic erythrocytes
PCNA	Proliferating cell nuclear antigen
PCOS	Polycystic ovary syndrome

PDD-NOS	Pervasive developmental disorder-not otherwise specified
PET	Positron emission tomography
PFC	Prefrontal cortex
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
PIM	Perceived insufficient milk (supply)
PIN	Prostatic intraepithelial neoplasia
PKA	Protein kinase-A
PND	Post-natal day
PNW	Post-natal week
POMC	Pro-opiomelanocortin
PPARY	Peroxisome proliferator-activated receptor gamma
PRL	Prolactin
PS	Prostasphere
PSO	Pumpkin seed oil
PVN	Paraventricular nucleus
pWAT	Perigonadal white adipose tissue
PXR	Pregnane X receptor
RAM	Radial arm maze
RCMAS	Revised Children's Manifest Anxiety Scale
ROS	Reactive oxygen species
RP	Reference point
rPen	Rostral periventricular area
RR	Relative Risk
rT3	Reverse triiodothyronine
RWT	Relative wall thickness
RXR	Retinoid-X-receptor
SAC	Spindle assembly checkpoint
SAT	Subcutaneous adipose tissue
SBS	Single base substitutions
SCD	Sperm chromatin dispersion
SCEs	Sister chromatid exchanges

SCSA	Sperm chromatin structure assay
SD	Sprague Dawley
SDF	Sperm DNA fragmentation
SDF1	Stromal cell-derived factor 1
SDQ	Strength and Difficulties Questionnaire
SEM	Standard error mean
SFA	Saturated fatty acid
SGA	Small for gestational age
SHBG	Sex hormone-binding globulin
SML	Specific migration limit
SMS	Social maturity scale
SNP	Single-nucleotide polymorphisms
SO	Sesame oil
SOD	Superoxide dismutase
SREBPs	Sterol regulatory element-binding proteins
SRS-2	Social responsiveness scale-2
StAR	Steroidogenic acute regulatory protein
STZ	Streptozotocin
SULT	Sulfotransferase
SVZ	Subventricular zone
T1DM	Type-1 diabetes mellitus
T2DM	Type-2 diabetes mellitus
T ₃	Triiodothyronine
T ₄	Thyroxine
TAG	Triacylglycerol
TAU	Taurine
TBARS	Thiobarbituric acid reactive substances
TBBPA	Tetrabromobisphenol A
TCC	T-type calcium channel
TCR	T-cell receptor
TD	Terminal duct
TDI	Tolerable daily intake

TEBs	Terminal end buds
TG	Test guideline
TJ	Tight junction
TLR	Toll-like receptor
Top1	Topoisomerase-I
TSH	Thyroid stimulating hormone
TSLP	Thymic stromal lymphopoietin
TT ₃	Total triiodothyronine
TT ₄	Total thyroxine
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
UF	Uncertainty factor
UGT	UDP-glucuronyl-transferase
US EPA	United States Environmental Protection Agency
VAT	Visceral adipose tissue
VM	Ventral mesencephalon
VMH	Ventromedial hypothalamus
VMWM	Virtual Morris water maze
WAT	White adipose tissue
WG	Working group
WGS	Whole genome sequencing
WHHL	Watanabe heritable hyper-lipidemic
WISC-IV	Wechsler intelligence scale for children IV
WoE	Weight of evidence
WPPSI-III	Wechsler primary and preschool scale of intelligence–III
XO	Xanthine oxidase
XOR	Xanthine oxidoreductase

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15138 **Appendices****Appendix A – Outcome of the call for data²⁰****Call for data relevant to the hazard assessment of Bisphenol A (BPA)**

Published: 9 Marzo 2018 Deadline: 15 Ottobre 2018 - 23:59 (CEST)



EFSA-Q-number: EFSA-Q-2018-00221

- Published: 09/03/2018
- Deadline for registering interest: 30/06/2018
- Deadline for submission of data: 31/08/2018
- Updated deadline: 15/10/2018

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15140 The data submitted through the call and the approach used for their consideration/exclusion are reported

15141 in Table A1.

15142 **Table A1:** Data submitted through the call for data relevant to the hazard assessment of BPA.

Data submitted	Notes (against exclusion and inclusion criteria)	RefID number in Distiller
Data submitted by Joseph Laakso on behalf of Angel Nadal from the Endocrine Society APPENDIX: Selected publications on the effects of BPA:		
Stahlhut RW, Myers JP, Taylor JA, Nadal A, Dyer JA and Vom Saal FS, 2018. Experimental BPA exposure and glucose-stimulated insulin response in adult men and women. <i>Journal of the Endocrine Society</i> , 2(10), 1173–1187. doi:10.1210/js.2018-00151	Included	RefID 13171
Li Q, Lawrence CR, Nowak RA, Flaws JA, Bagchi MK and Bagchi IC, 2018d. Bisphenol A and phthalates modulate peritoneal macrophage function in female mice involving SYMD2-H3K36 dimethylation. <i>Endocrinology</i> , 159(5), 2216–2228. doi:10.1210/en.2017-03000	Included	RefID 12475
Drobná Z, Henriksen AD, Wolstenholme JT, Montiel C, Lambeth PS, Shang S, Harris EP, Zhou C, Flaws JA, Adli M and Rissman EF, 2018. Transgenerational effects of bisphenol A on gene expression and DNA methylation of imprinted	Included	RefID 11853

²⁰ <https://www.efsa.europa.eu/it/consultations/call/180309-0>

genes in brain. <i>Endocrinology</i> , 159(1), 132–144. doi:10.1210/en.2017-00730		
Eckstrum KS, Edwards W, Banerjee A, Wang W, Flaws JA, Katzenellenbogen JA, Kim SH and Raetzman LT, 2018. Effects of exposure to the endocrine-disrupting chemical bisphenol A during critical windows of murine pituitary development. <i>Endocrinology</i> , 159(1), 119–131. doi:10.1210/en.2017-00565	Included	RefID 11874
Mahalingam S, Ther L, Gao L, Wang W, Ziv-Gal A and Flaws JA, 2017. The effects of in utero bisphenol A exposure on ovarian follicle numbers and steroidogenesis in the F1 and F2 generations of mice. <i>Reproductive Toxicology</i> , 74, 150–157. doi:10.1016/j.reprotox.2017.09.013	Included	RefID 4779
Olson MR, Su R, Flaws JA and Fazleabas AT, 2017. Bisphenol A impairs decidualization of human uterine stromal fibroblasts. <i>Reproductive Toxicology</i> , 73, 339–344. doi:10.1016/j.reprotox.2017.07.008	Included	RefID 5518
Patel S, Brehm E, Gao L, Rattan S, Ziv-Gal A and Flaws JA, 2017. Bisphenol A exposure, ovarian follicle numbers, and female sex steroid hormone levels: Results from a CLARITY-BPA study. <i>Endocrinology</i> , 158(6), 1727–1738. doi:10.1210/en.2016-1887	Included	RefID 5708
Ziv-Gal A and Flaws JA, 2016. Evidence for bisphenol A-induced female infertility: A review (2007–2016). <i>Fertility and Sterility</i> , 106(4), 827–856. doi:10.1016/j.fertnstert.2016.06.027	Included	RefID 9141
Li Q, Davila J, Kannan A, Flaws JA, Bagchi MK and Bagchi IC, 2016. Chronic exposure to bisphenol A affects uterine function during early pregnancy in mice. <i>Endocrinology</i> , 157(5), 1764–1774. doi:10.1210/en.2015-2031	Included	RefID 4128
Berger A, Ziv-Gal A, Cudiamat J, Wang W, Zhou C and Flaws JA, 2016. The effects of in utero bisphenol A exposure on the ovaries in multiple generations of mice. <i>Reproductive Toxicology</i> , 60, 39–52. doi:10.1016/j.reprotox.2015.12.004	Included	RefID 524
Heindel JJ, Newbold RR, Bucher JR, Camacho L, Delclos KB, Lewis SM, Vanlandingham M, Churchwell MI, Twaddle NC, Mclellen M, Chidambaram M, Bryant M, Woodling K, Gamboa da Costa G, Ferguson SA, Flaws J, Howard PC, Walker NJ, Zoeller RT, Fostel F, Favaro C and Schug TT, 2015. NIEHS/FDA CLARITY-BPA research program update. <i>Reproductive Toxicology</i> , 58, 33–44. doi:10.1016/j.reprotox.2015.07.075	Excluded, because it is a secondary review study. But included the Grantees studies it refers to.	RefID 2672
Zhou C, Wang W, Peretz J and Flaws JA, 2015 November. Bisphenol A exposure inhibits germ cell nest breakdown by reducing apoptosis in cultured	Included	RefID 9019

neonatal mouse ovaries. <i>Reproductive Toxicology</i> , 57, 87–99. doi:10.1016/j.reprotox.2015.05.012		
Ziv-Gal A, Wang W, Zhou C and Flaws JA, 2015. The effects of in utero bisphenol A exposure on reproductive capacity in several generations of mice. <i>Toxicology and Applied Pharmacology</i> 1284, 3(3), 354–362. doi:10.1016/j.taap.2015.03.003	Included	RefID 9143
Strakovsky RS, Wang H, Engeseth NJ, Flaws JA, Helferich WG, Pan YX and Lezmi S, 2015. Developmental bisphenol A (BPA) exposure leads to sex-specific modification of hepatic gene expression and epigenome at birth that may exacerbate high-fat diet-induced hepatic steatosis. <i>Toxicology and Applied Pharmacology</i> , 284(2), 101–112. doi:10.1016/j.taap.2015.02.021	Included	RefID 6914
Peretz J, Vrooman L, Ricke WA, Hunt PA, Ehrlich S, Hauser R, Padmanabhan V, Taylor HS, Swan SH, VandeVoort CA and Flaws JA, 2014. Bisphenol A and reproductive health: Update of experimental and human evidence, 2007–2013. <i>Environmental Health Perspectives</i> , 122(8), 775–786. doi:10.1289/ehp.1307728	Included	RefID 5778
Wang W, Hafner KS and Flaws JA, 2014a. In utero bisphenol A exposure disrupts germ cell nest breakdown and reduces fertility with age in the mouse. <i>Toxicology and Applied Pharmacology</i> , 276(2), 157–164. doi:10.1016/j.taap.2014.02.009	Included	RefID 7759
Peretz J, Neese SL and Flaws JA, 2013. Mouse strain does not influence the overall effects of bisphenol A-induced toxicity in adult antral follicles. <i>Biology of Reproduction</i> , 789(5), 108.	Included	RefID 5776
Ziv-Gal A, Craig ZR, Wang W and Flaws JA, 2013. Bisphenol A inhibits cultured mouse ovarian follicle growth partially via the aryl hydrocarbon receptor signaling pathway. <i>Reproductive Toxicology</i> , 42, 58–67. doi:10.1016/j.reprotox.2013.07.022	Included	RefID 9140
Ehrlich S, Williams PL, Hauser R, Missmer SA, Peretz J, Calafat AM and Flaws JA, 2013. Urinary bisphenol A concentrations and cytochrome P450 19 A1 (Cyp19) gene expression in ovarian granulosa cells: An <i>in vivo</i> human study. <i>Reproductive Toxicology</i> , 42, 18–23. doi:10.1016/j.reprotox.2013.06.071	Included	RefID 1770
Peretz J and Flaws JA, 2013. Bisphenol A down-regulates rate-limiting Cyp11a1 to acutely inhibit steroidogenesis in cultured mouse antral follicles. <i>Toxicology and Applied Pharmacology</i> , 271(2), 249–256. doi:10.1016/j.taap.2013.04.028	Included	RefID 5775
Ehrlich S, Williams PL, Missmer SA, Flaws JA, Ye X, Calafat AM, Petrozza JC, Wright D and Hauser R, 2012. Urinary bisphenol A concentrations and early reproductive health outcomes among women	Excluded, published in 2012	Not applicable

undergoing IVF. <i>Human Reproduction</i> , 27(12), 3583–3592. doi:10.1093/humrep/des328		
Brannick KE, Craig ZR, Himes AD, Peretz JR, Wang W, Flaws JA and Raetzman LT, 2012. Prenatal exposure to low doses of bisphenol A increases pituitary proliferation and gonadotroph number in female mice offspring at birth. <i>Biology of Reproduction</i> , 87(4), 82. doi:10.1095/biolreprod.112.100636	Excluded, published in 2012	Not applicable
Peretz J, Craig ZR and Flaws JA, 2012. Bisphenol A inhibits follicle growth and induces atresia in cultured mouse antral follicles independently of the genomic estrogenic pathway. <i>Biology of Reproduction</i> , 87(3), 63. doi:10.1095/biolreprod.112.101899	Excluded, published in 2012	Not applicable
Ehrlich S, Williams PL, Missmer SA, Flaws JA, Berry KF, Calafat AM, Ye X, Petrozza JC, Wright D and Hauser R, 2012. Urinary bisphenol A concentrations and implantation failure among women undergoing <i>in vitro</i> fertilization. <i>Environmental Health Perspectives</i> , 120(7), 978–983. doi:10.1289/ehp.1104307	Excluded, published in 2012	Not applicable
Peretz J, Gupta RK, Singh J, Hernández-Ochoa I and Flaws JA, 2011. Bisphenol A impairs follicle growth, inhibits steroidogenesis, and downregulates rate-limiting enzymes in the estradiol biosynthesis pathway. <i>Toxicological Sciences</i> , 119(1), 209–217. doi:10.1093/toxsci/kfq319	Excluded, published in 2011 and	Not applicable
Tucker DK, Hayes Bouknight S, Brar SS, Kissling GE and Fenton SE, 2018. Evaluation of pre-natal exposure to bisphenol analogues on development and long-term health of the mammary gland in female mice. <i>Environmental Health Perspectives</i> , 126(8). doi:10.1289/EHP3189, PubMed: 087003	Included	RefID 13275
Prins GS, Ye SH, Birch L, Zhang X, Cheong A, Lin H, Calderon-Gierszal E, Groen J, Hu WY, Ho SM and van Breemen RB, 2017. Prostate cancer risk and DNA methylation signatures in aging rats following developmental BPA exposure: A dose–response analysis. <i>Environmental Health Perspectives</i> , 125(7), 077007. doi:10.1289/EHP1050	Included	RefID 5930
Hass U, Christiansen S, Boberg J, Rasmussen MG, Mandrup K and Axelstad M, 2016. Low-dose effect of developmental bisphenol A exposure on sperm count and behaviour in rats. <i>Andrology</i> , 4(4), 594–607. doi:10.1111/andr.12176	Included	RefID 2610
Mandrup K, Boberg J, Isling LK, Christiansen S and Hass U, 2016. Low-dose effects of bisphenol A on mammary gland development in rats. <i>Andrology</i> , 4(4), 673–683. doi:10.1111/andr.12193	Included	RefID 4831

Tremblay-Franco M, Cabaton NJ, Canlet C, Gautier R, Schaeberle CM, Jourdan F, Sonnenschein C, Vinson F, Soto AM and Zalko D, 2015. Dynamic metabolic disruption in rats perinatally exposed to low doses of bisphenol-A. <i>PLOS ONE</i> , 10(10), e0141698. doi:10.1371/journal.pone.0141698	Included	RefID 7285
Christiansen S, Axelstad M, Boberg J, Vinggaard AM, Pedersen GA and Hass U, 2014. Low-dose effects of bisphenol A on early sexual development in male and female rats. <i>Reproduction</i> , 147(4), 477–487. doi:10.1530/REP-13-0377	Included	RefID 1224
Wadia PR, Cabaton NJ, Borrero MD, Rubin BS, Sonnenschein C, Shioda T and Soto AM, 2013. Low-dose BPA exposure alters the mesenchymal and epithelial transcriptomes of the mouse fetal mammary gland. <i>PLOS ONE</i> , 8(5), e63902. doi:10.1371/journal.pone.0063902	Included	RefID 7531
Cabaton NJ, Canlet C, Wadia PR, Tremblay-Franco M, Gautier R, Molina J, Sonnenschein C, Cravedi JP, Rubin BS, Soto AM and Zalko D, 2013. Effects of low doses of bisphenol A on the metabolome of perinatally exposed CD-1 mice. <i>Environmental Health Perspectives</i> , 121(5), 586–593. doi:10.1289/ehp.1205588	Included	RefID 776
Bernier MR and Vandenberg LN, 2017. Handling of thermal paper: Implications for dermal exposure to bisphenol A and its alternatives. <i>PLOS ONE</i> , 12(6), e0178449. doi:10.1371/journal.pone.0178449	Excluded, it addresses BPA exposure	RefID 534
Vandenberg LN, Schaeberle CM, Rubin BS, Sonnenschein C and Soto AM, 2013. The male mammary gland: A target for the xenoestrogen bisphenol A. <i>Reproductive Toxicology</i> , 37, 15–23. doi:10.1016/j.reprotox.2013.01.002	Included	RefID 7405
Acevedo N, Davis B, Schaeberle CM, Sonnenschein C and Soto AM, 2013. Perinatally administered bisphenol A as a potential mammary gland carcinogen in rats. <i>Environmental Health Perspectives</i> , 121(9), 1040–1046. doi:10.1289/ehp.1306734	Included	RefID 32
Tang WY, Morey LM, Cheung YY, Birch L, Prins GS and Ho SM, 2012. Neonatal exposure to estradiol/bisphenol A alters promoter methylation and expression of <i>nsbp1</i> and <i>hpcal1</i> genes and transcriptional programs of <i>dnmt3a/b</i> and <i>MBD2/4</i> in the rat prostate gland throughout life. <i>Endocrinology</i> , 153(1), 42–55. doi:10.1210/en.2011-1308	Excluded, published in 2012	Not applicable
Tharp AP, Maffini MV, Hunt PA, Vandervoort CA, Sonnenschein C and Soto AM, 2012. Bisphenol A alters the development of the rhesus monkey mammary gland. <i>Proceedings of the National</i>	Excluded, published in 2012	Not applicable

Academy of Sciences of the United States of America, 109(21), 8190–8195. doi:10.1073/pnas.1120488109		
Betancourt AM, Wang J, Jenkins S, Mobley J, Russo J and Lamartiniere CA, 2012. Altered carcinogenesis and proteome in mammary glands of rats after prepubertal exposures to the hormonally active chemicals bisphenol a and genistein. <i>Journal of Nutrition</i> , 142(7), 1382S–1388S. doi:10.3945/jn.111.152058	Excluded, published in 2012	Not applicable
Hunt PA, Lawson C, Gieske M, Murdoch B, Smith H, Marre A, Hassold T and VandeVoort CA, 2012. Bisphenol A alters early oogenesis and follicle formation in the fetal ovary of the rhesus monkey. <i>Proceedings of the National Academy of Sciences of the United States of America</i> , 109(43), 17525–17530. doi:10.1073/pnas.1207854109	Excluded, published in 2012	Not applicable
Cabaton NJ, Wadia PR, Rubin BS, Zalko D, Schaeberle CM, Askenase MH, Gadbois JL, Tharp AP, Whitt GS, Sonnenschein C and Soto AM, 2011. Perinatal exposure to environmentally relevant levels of bisphenol A decreases fertility and fecundity in CD-1 mice. <i>Environmental Health Perspectives</i> , 119(4), 547–552. doi:10.1289/ehp.1002559	Excluded, published in 2011	Not applicable
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Jenkins S, Wang J, Eltoum I, Desmond R and Lamartiniere CA, 2011. Chronic oral exposure to bisphenol A results in a nonmonotonic dose response in mammary carcinogenesis and metastasis in MMTV-erbB2 mice. <i>Environmental Health Perspectives</i> , 119(11), 1604–1609. doi:10.1289/ehp.1103850	Excluded, published in 2011	Not applicable
Betancourt AM, Eltoum IA, Desmond RA, Russo J and Lamartiniere CA, 2010. In utero exposure to bisphenol A shifts the window of susceptibility for mammary carcinogenesis in the rat. <i>Environmental Health Perspectives</i> , 118(11), 1614–1619. doi:10.1289/ehp.1002148	Excluded, published in 2010	Not applicable
Betancourt AM, Mobley JA, Russo J and Lamartiniere CA, 2010. Proteomic analysis in mammary glands of rat offspring exposed in utero to bisphenol A. <i>Journal of Proteomics</i> , 73(6), 1241–1253. doi:10.1016/j.jprot.2010.02.020	Excluded, published in 2010	Not applicable
Jenkins S, Raghuraman N, Eltoum I, Carpenter M, Russo J and Lamartiniere CA, 2009. Oral exposure to bisphenol A increases dimethylbenzanthracene-induced mammary cancer in rats. <i>Environmental</i>	Excluded, published in 2009	Not applicable

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Vandenberg LN, Maffini MV, Schaeberle CM, Ucci AA, Sonnenschein C, Rubin BS and Soto AM, 2008. Perinatal exposure to the xenoestrogen bisphenol-A induces mammary intraductal hyperplasias in adult CD-1 mice. <i>Reproductive Toxicology</i> , 26(3–4), 210–219. doi:10.1016/j.reprotox.2008.09.015	Excluded, published in 2008	Not applicable
Moral R, Wang R, Russo IH, Lamartiniere CA, Pereira J and Russo J, 2008. Effect of pre-natal exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression signature. <i>Journal of Endocrinology</i> , 196(1), 101–112. doi:10.1677/JOE-07-0056	Excluded, published in 2008	Not applicable
Vandenberg LN, Maffini MV, Wadia PR, Sonnenschein C, Rubin BS and Soto AM, 2007. Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters development of the fetal mouse mammary gland. <i>Endocrinology</i> , 148(1), 116–127. doi:10.1210/en.2006-0561	Excluded, published in 2007	Not applicable
Murray TJ, Maffini MV, Ucci AA, Sonnenschein C and Soto AM, 2007. Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. <i>Reproductive Toxicology</i> , 23(3), 383–390. doi:10.1016/j.reprotox.2006.10.002	Excluded, published in 2007	Not applicable
Durando M, Kass L, Piva J, Sonnenschein C, Soto AM, Luque EH and Muñoz-de-Toro M, 2007. Prenatal bisphenol A exposure induces pre-neoplastic lesions in the mammary gland in Wistar rats. <i>Environmental Health Perspectives</i> , 115(1), 80–86. doi:10.1289/ehp.9282	Excluded, published in 2007	Not applicable
Wadia PR, Vandenberg LN, Schaeberle CM, Rubin BS, Sonnenschein C and Soto AM, 2007. Perinatal bisphenol A exposure increases estrogen sensitivity of the mammary gland in diverse mouse strains. <i>Environmental Health Perspectives</i> , 115(4), 592–598. doi:10.1289/ehp.9640	Excluded, published in 2007	Not applicable
Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM and Soto AM, 2006. Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. <i>Endocrinology</i> , 147(8), 3681–3691. doi:10.1210/en.2006-0189	Excluded, published in 2006	Not applicable
Muñoz-de-Toro M, Markey CM, Wadia PR, Luque EH, Rubin BS, Sonnenschein C and Soto AM, 2005. Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. <i>Endocrinology</i> , 146(9), 4138–4147. doi:10.1210/en.2005-0340	Excluded, published in 2005	Not applicable
Markey CM, Wadia PR, Rubin BS, Sonnenschein C and Soto AM, 2005. Long-term effects of fetal exposure to low doses of the xenoestrogen	Excluded, published in 2005	Not applicable

bisphenol-A in the female mouse genital tract. <i>Biology of Reproduction</i> , 72(6), 1344–1351. doi:10.1095/biolreprod.104.036301		
Markey CM, Coombs MA, Sonnenschein C and Soto AM, 2003. Mammalian development in a changing environment: Exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. <i>Evolution and Development</i> , 5(1), 67–75. doi:10.1046/j.1525-142x.2003.03011.x	Excluded, published in 2003	Not applicable
Markey CM, Luque EH, Munoz De Toro M, Sonnenschein C and Soto AM, 2001. In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. <i>Biology of Reproduction</i> , 65(4), 1215–1223. doi:10.1093/biolreprod/65.4.1215	Excluded, published in 2001	Not applicable
Papers on BPA replacement chemicals:		
Kolla S, Morcos M, Martin B and Vandenberg LN, 2018. Low dose bisphenol S or ethinyl estradiol exposures during the perinatal period alter female mouse mammary gland development. <i>Reproductive Toxicology</i> , 78, 50–59. Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. <i>Endocrinology</i> doi:10.1016/j.reprotox.2018.03.003	Excluded, it does not investigate BPA	Not applicable
LaPlante CD, Catanese MC, Bansal R and Vandenberg LN, 2017. Bisphenol S alters the lactating mammary gland and nursing behaviors in mice exposed during pregnancy and lactation. <i>Endocrinology</i> , 158(10), 3448–3461. doi:10.1210/en.2017-00437	Excluded, it does not investigate BPA	Not applicable
Hill CE, Sapouckey SA, Suvorov A and Vandenberg LN, 2017. Developmental exposures to bisphenol S, a BPA replacement, alter estrogen-responsiveness of the female reproductive tract: A pilot study. <i>Cogent Medicine</i> , 4(1). doi:10.1080/2331205X.2017.1317690, PubMed: 31231671	Excluded, it does not investigate BPA	Not applicable
Catanese MC and Vandenberg LN, 2017. Bisphenol S (BPS) alters maternal behavior and brain in mice exposed during pregnancy/lactation and their daughters. <i>Endocrinology</i> , 158(3), 516–530. doi:10.1210/en.2016-1723	Excluded, it does not investigate BPA	Not applicable
Kim B, Colon E, Chawla S, Vandenberg LN and Suvorov A, 2015. Endocrine disruptors alter social behaviours and indirectly influence social hierarchies via changes in body weight. <i>Environmental Health: A Global Access Science Source</i> , 14, 64. doi:10.1186/s12940-015-0051-6	Excluded, it does not investigate BPA	Not applicable
2. Ninja Reineke on behalf of CHEM Trust (report)	Notes	RefID in Distiller

CHEMTrust (2018). 'From BPA to BPZ a toxic soup?: How companies switch from a known hazardous chemical to one with similar properties, and how regulators could stop them.' CHEM Trust Report	Excluded, secondary publication	RefID 13775
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3. Submission by Hanane Yasmina Anteur (2 articles)	Notes	Distiller
Anteur HY, Bendahmane M and Khan NA, 2016. Perinatal exposure to bisphenol A affects body weight and the reproductive function of Wistar rat. <i>Journal of Applied Environmental and Biological Sciences.</i> , 6(3), 1–8.	Included	RefID 13773
Anteur HY, Bendahmane M, Mehida H, Beghdadli B, Aboubekr FA, Khan NA and Kandouci BA, 2016. Is bisphenol A exposure associated with uterine leiomyoma in Western Algerian women of childbearing potential? <i>Journal of Disease and Global Health</i> , 8(3), 131–140.	Included	RefID 13774

4. Submission by Plastics Europe (10 submissions)	Notes	Distiller
2. Comments from the PlasticsEurope EU PC/BPA Group in Response to EFSA's Call for Data on BPA	Excluded, they are just a description of the CLARITY-BPA NTP study design.	Not applicable
3. Final Report 'Analysis of Draft Results for the CLARITY-BPA Core Study' Exponent, August 31, 2018	Excluded, secondary study	RefID 1380
5. Final Report: 'Three-generation reproductive toxicity evaluation of Bisphenol A administered in the feed to CD (Sprague–Dawley) Rats', RTI Identification Number: 65C-07036-000 (full study report 17 pdf files submitted)	Unpublished study report, date of completion May 12, 2000 Excluded: Study report of Tyl et al., 2002; Study already evaluated in the 2006, 2008 and 2015 EFSA BPA opinions	Not applicable
6. Final Report: 'A dietary developmental neurotoxicity study of bisphenol A in rats' Study Number WIL-186056; Donald G. Stump, PhD, DABT Full study report: 20 pdf files submitted Study initiation date: 26 June 2008 Study completion date: 30 September 2009	Excluded, already evaluated in CEF Panel, (2010)	Not applicable
7. Extended abstract: 'Pharmacokinetics of Bisphenol A in humans following dermal administration', Kristina A Thayer, Division of the National Toxicology Program. Kristina A. Thayer, Daniel R. Doerge, Dawn Hunt, Shepherd Schurman, Nathan C. Twaddle, Mona I. Churchwell, Stavros Garantziotis, Grace E. Kissling, Michael R. Easterling, John R. Bucher, Linda S. Birnbaum	Preliminary results: These findings have not been peer-reviewed and should not be considered to represent NTP opinion. Excluded, final data reported in RefID 7183, considered by the CEP Panel	Not applicable
8. Final Report: 'Two-generation reproduction study of Bisphenol A in rats', Study No: SR-98101, Makoto Ema, National Institute of Health Sciences, Osaka	Raw data of: Ema M, Fujii S, Furukawa M, Kiguchi M, Ikka T, Harazono A, 2001. Rat two-generation reproductive toxicity study of	Not applicable

<p>Full study report submitted (7 pdf files 'CCSRI 2000 – Part X')</p> <p>Date of commencement: December 17, 1998</p> <p>Date of completion: May 12, 2000</p>	<p>bisphenol A. Reproductive Toxicology, 15(5), 505–523:</p> <p>https://www.ncbi.nlm.nih.gov/pubmed/11780958</p> <p>Excluded, Published in 2001</p>	
<p>9. Draft report: 'Draft NTP Research Report on The CLARITY-BPA Core Study: A Perinatal and Chronic Extended-Dose-Range Study of Bisphenol A in Rats', February 2018</p>	<p>This is the draft CLARITY-BPA report. The final report of 2018 and the Camacho et al., 2019 paper (both assigned to RefID 11370) were considered for the assessment</p>	<p>It corresponds to RefID 11370</p>
<p>10. NCTR GLP/NTP Technical report: 'Evaluation of the toxicity of Bisphenol A (BPA) in male and female Sprague–Dawley rats exposed orally from gestation day 6 through PND 90', PI: Barry Delclos, 2013</p> <p>Technical report: Finalised July 2013</p> <p>Date of Original Technical Report: March 4, 2013</p> <p>Date of Technical Report Amendment #1: May 31, 2013</p> <p>Date of Amendment #2 to Technical Report: July 11, 2013</p> <p>Data probably for the study Delclos 2013. 50 pdf files submitted</p>	<p>Excluded, already evaluated in the 2015 BPA EFSA opinion</p>	<p>Not applicable</p>
<p>5. Gail Prins – University of Illinois</p>		
<p>Prins GS., Hu WY, Xie L, Shi GB, Hu DP, Birch L and Bosland MC, 2018. Evaluation of Bisphenol A (BPA) exposures on prostate stem cell homeostasis and prostate cancer risk in the NCTR-Sprague-Dawley rat: an NIEHS/FDA CLARITY-BPA Consortium Study. Environmental Health Perspectives, 126(11), 117001. doi:10.1289/EHP3953</p>	<p>Manuscript Draft</p> <p>The final study is now available online. 'Received 25 May 2018; Revised 4 October 2018; Accepted 11 October 2018; Published 2 November 2018</p> <p>Supplemental Material is available online (https://doi.org/10.1289/EHP3953).'</p> <p>Included</p>	<p>RefID 13779</p>
<p>6. Submission by Michal Filipiak</p>		
<p>EFSA-Q-2018-00221 Toxicological data derived from human studies</p> <p>[toxicity of BPA on my own example] Concerns: observed and confirmed: reproductive, endocrine system, metabolic systems problems. In response to:</p> <p>www.efsa.europa.eu/en/consultations/call/180309-0</p>	<p>Notes</p> <p>Excluded, self-report of a personal pathological situation, not a scientific study</p>	<p>Distiller</p> <p>Not applicable</p>

15143

Appendix B – The Montévil et al. (2020) study: Consideration of low-dose effects and non-monotonic dose–response reported in that study

15144 Examining the relationship between gestational exposure BPA and offspring mammary gland development
 15145 (different morphological features) in Sprague–Dawley rats, Montévil et al. (2020) [RefID 13788] concluded
 15146 that the relationship followed a non-monotonic dose–response (NMDR) curve. This conclusion was based
 15147 on the author’s statistical evaluation by fitting a linear step function with the unit function placed either
 15148 between the 25 and 250; or the 250 and 2500 µg/kg bw dose per day.

15149 Such functions are commonly used in engineering to explain time-dependent processes where a sudden
 15150 (binary) shift in response is initiated (e.g. sudden changes in voltage or market conditions, respectively).
 15151 The use of step functions to describe biological process is neither common nor conventional.

15152 As the developmental effects examined in this study are biologically relevant and potentially adverse, the
 15153 WG evaluated the findings from this study using a statistical approach that is more conventional for
 15154 assessing the dose–response in toxicological studies.

15155 The outcomes evaluated were those reported in Figures 8 and Figure 9 of the study (i.e. weight, gland
 15156 density, Dimension 3D and angles of branches between beginning and end, thickness of epithelium,
 15157 variation of ductal thickness and aspect ratio). Raw data for Figure 8, Panel E, could not be assessed as
 15158 they were not included in the publicly available dataset for the study.

15159 In the WG assessment the presence of any dose–response information in the data was first evaluated by
 15160 using a simple F-test under null hypothesis that the mean response in all groups is the same. For
 15161 transparency the test results, including total sum of squares (SSQ), model sum of squares, the
 15162 corresponding degrees of freedom (DF) and the resulting p-value (F-test) are shown in Table B1.

15163 **Table B1:** Examining the presence of dose–response in figures 8 and 9 in the Montévil et al.
 15164 (2020) [RefID 13788] study under the NULL hypothesis that all groups are equal.

	Total		Model		p-value ¹
	SSQ	DF	SSQ	DF	
Figure 8	SSQ	DF	SSQ	DF	
A – SD with 3D	2098	58	420	5	0.033
B – Thickness mm	23941	58	4263	5	0.058
C – Dimension 3D	0.4886	58	0.0545	5	0.266
D – Angle between beginning and end	149.4	58	12.8	5	0.431
E – Dim3	Data not available				
F – Aspect Ratio	14.92	58	2.36	5	0.095
Figure 9	SSQ	DF	SSQ	DF	
A – Gland weight, PND90, stop dose	4.990	55	0.751	5	0.136
B – Density analysis PND90, continuous dose	14250	58	1580	5	0.269

C – Density analysis, 6 months, continuous dose	11975	59	1235	5	0.303
D – Density analysis, 9 months, stop dose	9358	59	1208	5	0.176

15165

15166 Only the mean variation of ductal thickness (SD with 3D, figure 8A) reached formal significance ($p < 0.05$)
 15167 meaning that the mean response across all groups was significantly different. This significance was driven
 15168 by higher response in the 25 $\mu\text{g}/\text{kg}$ bw per day dose group compared with controls. For figure 8B there
 15169 was a borderline significant effect ($p = 0.058$) for the mean thickness of the epithelium, again driven by
 15170 higher response in the 25 $\mu\text{g}/\text{kg}$ bw dose group. For the aspect ratio (figure 8F), the 250 $\mu\text{g}/\text{kg}$ bw per day
 15171 dose groups was significantly different from the controls ($p = 0.02$, t-test) but the F-test for differences
 15172 across dose groups did not reach formal significance. Visual inspection of model residuals confirmed that
 15173 the use of F-test was appropriate.

15174 Taking figure 8A (the mean variation of ductal thickness) as an example of an outcome showing a potential
 15175 dose–response, the shape of the dose–response was evaluated by modelling the data in PROAST using the
 15176 following set of models (Table B2).

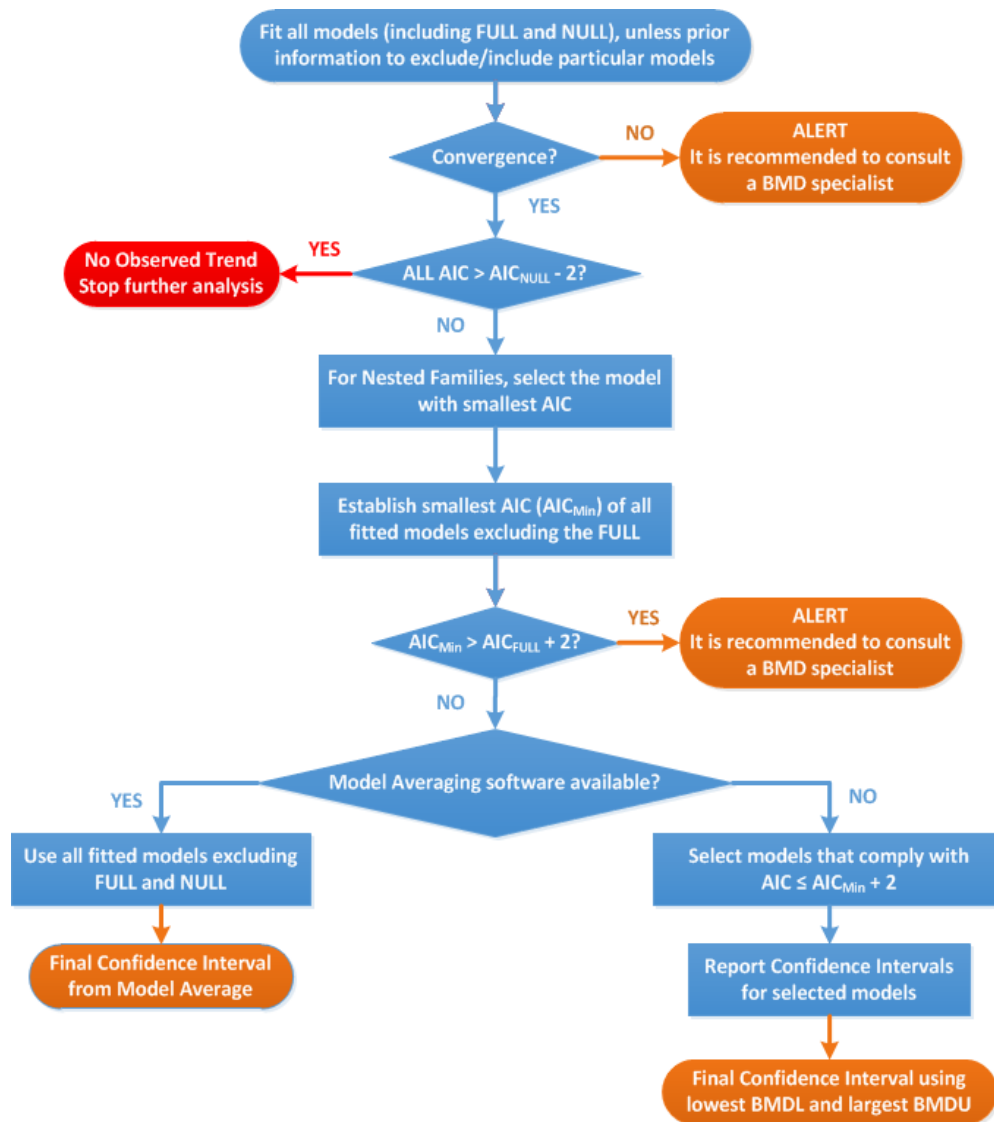
15177 **Table B2:** Modelling of the data in PROAST.

Model	Number of parameters	Formula
Null	1	$y = a$
Full	no. of groups	$y = \text{group mean}$
Exp model 3	3	$y = a \cdot \exp(bx^d)$
Exp model 4	4	$y = a \cdot (c - (c - 1)\exp(-bx^d))$
Hill model 3	3	$y = a \cdot \left(1 - \frac{x^d}{b^d + x^d}\right)$
Hill model 4	4	$y = a \cdot \left(1 - \frac{(c - 1) \cdot x^d}{b^d + x^d}\right)$
Inverse exponential	4	$y = a \cdot (1 + (c - 1)\exp(-bx^{-d}))$
Log-normal family	4	$y = a \cdot (1 + (c - 1)\Phi(\ln b + d \ln x))$

15178

15179 These functions are sufficiently flexible to capture presence of non-monotonicity and have been used
 15180 previously for that purpose (Beausoleil et al., 2016; Badding et al., 2019).

15181 The criteria for evaluating if there is a significant dose–response is then as follows (Figure B1).



15182

15183

15184 **Figure B1:** Criteria for evaluating if there is a significant dose–response.

15185 In short, none of the models deviated significantly from the NULL model. The PROAST output is shown in
 15186 Table B3 below (based on the model log likelihood the p-value for the model fit (Chi-square test), can for
 15187 example be derived).

15188 **Table B3:** PROAST output based on the model log likelihood the p-value for the model fit (chi-
 15189 squared test).

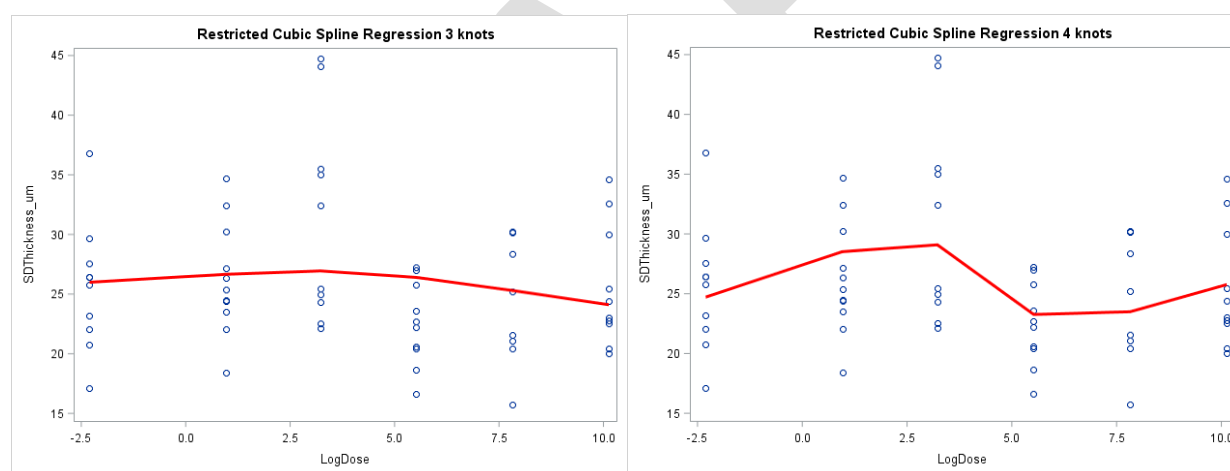
model	converged	loglik	npar	AIC
full model	Yes	10.09	9	-2.18

null model	Yes	2.23	2	-0.46
Expon. m3-	Yes	3.39	4	1.22
Expon. m5-	Yes	3.52	5	2.96
Hill m3-	Yes	3.39	4	1.22
Hill m5-	Yes	3.67	5	2.66
Inv.Expon. m3-	Yes	2.37	4	3.26
Inv.Expon. m5-	Yes	4.00	5	2.00
LN m3-	Yes	3.45	4	1.10
LN m5-	Yes	3.77	5	2.46

15190

15191 Similar conclusions were also reached for all other outcomes in figures 8 and 9 (data not shown).

15192 Additional analyses using different models, including splines, did not reveal any significant dose-response
 15193 in the data in figures 8 and 9. Significant dose-response could only be generated for figure 8B by overfitting
 15194 the data using a restricting cubic spline as implemented in 'proc glmselect' in SAS (v9.2). This example is
 15195 shown in Figure B2 below where a non-significant fit is obtained when fitting a spline function using 3 knots
 15196 ($p = 0.48$). However, when fitting a spline function using 4 knots, which follows changes in response
 15197 between every dose groups, a significant model fit was obtained ($p = 0.03$). This is however a clear
 15198 example of overfitting the data and cannot be considered as a reasonable reflection of dose-response that
 15199 could be replicated in different experimental setting.



15200

15201 **Figure B2:** Results from fitting a restricted cubic spline using 3 and 4 knots to the data from figure 8A
 15202 (Montévil et al., 2020 [RefID 13788]).

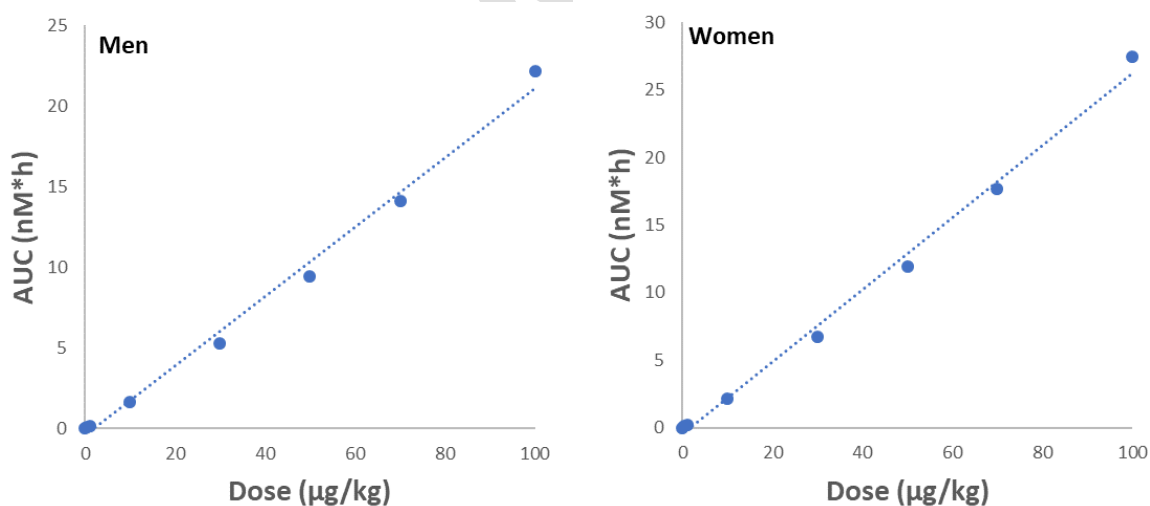
15203 For the left figure (3 knots) the model sum of square was 2098 (DF = 58) and model sum of square was
 15204 55 (DF = 2). The resulting p-value was 0.48 (F-test). For the right-hand figure (4 knots) the model sum of
 15205 square was 307 (DF = 2) and the resulting p-value was 0.032. As can be seen in the 4 knots result in exact
 15206 fit between each pair of dose groups result in clear overfitting of the data²¹.

²¹ Overfitting is the term used for model fit that corresponds too closely or exactly to a particular set of data, which may therefore fail to fit additional data or predict future observations reliably. Such models usually contain parameters than can be justified by the data.

15207 In summary, based on a simple F-test, only the results from figure 8A show any indication of significant
15208 dose–response. However, when fitting flexible biologically based non-linear models though the data (in
15209 PROAST) or spline functions (without overfitting the data) no indication of any meaningful dose–response
15210 is observed. A significant NMDR could only be generated when overfitting the data using splines or by using
15211 a step function designed around the dose where pairwise significance was reached. Based on the WG
15212 analyses the significant NMDR that the authors (Montévil et al., 2020 [RefID 13788]) generated appears,
15213 not to be very robust; one may equally assume that the significant difference for the 25 µg/kg bw per day
15214 dose group is a chance finding as no clear biologically recognisable pattern is observed in the data. In
15215 biostatistical terms, the evidence for NMDR in the Montévil et al. study appears weak and inconclusive.

Appendix C – Application of PBPK model of Karrer et al. (2018) to check linearity

15216 The PBPK model of Karrer et al. (2018) [RefID 12289] was used to check the linearity of the AUC with the
15217 administered dose. A bolus oral exposure was considered, and the AUC was computed 24 h after the
15218 administration. Few modifications were made to the PBPK model (model code in R given in the
15219 supplementary materials of the paper by Karrer et al. (2018) [RefID 12289]): the flow values were changed
15220 to correspond to plasma flows and not to blood flows by means of the haematocrit, the intake scheme was
15221 adapted to our simulation (a unique oral dose) and the computation of the AUC of BPA in plasma was
15222 added (Figure C1). Several doses were tested: 0.5, 1, 10, 30, 50, 70 and 100 µg/kg bw. Simulations were
15223 run for both men (bw = 73 kg) and women (bw = 60 kg).



15224
15225 **Figure C1:** Application of PBPK model of Karrer et al. (2018) [RefID 12289] to check linearity.

15226

Appendix D – Uncertainty analysis outcome

15227

15228 The purpose of the uncertainty analysis was to assess whether other effects of BPA may potentially occur
15229 after exposure to lower doses than the endpoint on which the reference point (RP) is based and, if so,
15230 inform a decision on what size of additional UF would be suitable to take those effects into account. This
15231 was carried out in the series of steps described in Chapter 2.3.4, and the results are presented below.

15232 **D.1. Assessment of clusters by expert judgement**

15233 Five HOCs were considered in the main part of the uncertainty analysis: Immunotoxicity, Metabolic effects,
15234 Neurotoxicity and developmental neurotoxicity, Reproductive and developmental toxicity, and
15235 Carcinogenicity and mammary gland proliferative effects (the HOC for General toxicity was considered later
15236 and cardiotoxicity was not considered in the uncertainty analysis because the evidence was either judged
15237 as Not Likely or as Inadequate). Within these five HOCs, the uncertainty analysis focused on 21 clusters of
15238 endpoints that were rated ALAN, Likely or Very Likely in the WoE assessment. Uncertainty was assessed
15239 by quantitative expert judgements of two questions for each cluster, elicited from two or three experts per
15240 cluster, who were chosen for their expertise on the endpoints in that cluster (11 experts in total). Experts
15241 were invited to revise their judgements, if they wished, after discussing them with the other expert(s)
15242 assessing the same cluster.

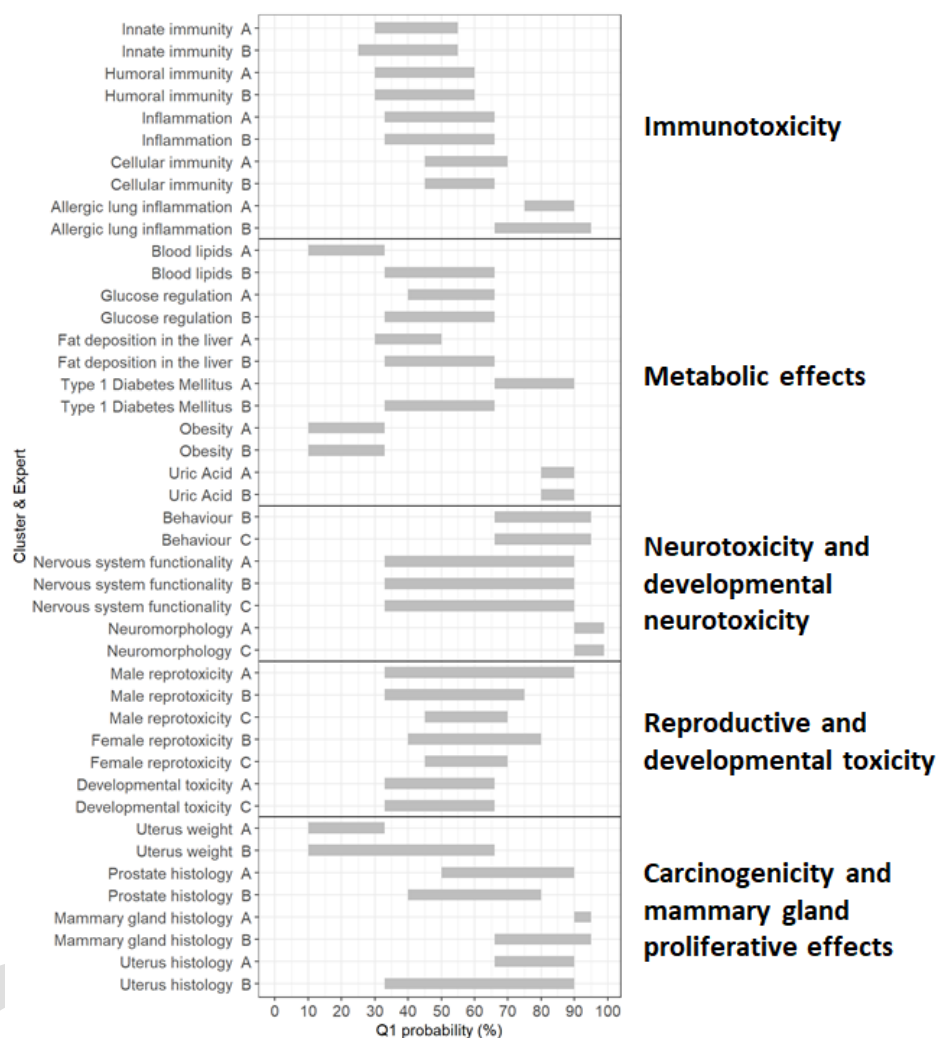
15243 Question 1 was 'What is your probability that there is at least one endpoint in the WoE table for this cluster
15244 that occurs in animals tested with BPA and is relevant and adverse in humans?' In effect, this quantifies
15245 uncertainty in hazard identification for each cluster. The experts gave their judgement on this in the form
15246 of approximate probabilities, i.e. ranges of probabilities, and their responses are presented graphically in
15247 Figure D1.

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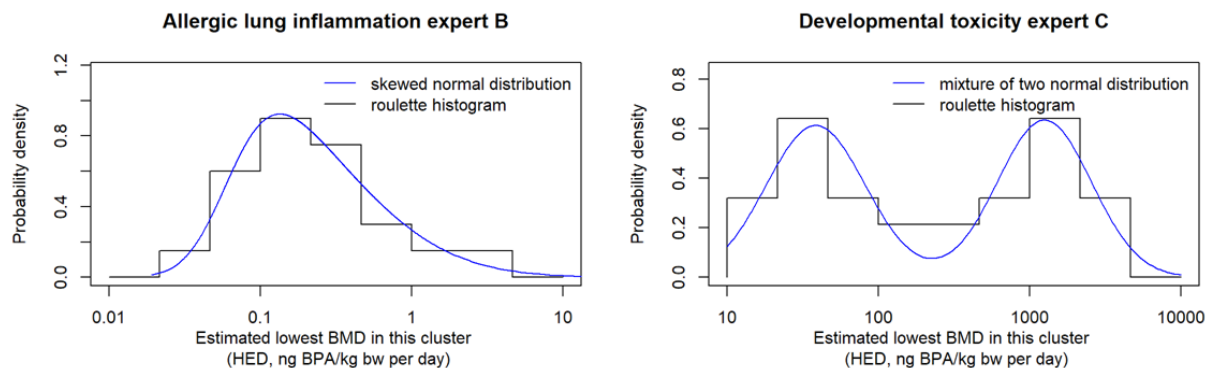


15252
 15253 Each bar represents the range of % probability for the specified cluster and expert. Experts were identified as A, B, C for each
 15254 cluster.
 15255

15256 **Figure D1:** Experts' approximate probabilities, for each cluster, that there is at least one endpoint in
 15257 the WoE table for the cluster that occurs in animals tested with BPA and is relevant and adverse in
 15258 humans.

15259 Question 2 was 'If one or more endpoints in the WoE table for this cluster occurs in animals tested with
 15260 BPA and is both relevant and adverse for humans, what is your prediction for the lowest BMD of those
 15261 endpoints, expressed as HED?'. In effect, this quantifies uncertainty in hazard characterisation (dose-
 15262 response) for each cluster. The experts gave their judgement on this in the form of a probability distribution
 15263 for the estimated lowest BMD in each cluster. Two examples of these distributions are shown in Figure D2,
 15264 together with the parametric distributions that were fitted to them. The examples in Figure D2 were chosen
 15265 to illustrate two different types of distributions that were considered to find a good fit for each cluster and
 15266 expert (a single unskewed or skewed distribution, or a mixture of two unskewed distributions).

15267 Histograms and fitted distributions for all the clusters and experts are presented in Annex K. A summary of
15268 the fitted distributions is shown in Figure D3, to facilitate comparisons between clusters and between
15269 experts for the same cluster.

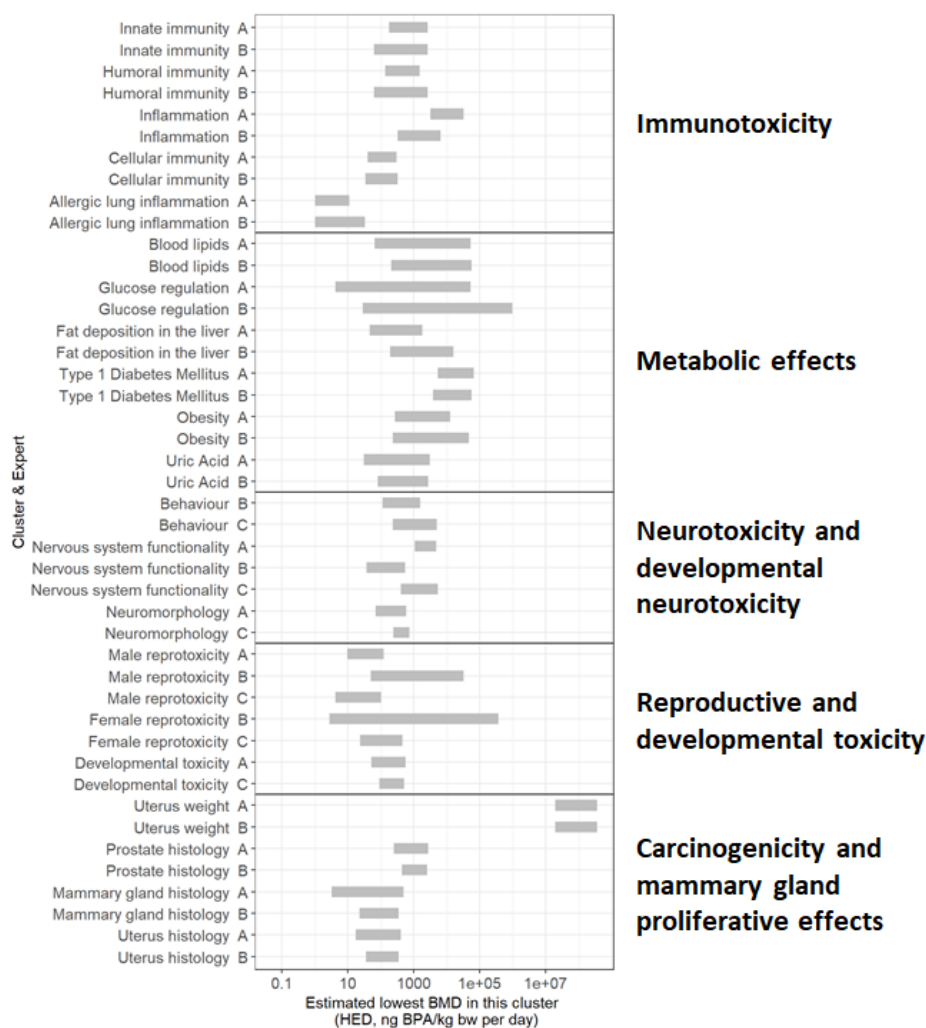


15270

15271 Each graph shows the histogram provided by the experts, and the parametric distribution that was subsequently fitted to their
15272 judgements.

15273

15274 **Figure D2:** Two examples, for different clusters, of probability distributions elicited from experts
15275 for the estimated lowest BMD of those endpoints in the cluster that occur in animals tested with BPA
15276 and is both relevant and adverse for humans.



15277
 15278 Each bar represents the 90% probability interval of the distribution for the specified cluster and expert. Experts were identified as A,
 15279 B, C for each cluster.

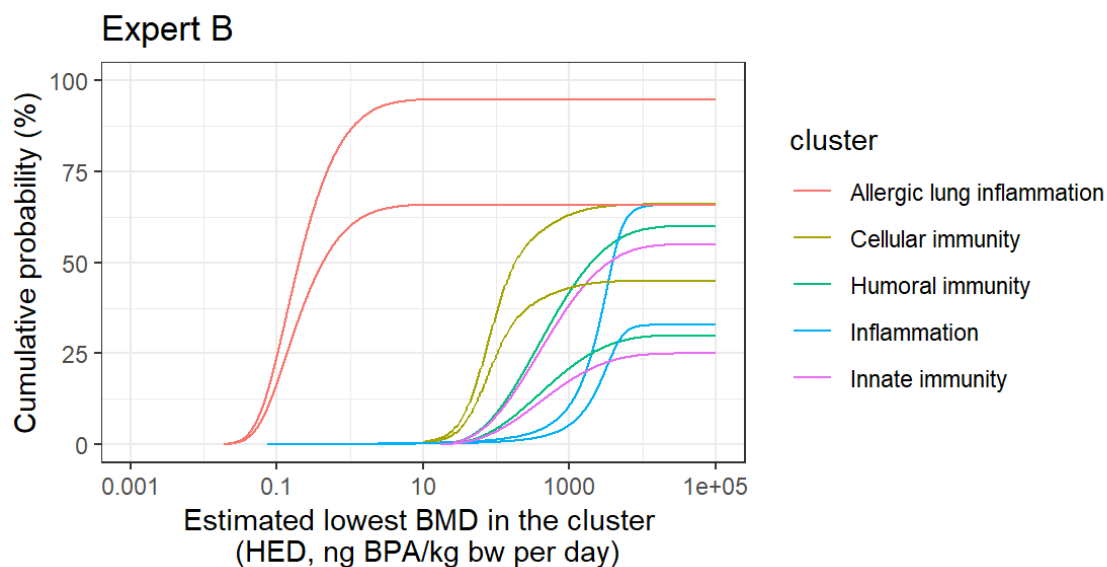
15280 **Figure D3:** Summary of distributions fitted to each experts' judgements, for each cluster, for the
 15281 estimated lowest BMD in the cluster for endpoints that occur in animals tested with BPA and is
 15282 relevant and adverse in humans.

15283 **D.2. Combining judgements for Questions 1 and 2**

15284 For each cluster and expert, the WG expert's probability from Question 1 was combined with their
 15285 distribution from Question 2 by multiplication. This provides, for each cluster and expert, a Cpf for the
 15286 estimated lowest BMD of effects in that cluster that occur in animals and are relevant and adverse for
 15287 humans.

15288 Figure D4 shows examples of these cpfs for one expert's judgements on five clusters in the HOC
 15289 Immunotoxicity. As their probability for Question 1 was expressed as a range, each cpf has a lower and

15290 upper bound, shown as two curves of the same colour. The lower and upper bound of each cpf resulted
 15291 from multiplying the distribution for Question 2 by, respectively, the lower and upper bound of the
 15292 probability for Question 1. The lower and upper bounds of the cpf show, on the vertical axis, a range for
 15293 expert's judgement of the probability that the cluster contains an endpoint that occurs in animals *and* is
 15294 relevant and adverse in humans *and* has a BMD that is *lower* than the dose shown on the horizontal axis.



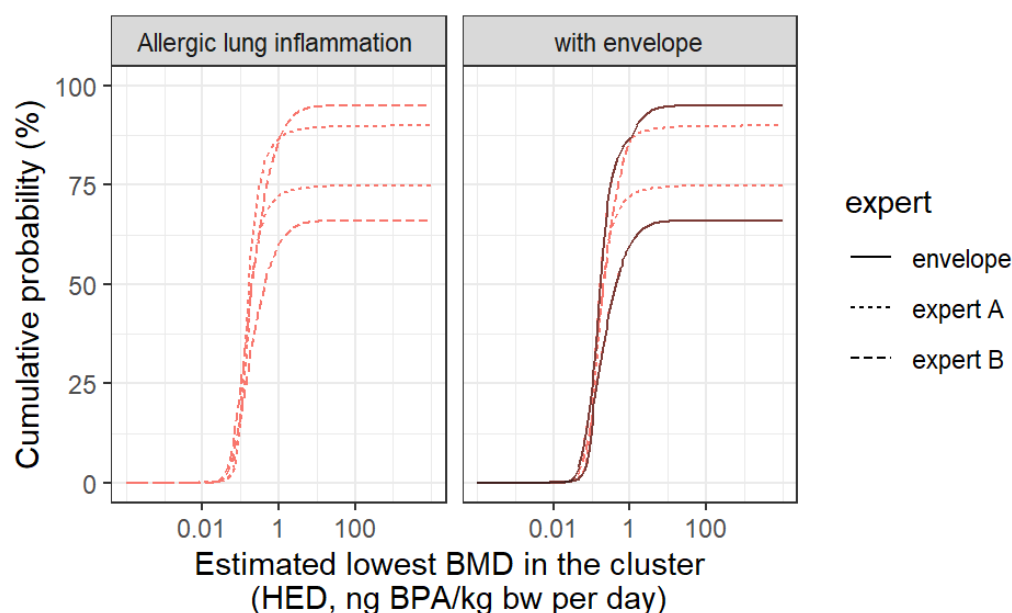
15295
 15296 **Figure D4:** Example of the results of combining one expert's judgements on Questions 1 and 2 for five
 15297 immunotoxicity clusters; see text for explanation.

15298 D.3. Combining judgements of different experts for the same cluster

15299 The cpfs for different experts were combined by enveloping, i.e., taking the minimum and maximum
 15300 cumulative probability at each dose. This reduces the 2 or 3 cpfs for the different experts assessing the
 15301 cluster to a single cpf with a wider gap between its lower and upper bounds, reflecting the combined
 15302 uncertainty arising from (a) the approximate nature of the Question 1 probabilities and (b) the range of
 15303 opinion between the experts for both Questions 1 and 2.

15304 Figure D5 shows an example of this process for one immunotoxicity cluster: allergic lung inflammation. The
 15305 left-hand graph shows the separate cpfs for the two experts who assessed this cluster, and the right-hand
 15306 graph is the same but with the addition of the lower and upper bounds for the combined cpf (solid black
 15307 curves).

15308 Interpretation of Figure D5 is similar to Figure D4, but for multiple experts. The lower and upper bound of
 15309 the enveloped cpf shows, on the vertical axis, the range of probabilities (combined across experts) that the
 15310 cluster contains an endpoint that occurs in animals *and* is relevant and adverse in humans *and* has a BMD
 15311 that is *lower* than the dose shown on the horizontal axis.



15312

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Figure D5: Example illustrating how different experts' judgements for the same cluster were combined by enveloping; see text for explanation.

15315

15316 **D.4. Combining judgements for different clusters**

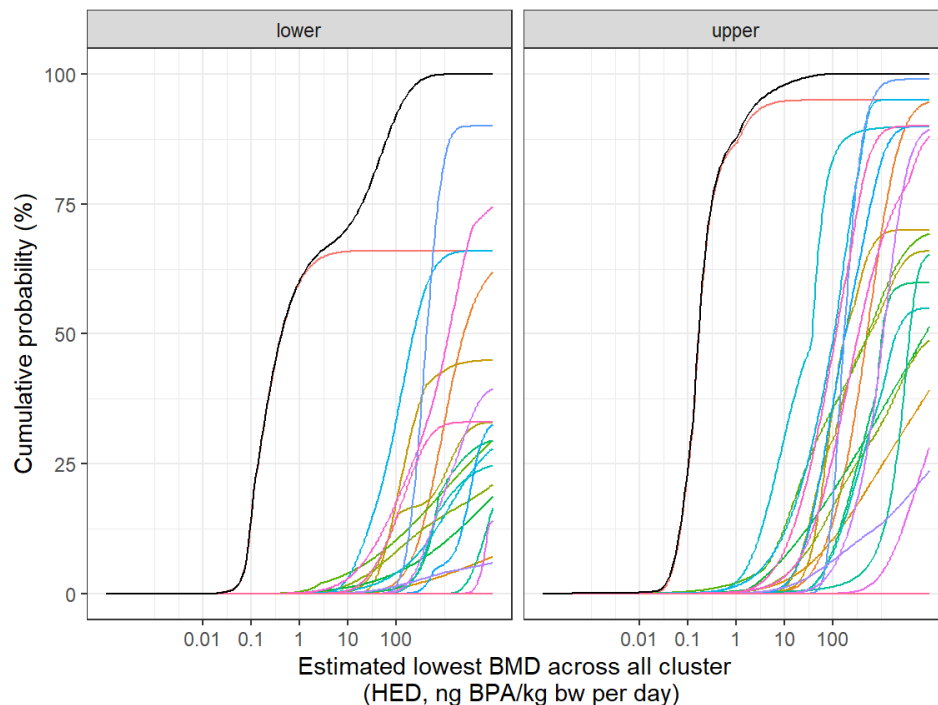
15317 The cdfs for different clusters were combined by the probability calculation described in Annex K to produce
 15318 a cdf for the estimated lowest BMD across all 21 clusters for endpoints that occur in animals and are
 15319 relevant and adverse for humans, assuming that the judgements for different clusters are independent. As
 15320 the cdf for each cluster had a lower and upper bound, the cdf for all 21 clusters also has a lower and upper
 15321 bound.

15322 Figure 6 shows the inputs and outputs of this calculation. The coloured curves are the lower (left-hand
 15323 graph) or upper (right-hand graph) bounds of the cdfs for the 21 different clusters, and the black curves
 15324 are the lower and upper bounds of the cdf for the estimated lowest BMD across all clusters.

15325 Interpretation of Figure D6 is similar to Figures D4 and D5 but combining all the clusters as well as the
 15326 different experts. The lower and upper bounds for the combined cdf show, on the vertical axis, the range
 15327 of probabilities (combined across experts) that *one or more* of the 21 clusters contains an endpoint that
 15328 occurs in animals *and* is relevant and adverse in humans *and* has a BMD that is *lower* than the dose shown
 15329 on the horizontal axis. This is relevant because the purpose of the uncertainty analysis is to assess whether
 15330 the probability of one or more effects in the 21 clusters occurring after exposure to lower doses than the
 15331 endpoint used for the RP is high enough to warrant an additional UF and, if so, determine how large the
 15332 UF should be to reduce that probability of such effects to an appropriately low level.

15333 It can be seen from Figure 6 that both the lower and upper bounds of the combined cdf are determined
 15334 mainly by the cluster for allergic lung inflammation. The lower part of each black curve follows the coloured

15335 curve for allergic lung inflammation, because there is virtually no probability of effects in other clusters in
 15336 that dose range. The cpfs for other clusters are at higher doses (further to the right) so, at those doses,
 15337 the combined cpf rises above the cpf for allergic lung inflammation due to the added probability of effects
 15338 from one or more of those clusters.



- Allergic lung inflammation
- Behaviour
- Blood lipids
- Cellular immunity
- Developmental toxicity
- Fat deposition in the liver
- Female reprotoxicity
- Glucose regulation
- Humoral immunity
- Inflammation
- Innate immunity
- Male reprotoxicity
- Mammary gland histology
- Nervous system functionality
- Neuromorphology
- Obesity
- Prostate histology
- Type 1 Diabetes Mellitus
- Uric Acid
- Uterus histology

15339
 15340 The coloured curves show the lower and upper bounds of the cpfs for the individual clusters, from which the combined cpf is
 15341 calculated.
 15342

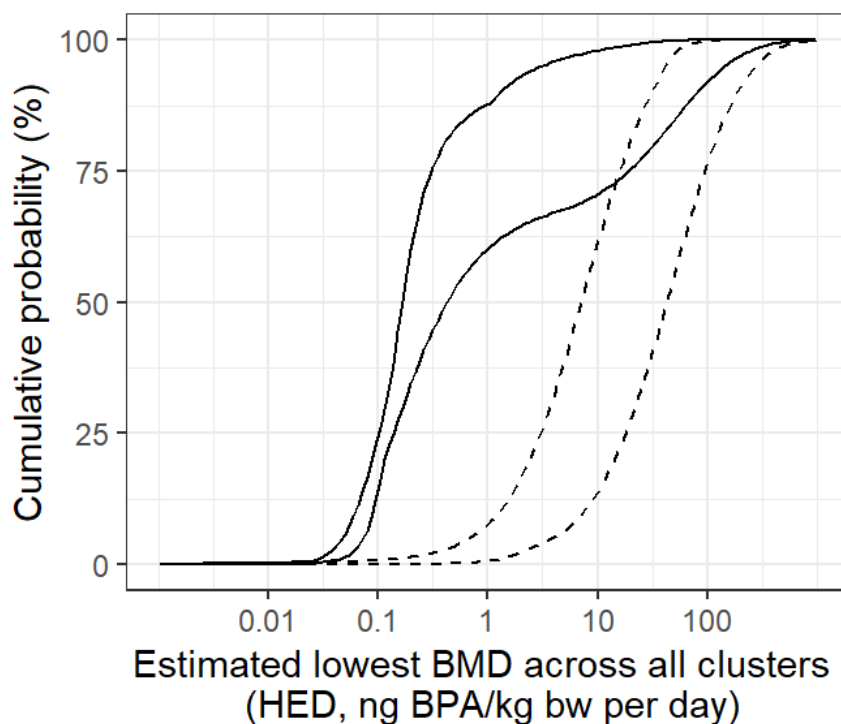
Figure D6: The black curves show the lower and upper bounds of the cpf for the estimated lowest BMD across all clusters for endpoints that occur in animals and are relevant and adverse for humans, assuming that the experts' judgements for different clusters are independent. The coloured curves show the lower and upper bounds of the cpfs for the individual clusters, from which the combined cpf is calculated.

15348 **D.5. Initial sensitivity analysis**

15349 A series of calculations was done to explore the sensitivity of the combined cpf to different factors. First,
 15350 the calculations were repeated including and excluding the cluster for allergic lung inflammation, to confirm

15351 its influence on the combined cpf. Figure D7 shows that, when allergic lung inflammation is excluded, the
 15352 combined cpf shifts to the right by about two orders of magnitude. In other words, when allergic lung
 15353 inflammation is excluded, the doses at which any effects are expected increase by about two orders of
 15354 magnitude.

15355



15356

15357 Lower and upper bound curves are shown for each cpf. See text for explanation.

15358 **Figure D7:** Comparison of cpfs for the estimated lowest BMD combining all the assessed
 15359 clusters including (solid curves) and excluding (dashed curves) allergic lung inflammation.

15360 A second sensitivity analysis showed very little change in the combined cpfs when the parametric
 15361 distributions fitted to each expert's judgements for Question 2 were replaced by the histograms provided
 15362 by each expert.

15363 D.6. Expert review and revision of calculation results

15364 As allergic lung inflammation had much more influence on the combined cpf than other clusters, the
 15365 assessment of this cluster was reviewed by the whole WG. There was a detailed discussion of the quality
 15366 and results of the studies considered for allergic lung inflammation and of its relevance to an adversity in
 15367 humans. In the light of this discussion, the two experts responsible for assessing this cluster both revised
 15368 their judgements for Question 1 to 50–90% probability but retained their original judgements for Question
 15369 2. When the revision for Question 1 was shared with the rest of the WG, three other experts proposed a

15370 lower bound probability of 33% rather than 50%, and it was agreed to explore the effect of this difference
15371 of opinion when revising the calculations.

15372 In the same meeting, the WG experts considered whether it was necessary to elicit judgements for the
15373 General toxicity clusters, which were excluded from the earlier assessment. It was noted that no Likely or
15374 Very Likely clusters were identified for this HOC, and the lowest effect level reported was 8 ng/kg bw per
15375 day (HED) for relative liver weight (Ke et al., 2016 [RefID 3447]; single dose study) with an effect size of
15376 about 4% which was considered non-adverse. Therefore elicitation was not needed for any of the General
15377 toxicity clusters.

15378 **D.7. Elicitation of additional judgements for the most influential cluster**

15379 In the light of the range of opinion expressed when the WG experts discussed allergic lung inflammation,
15380 judgements on Question 2 for this cluster were elicited from all the experts in the WG. Before eliciting the
15381 judgements, the key endpoints from the dose-response studies for this cluster were reviewed (Table D1)
15382 and uncertainties affecting these studies were discussed in detail. These included issues relating to litter
15383 control, missing animals for some endpoints, and lack of information about cross-contamination precautions
15384 for O'Brien et al., 2014a [RefID 5462]; a single control litter with only three mice and only males or females
15385 in two of the dose groups and differing proportions of male and female animals in control and treated
15386 groups for O'Brien et al., 2014b [RefID 5463]; no details of analysis of diet for BPA in either RefID 5462 or
15387 5463; unclear group sizes and insufficient information on blinding in Tajiki-Nishino et al., 2018 [RefID
15388 13211].

15389 **Table D1:** Summary of key endpoints considered for elicitation of judgements on Question 2 for
15390 allergic lung inflammation.

RefID	Endpoint	NOAEL (ng/kg bw per day) HED	LOAEL (ng/kg bw per day) HED	Effect size compared with the control
5462 O'Brien et al., 2014a	anti-OVA IgE in females	n.a.	0.116	39% (at the LOAEL)
5462 O'Brien et al., 2014a	anti-OVA IgE in males	0.116	116	271% (at the NOAEL)
5463 O'Brien et al., 2014b	cysteinyl leukotrienes	n.a.	0.116	1693% (at the LOAEL)
5463 O'Brien et al., 2014b	prostaglandin D2	116	116000	89% (at the NOAEL)
13211 Tajiki-Nishino et al., 2018	score of red colour	n.a.	930	140% (at the LOAEL)
13211 Tajiki-Nishino et al., 2018	eosinophil infiltration in BALF	930	3100	67% (at the NOAEL)

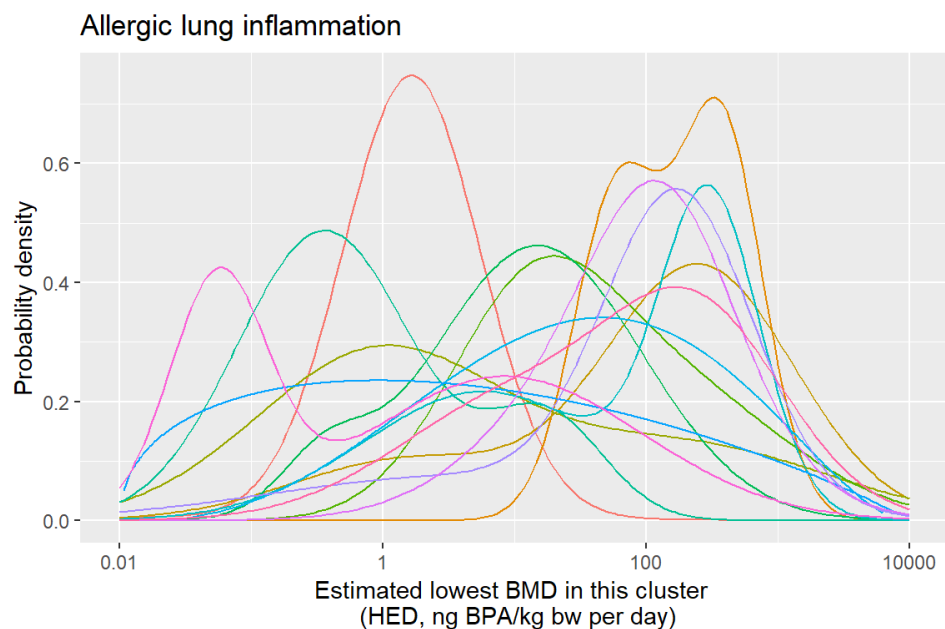
15391 LOAEL: Lowest observed adverse effect level

15392 NOAEL : No observed adverse effect level

15393 n.a: not available

15394
15395 The experts were then asked to consider individually their plausible limits for Question 2 for this cluster,
15396 and it was established that all of these lay within the range from 0.01 ng/kg bw per day HED to 3000 ng/kg
15397 bw per day HED. Distributions within this range were then elicited from 14 WG experts including the two
15398 experts that made the original assessment for this cluster, who revised their earlier judgements in the light

15399 of the discussion. The resulting judgements are presented in Table 28 in Annex K and the fitted distributions
 15400 are shown in Figure D8.



15401
 15402 The raw judgements are shown in Table 1 of Annex K.

15403 **Figure D8:** Parametric distributions fitted to the judgements of 14 WG experts for Question 2 for the
 15404 cluster allergic lung inflammation.

15405 Reasons for the wide range of opinion shown in Figure D8 were explored by discussing and listing the
 15406 evidence and reasoning for the lower and upper ends of the range covered by the experts' individual
 15407 distributions. The outcome of this is summarised in Table D2.

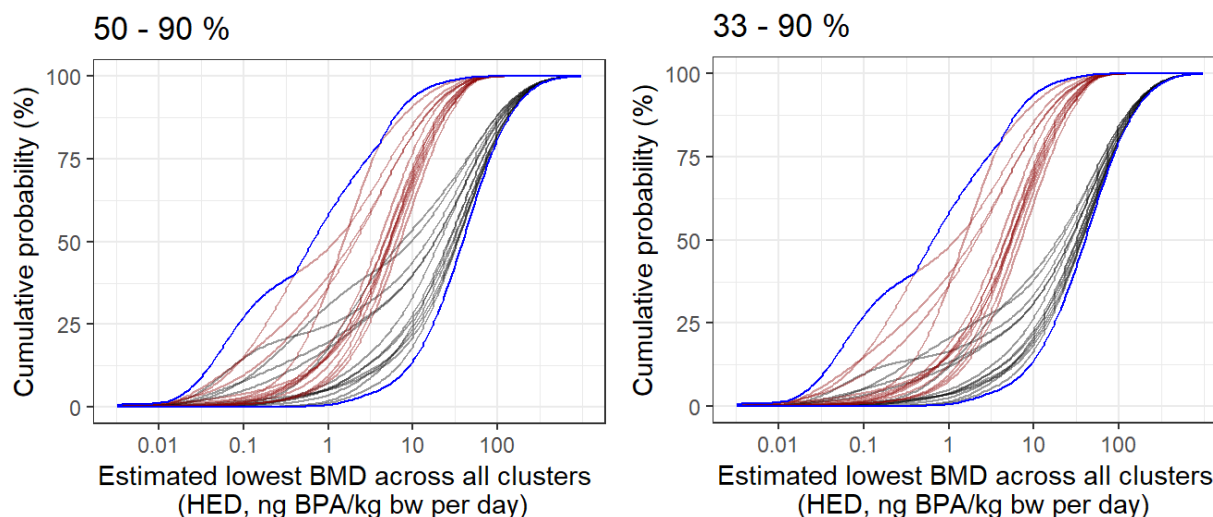
15408 **Table D2:** Key considerations identified by the WG experts as influencing the lower and upper ends
 15409 of their distributions for Question 2 for allergic lung inflammation (shown in Table D1 and Figure
 15410 D8).

Key considerations influencing the <i>lower ends</i> of the experts' distributions (lower BMDs)	Key considerations influencing the <i>upper ends</i> of the experts' distributions (higher BMDs)
<ul style="list-style-type: none"> • Anti-OVA IgE in females, anti-OVA IgE in males, cysteinyl leukotrienes seen in this dose range and are likely to be adverse • More downstream effects, e.g. eosinophil infiltration in BALF more apical and increase probability that IgE effect is adverse, although appearing at higher doses • If you are sensitised to one or multiple allergens, BPA increases the IgE levels on your mast cells, which increases the potential for adverse/apical effects to follow from subsequent exposure to the same allergens 	<ul style="list-style-type: none"> • Effect on eosinophil infiltration in BALF in better-rated studies than the effects at lower doses • Sex difference in IgE effects may raise uncertainty about those → more weight to effects seen at mid-range (but there are some reasons for expecting greater sensitivity in females) • Small effect in females significant but larger effect in males is not significant – may be due to higher variability in males

- | | |
|--|---|
| <ul style="list-style-type: none"> • Several parameters in these studies show effects, some with clear dose-response, adding to the weight for the cluster as a whole • 30% of people in EU population are sensitised so significant group at potentially increased risk | <ul style="list-style-type: none"> • Doubts about doses in studies at very low range (not measured, very difficult to achieve homogeneously), lack of explanation of different group sizes, small groups and litter effect, very large ($\times 1000$) dose intervals, only single study so reproducibility is unknown – this all leads to doubt whether the effect at the lowest dose is real or chance result of multiple testing and/or publication bias • Effects at higher doses are more clearly adverse |
|--|---|

15411

15412 The probability calculations to obtain a combined cpf for the estimated lowest BMD across all clusters was
 15413 performed separately with each expert's fitted distribution for Question 2 for allergic lung inflammation.
 15414 Figure D9 shows the resulting upper (red curves) and lower bounds (black curves) of the cpfs for each
 15415 expert, and the lower and upper bound of a combined cpf (blue curves), obtained by enveloping the
 15416 individual cpfs. Comparing the left and right-hand graphs shows the effect of the alternative lower bound
 15417 probability for Question 1 on allergic lung inflammation, moving the lower bounds of the cpfs downwards
 15418 and to the right.



15419

15420 The red and black curves show the upper and lower boundaries for the individual experts' cpfs, from which the lower and upper
 15421 bounds for combined cpf are obtained. Question 1 approximate probability for allergic lung inflammation is 50–90% in the left-hand
 15422 graph and 33–90% in the right-hand graph.

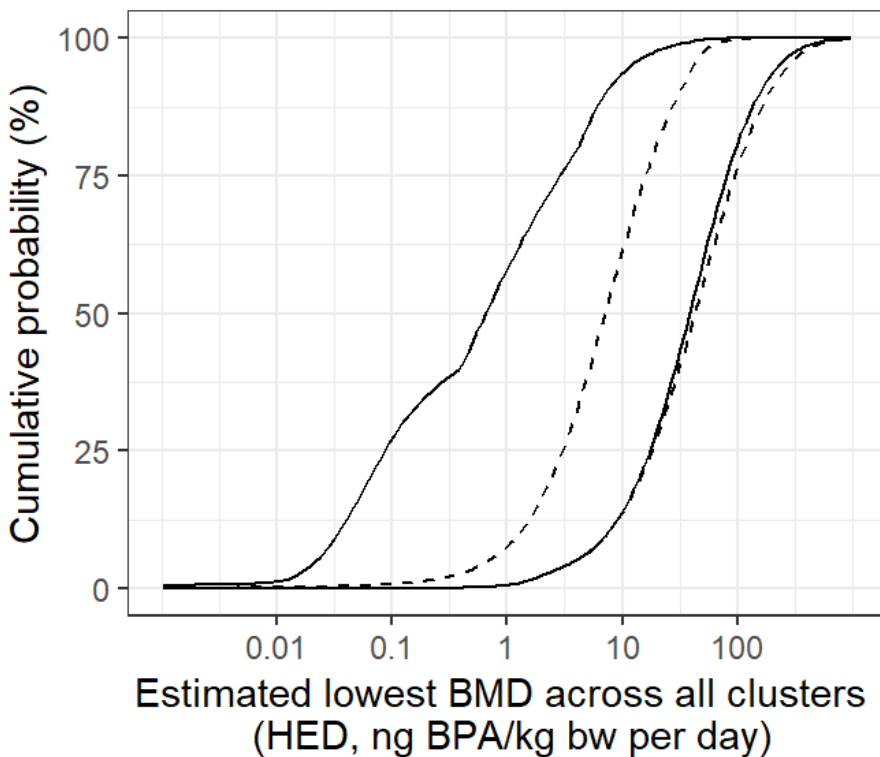
15423 **Figure D9:** The blue curves show the lower and upper bounds for the cpf for the estimated lowest
 15424 BMD across all clusters and all experts for endpoints that occur in animals and are relevant and
 15425 adverse for humans, assuming that the experts' judgements for different clusters are independent.

15426 D.8. Revised sensitivity analysis

15427 More extensive sensitivity analysis was conducted at this point, to inform the WG experts' assessment of
 15428 additional uncertainties and dependencies. Comparison of Figure D10 with Figure D7 shows that when the
 15429 original judgements for allergic lung inflammation are replaced with the distributions shown in Figure D8,

15430 allergic lung inflammation still has a very large influence on the upper bound of the combined cpf but much
 15431 less on the lower bound.

15432

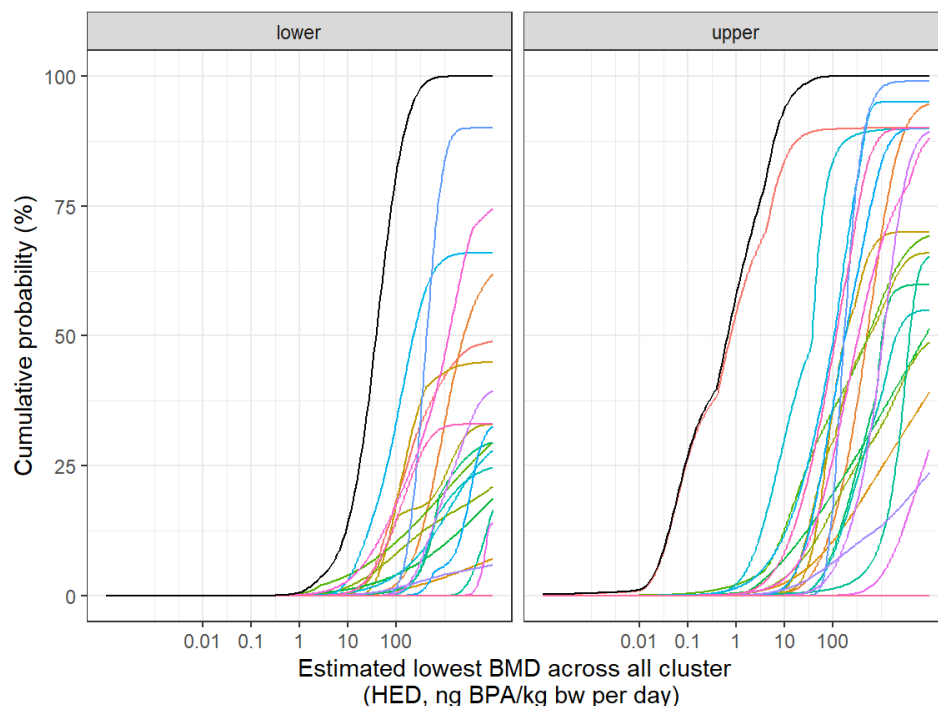


15433

15434 The solid curves are lower and upper boundaries for the combined cpf including allergic lung inflammation, based on the expert
 15435 judgements shown in Table D2, while the dashed curves exclude this cluster.

15436 **Figure D10:** Revised comparison of cpfs combining all the assessed clusters, including and excluding
 15437 allergic lung inflammation.

15438 The contributions of different clusters to the combined cpf including allergic lung inflammation are
 15439 examined further in Figures D11 and D12. In Figure D11, the upper bound of the combined cpf (black
 15440 curve in right-hand graph) is driven entirely by allergic lung inflammation (red) at the lower end, with a
 15441 small but increasing contribution from other clusters at the upper end. In contrast with this, the lower
 15442 bound for the combined cpf (black, left-hand graph) is entirely a combination of multiple clusters (various
 15443 colours) with only a minor contribution from allergic lung inflammation.

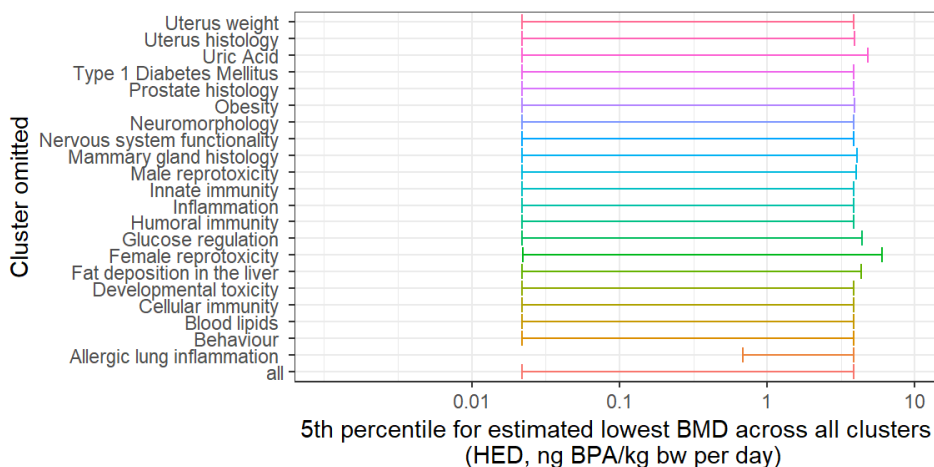


- Allergic lung inflammation
- Behaviour
- Blood lipids
- Cellular immunity
- Developmental toxicity
- Fat deposition in the liver
- Female reprotoxicity
- Glucose regulation
- Humoral immunity
- Inflammation
- Innate immunity
- Male reprotoxicity
- Mammary gland histology
- Nervous system functionality
- Neuromorphology
- Obesity
- Prostate histology
- Type 1 Diabetes Mellitus
- Uric Acid
- Uterus histology

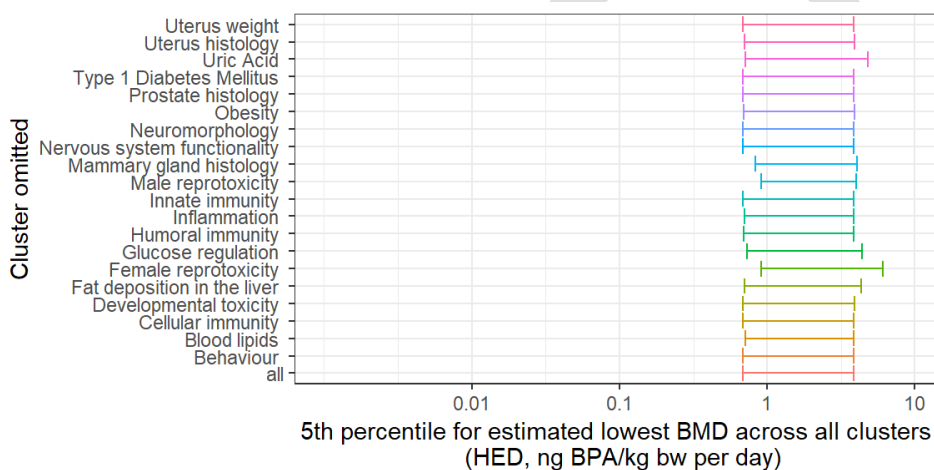
15444
 15445 The black curves in the left and right-hand graphs show respectively the lower and upper bounds of the combined cpf. The coloured
 15446 curves show the lower and upper bounds of the cpfs for the individual clusters, from which the combined cpf is calculated.
 15447 Comparing these graphs with Figure D6 shows the effect of the revised distributions for Question 2 for allergic lung inflammation,
 15448 shown in Figure D8.

15449 **Figure D11:** Graph indicating the contributions of different clusters to the combined cpf for the
 15450 estimated lowest BMD across all clusters.

15451 Figure D12 shows two sensitivity analyses in which different clusters were excluded from the calculations
 15452 in turn to assess their contribution to the fifth percentile of the lower and upper bounds of the combined
 15453 cpf. The left-hand graph shows that the lower bound for the fifth percentile increases markedly when
 15454 allergic lung inflammation is excluded, while the upper bound fifth percentile is affected similarly by all
 15455 clusters, although slightly more for the clusters uric acid, mammary gland histology, glucose regulation,
 15456 female reproductive toxicity and fat deposition in the liver. The right-hand graph shows that when both
 15457 allergic lung inflammation and a second cluster are excluded, the choice of second cluster to exclude has
 15458 much less effect, although slightly larger for the clusters uric acid, mammary gland histology, male
 15459 reproductive toxicity, glucose regulation, female reproductive toxicity and fat deposition in the liver.



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Each bar shows the lower and upper bound for the fifth percentile when a different cluster is omitted. In the upper graph a single cluster is omitted while, in the lower graph, allergic lung inflammation plus a second cluster is omitted.

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Figure D12: Impact of omitting different clusters on the fifth percentile of the combined cpf for the estimated lowest BMD across clusters.

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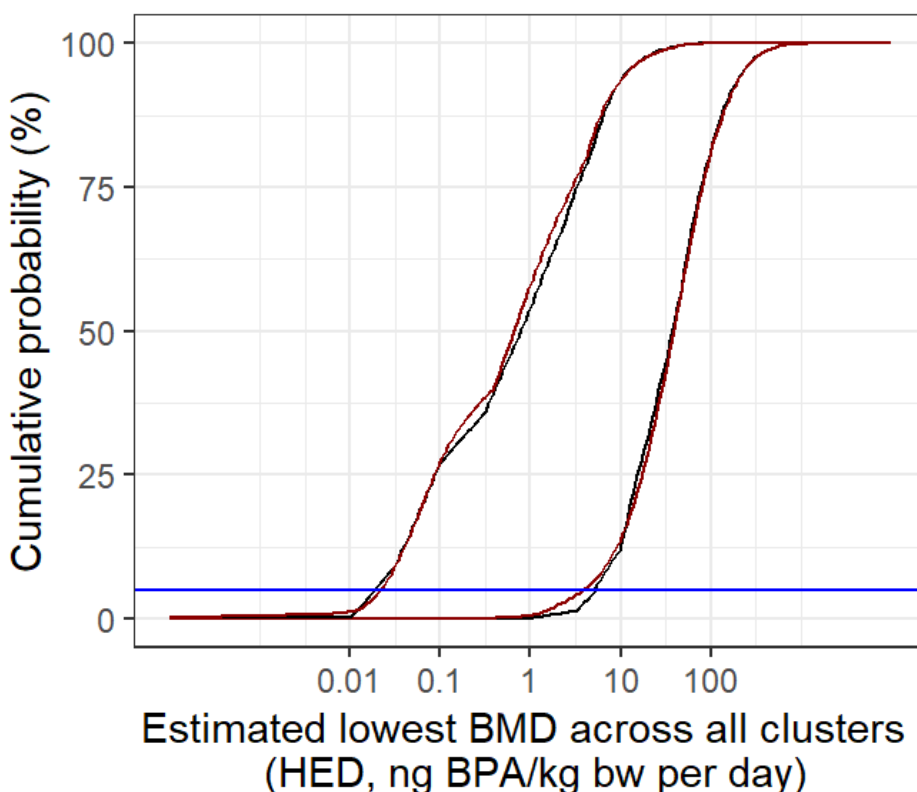
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Figure D13 shows that, with the revised judgements, there was still little change in the combined cpf when the parametric distributions fitted to each expert's judgements for Question 2 were replaced with the histograms provided by each expert. The difference is larger below the fifth percentile, but as this is below the probability that is conventionally used for the BMDL it is less likely to be important for deciding on whether an additional UF is needed.



15471

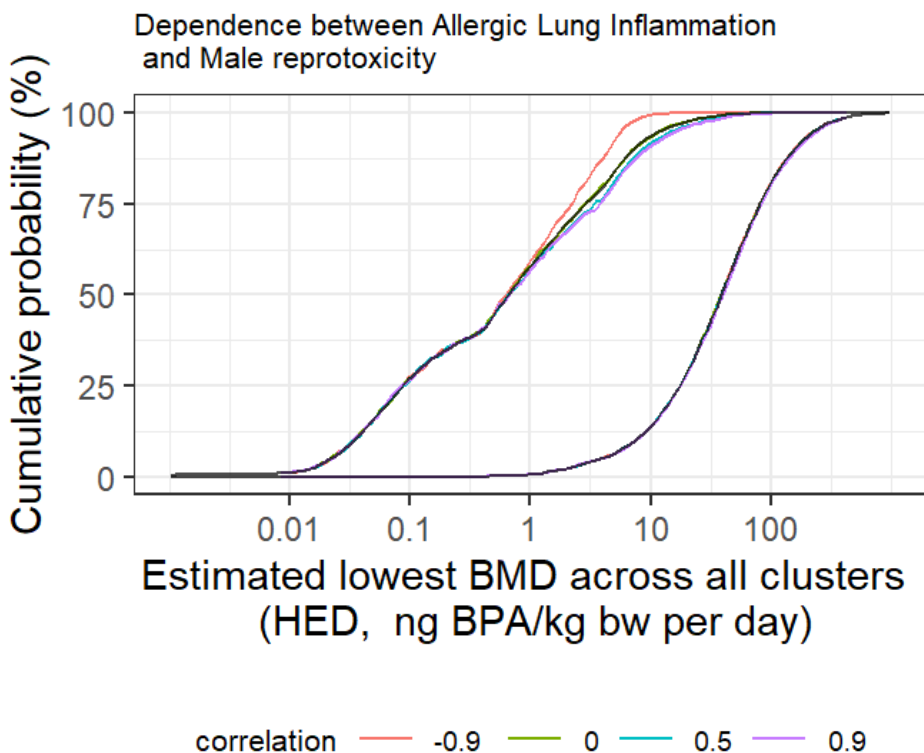
15472 The blue horizontal line marks the fifth percentile of cumulative probability.

15473 **Figure D13:** Sensitivity analysis assessing the difference between using fitted parametric distributions
15474 or the histograms provided by the experts for Question 2.

15475 The calculations performed to combine clusters assumed that the distributions for different clusters were
15476 independent of one another, so it was important to consider whether dependencies might be present and
15477 take account of this when assessing overall uncertainty. Distributions quantifying uncertainty for two
15478 different clusters would be dependent if obtaining more information (e.g. new studies) for one cluster might
15479 alter the experts' judgements and distributions for the other cluster. As such dependencies could not be
15480 ruled out for all combinations of clusters, a sensitivity analysis was performed to explore the potential
15481 impact of hypothetical dependencies between allergic lung inflammation and either male or female
15482 reproductive toxicity. These three clusters were chosen not because a biological relationship between them
15483 was assumed but because they had more influence on the assessment than other clusters and therefore
15484 more potential to show an impact of dependency. Figure D14 shows that hypothetical strong positive
15485 dependencies (correlation coefficients 0.5 and 0.9) or negative dependency (correlation coefficient -0.9)
15486 between allergic lung inflammation and male reproductive toxicity would cause only small changes in the
15487 upper third of the cpf. There was negligible change in the lower part of the cpf, which is more relevant for
15488 deciding whether an additional UF is needed. Hypothetical dependencies between allergic lung
15489 inflammation and female reproductive toxicity also had little impact. It was provisionally concluded that

15490 using parametric distributions and assuming independence between clusters are not important sources of
 15491 uncertainty.

15492



15493

15494 **Figure D14:** Sensitivity analysis assessing the impact of hypothetical dependencies between allergic
 15495 lung inflammation and male reproductive toxicity. See text for explanation.

15496 **D.9. Consideration of additional uncertainties and dependencies**

15497 As preparation for assessing overall uncertainty (EFSA Scientific Committee, 2018a), the WG experts
 15498 reviewed the results of the revised calculations and sensitivity analysis and held a structured discussion to
 15499 elicit a list of additional sources of uncertainty potentially affecting the hazard assessment. The WG experts
 15500 also identified which of these uncertainties had lower or higher potential to contribute to the overall
 15501 uncertainty of the assessment. The resulting list is presented in Table D3.

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Table D3: Sources of uncertainty considered as potentially affecting the hazard assessment (excluding the genotoxicity assessment). The two parts of the table distinguish uncertainties affecting different aspects of the hazard and uncertainty assessments.

Sections of the opinion	Factors of uncertainty	Potential contribution to overall uncertainty
Uncertainty analysis	Differences in judgement between experts because the dose range spanned several orders of magnitude (see figure X)	high
	Number of experts per cluster: The number of experts per cluster was considered as a possible source of uncertainty. The EKE for allergic lung inflammation was addressed by the whole WG (14 experts) but for the other clusters (two or three experts), this factor might have more effect. However, each cluster was assessed by WG members with special expertise on the endpoints discussed	low
	Difficulty in the EKE judgements: The EKE judgements (including translation into probabilities) were considered difficult to perform. This was taken into account for Q1 by using approximate probabilities. For Q2, the experts addressed uncertainty in their judgements by expanding the width of the distributions	low
	Calculations: Dependencies between clusters and the set of distributions considered and the rules used for selecting the best fit have much smaller impact than the expert differences	low
Methodology, inclusion criteria, choices and decisions in different parts of the assessment	Literature search: The literature search was done according to the protocol; the impact of the language (only English) was investigated (see Appendix D of Annex A) and considered minor. The journals and search strategy used were considered to provide a high coverage. The time span was done according to the terms of references	low
	Publication bias in animal studies: more likely to not publish negative results	high
	Publication bias in epidemiological studies: some negative findings on BPA may not have been published. For longitudinal studies less impact is expected	low
	Screening of title and abstract and full text: The criteria for the screening were well defined and the screening was double-checked	low
	Internal validity: Three key questions were defined in the assessment of the internal validity in animal studies; if one of them was scored negatively a study would be allocated directly to Tier 3 (low internal validity and high risk of bias (see Annex A, Section 6)) Some variation in answering to these key questions in different clusters may be possible. This variation represents a possible source of uncertainty because it would imply a variation in the tier allocation of the study, which, in turn, could have an impact on the selection of the studies reporting relevant endpoints and, therefore, to be considered in the WoE.	high
	Lack of consistent terminology in the publications: A source of uncertainty could be linked to the choice of relevant endpoints for each HOC by the experts, for which there was lack of consistent terminology of the endpoints in different published studies (e.g. for cardiotoxicity, where 'cardiomyopathy' comprises several endpoints, hampering their individual assessments, and for Carcinogenicity and mammary gland proliferative effects, for which e.g. ductal growth has not a unique definition)	low
	Grouping of endpoints into clusters: the experts' choices on how to group the relevant endpoints into clusters may have affected the outcome of the WoE (for example, neoplastic lesions in mammary gland that was considered a likely endpoint in a overall ALAN cluster). However, the impact of this is low, because the framing of the elicitation questions (assessing the estimated lowest BMD for any endpoint in each cluster) ensured that all endpoints were considered regardless of to which cluster they were assigned	low
	Statistical analysis: the data of three Grantee studies (Greenberg, 2018 [RefID 13785]; Ben-Jonathan, 2018, [RefID 13786]; Gonzalez-Cadauid, 2018 [RefID 13787]) of the Clarity FDA program were not yet published in peer-reviewed journals when this opinion was	low

	completed. The raw data were available on the FDA website and were considered by EFSA in conformity to the ToR. A statistical analysis was conducted by EFSA following the material and methods provided on the FDA website	
	Statistical analysis: in one case (Montévil et al. 2020 [RefID 13788]), the study authors used an unconventional methodology to assess dose-response and the CEP Panel made its own analysis	low
	Knowledge of the endpoints: limited knowledge on how certain intermediate endpoints are related to apical endpoints and how much is known about their MoA is a source of uncertainty for some clusters but was taken into account in Q1 in the UA	low
	Choice of BMRs (for BMD analysis and uncertainty analysis): the choice of BMRs was based on expert judgement; for some endpoints there was little specific evidence available on this, so default values were used. This aspect was taken into account in Q2 of the UA	low
	BMD analysis: If the BMDL was more than 10 times lower than the lowest non-zero dose, the study was not considered for the selection of the RP. In addition, for some endpoints a BMD analysis was not possible. In the uncertainty analysis all Tier 1 and Tier 2 studies in the WoE table were considered, taking into account the NOAELs, LOAELs and effect sizes and also BMDL and BMDU values, when available	low
	Exposure factors: data for conversion from BPA in feed/drinking water to mg/kg bw per day during gestation/lactation period were not available for few animal species (Rhesus Monkey and Marmoset monkey, see Chapter 3.1.1), therefore, some papers could not be considered in the UA	low
	HED factors: toxicokinetic data on BPA in mice were based on a study (Doerge et al., 2011) where some of the measured values were below the level of detection. The CEP Panel decided, as in 2015, to make assumptions on where the AUC level could be, based on this study and the interspecies comparison (Doerge et al., 2011) TKs data on BPA in humans were available in two experimental studies with different estimates of the BPA AUCs; the CEP Panel decided to use the median of the values from the two studies, in which BPA was administered in two different foods (soup vs. cookies)	high for mice, low for the other species
	AUC for liver: no data were available on the proportion of the pre-systemic elimination of BPA from enterocytes in the gut wall and from the liver cells, which would be needed for parameterising a PBPK model that would allow prediction of the concentration in the liver and the related AUC. In the absence of such data, the AUC in the peripheral blood was used also for the liver, to substitute the toxicokinetic part of the UF by an HEDF (see more detail in the Chapter 3.1.1.4). However, as the liver effects were not identified as critical endpoints, this uncertainty was considered low	low
	MoA studies: the MoA studies were not subjected to the same formal evaluation processes applied to the other studies so variation in their judgments may be possible in different HOCs. However, these studies were not considered for the assessment of the likelihood of the effects	low
	WoE assessment: Some endpoints were excluded from the WoE because occurring only in tier 3 studies (e.g. fat pad weights and liver fat % in HOC Metabolic effects or bronchial reactivity to methacholine in lung inflammation studies)	high
	WoE assessment: in clusters with a small number of studies (e.g. for allergic lung inflammation in Immunotoxicity, uric acid in Metabolic effects) there is a smaller opportunity to detect inconsistent results and, therefore, there is a higher chance to consider them as Likely or Not Likely. In contrast, clusters with more studies (e.g. obesity in Metabolic effects) are more prone to be considered as ALAN. This was considered as a potential source of high uncertainty but was taken into account in the uncertainty analysis for the most influential endpoint, allergic lung inflammation	low

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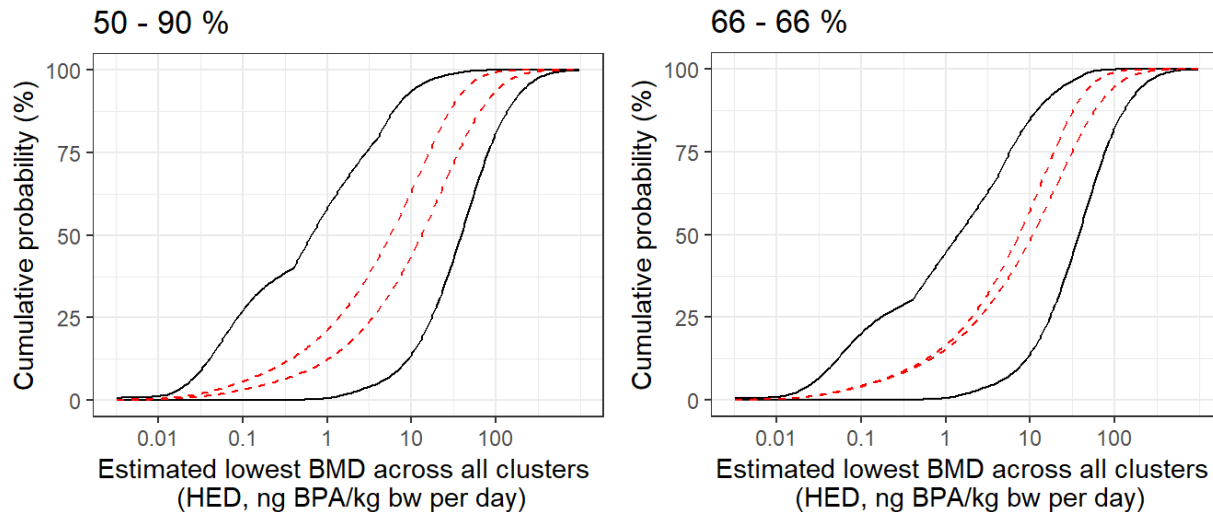
15506 Reconsideration of previous publications already assessed by EFSA, and the additional literature produced
15507 between the ending date of the literature search (15 October 2018) and the publication date of this opinion,
15508 were not considered additional sources of uncertainty because they are outside the scope of this opinion
15509 as defined by the Terms of Reference.

15510 Reviewing the uncertainties listed in Table D3 and the results of the sensitivity analyses conducted earlier,
15511 the WG experts judged that they would not alter the assessment of overall uncertainty provided already by
15512 the wide range of distributions (shown in Figure D9), resulting from the judgements of different experts
15513 for the most influential cluster. The WG experts also noted that they had already considered many of the
15514 listed uncertainties when making their personal judgements earlier in the uncertainty analysis, and they
15515 judged that the additional uncertainties would not have altered those judgements. The WG experts
15516 therefore agreed to use the range of distributions in Figure D9 as the basis for assessing overall uncertainty
15517 about the estimated lowest BMD across all clusters, and to explore options for integrating or averaging
15518 individual judgements to assist in developing consensus conclusions on the overall uncertainty.

15519 **D.10. Averaging cpfs of different experts for the same cluster**

15520 The dashed red curves in the left-hand graph in Figure D15 show the results of revised calculations in
15521 which the cpfs of different experts for each cluster were averaged by taking the linear pool. Comparing
15522 them with the previous cpfs (black solid curves), for which the experts were aggregated by enveloping, it
15523 is evident that averaging over experts greatly reduces the distance between the lower and upper bounds.
15524 This confirms that differences between experts (especially for allergic lung inflammation) are the largest
15525 contributor to the quantified uncertainty. The remaining distance between the upper and lower bounds of
15526 the averaged cpfs reflects the combined effect of the differences between lower and upper bounds of the
15527 approximate probabilities for Question 1 for all the clusters and experts.

15528 A sensitivity analysis (not shown) demonstrated that the remaining gap between the lower and upper
15529 bounds of the cpfs would be significantly reduced if the WG experts were to agree on a single consensus
15530 probability for Question 1 for allergic lung inflammation, replacing the range by a single probability value.
15531 The reasoning of the two experts with specialist knowledge of this cluster for their judgements on Question
15532 1 was displayed. After a short discussion, the WG experts agreed on a consensus of 66% probability. The
15533 effect of this on the gap between the lower and upper bounds of the cpf for the estimated lowest BMD
15534 across all clusters is shown in the right-hand graph in Figure 25.



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Figure D15: Effect on the lower and upper bounds of the cpf for the estimated lowest BMD across all clusters when the cpfs of different experts for each cluster were aggregated by averaging (dashed red curves) instead of being enveloped (solid black curves) and when the approximate probability of 50–90% for Question 1 for allergic lung inflammation (left-hand graph) was replaced with the WG consensus of 66% (right-hand graph).

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The WG experts agreed to take the results of averaging cpfs across experts in each cluster (red dashed curves in right-hand graph of Figure 25) as their consensus assessment of overall uncertainty (including the uncertainties listed in Table 30) for the estimated lowest BMD across all clusters including General toxicity clusters (for the reasons given earlier) and to use this as the basis for considering whether an additional UF is needed when deriving the TDI. They agreed to present also the lower and upper bounds of the combined cpf obtained by enveloping the individual distributions (black solid curves in right-hand graph of Figure D15), to make clear the range of opinion between experts.

15548 **D.11. Sensitivity analysis for consensus distribution**

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The sensitivity analyses were repeated using the consensus probability of 66% for Question 1 on allergic lung inflammation and the averaged individual cpfs for each cluster. The purpose of this was to check which clusters had most influence on the final consensus cpf for the estimated lowest BMD across all clusters and to check whether the consensus cpf was more sensitive to the method of distribution fitting and hypothetical dependencies between clusters than was the case in earlier sensitivity analysis. The revised sensitivity analyses focused on the 15th percentile of the consensus cpf, as this was close to the RP of 0.93 ng BPA/kg bw per day.

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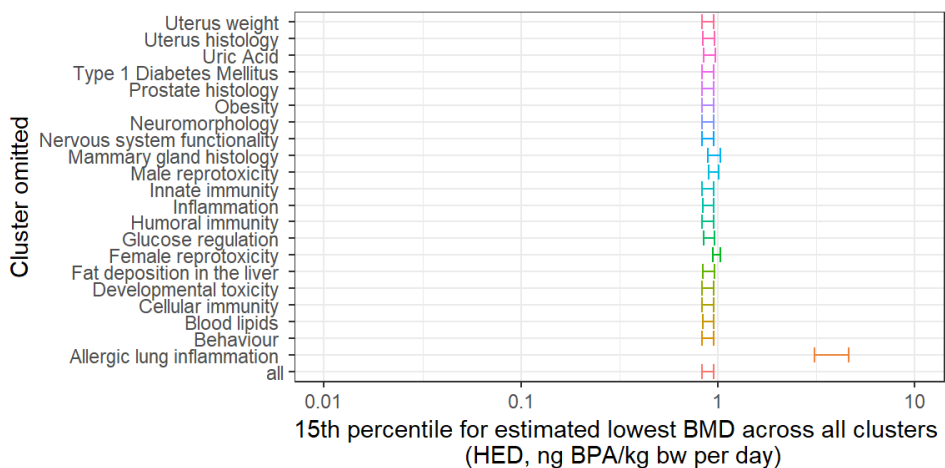
Figure D16 shows that, as in the earlier sensitivity analysis, the consensus cpf is determined mainly by allergic lung inflammation. When both allergic lung inflammation and a second cluster are excluded, the choice of second cluster to exclude has much less effect, although the clusters mammary gland histology, male reproductive toxicity and female reproductive toxicity have more influence than other clusters.

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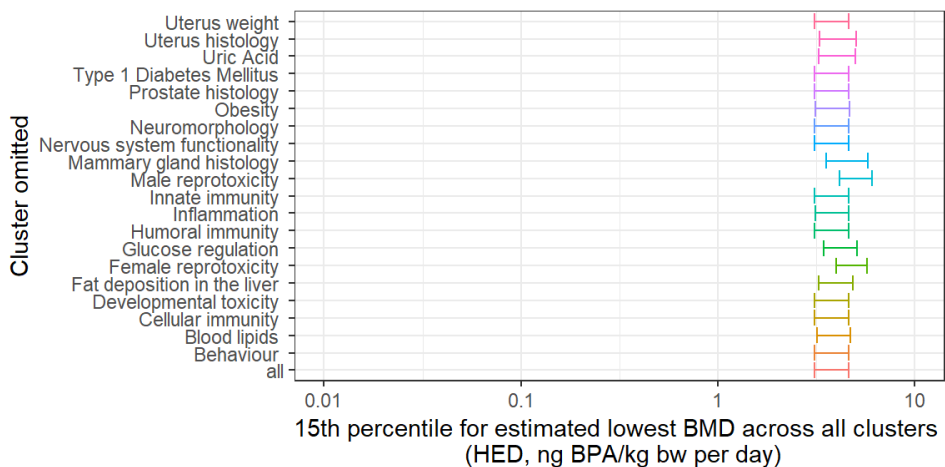
Figure D17 shows that using the histograms provided by the experts for Question 2 shifts the lower and upper bounds of the consensus cpf slightly to the right when compared with those obtained with fitted

15562 parametric distributions, including in the region of the 15th percentile. As the histograms more directly
 15563 reflect the experts' judgements, it was agreed to take account of both cpfs in Figure D17 when considering
 15564 whether an additional UF is needed.

15565 The need to consider potential dependencies and the method used for this are explained in the text
 15566 preceding Figure D14. Figure D18 shows that hypothetically large positive and negative dependencies
 15567 between allergic lung inflammation and male reproductive toxicity have little impact on the lower part of
 15568 the consensus cpf, including at the 15th percentile. The same result was obtained for hypothetical
 15569 dependencies between allergic lung inflammation and female reproductive toxicity, and between male and
 15570 female reproductive toxicity. As other clusters have less influence on the consensus cpf, any dependency
 15571 between them would also be negligible. The CEP Panel concluded that, if there were dependencies between
 15572 clusters, they would have negligible impact on the consensus cpf.



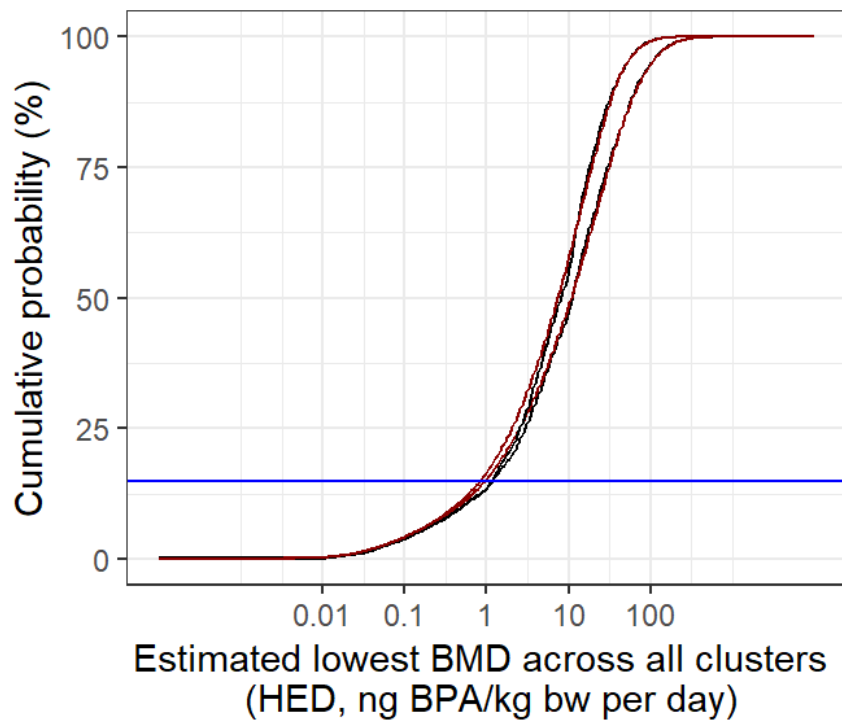
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15575 Each bar shows the lower and upper bound for the 15th percentile when a different cluster is omitted. In the upper graph a single
 15576 cluster is omitted while, in the lower graph, allergic lung inflammation plus a second cluster is omitted.

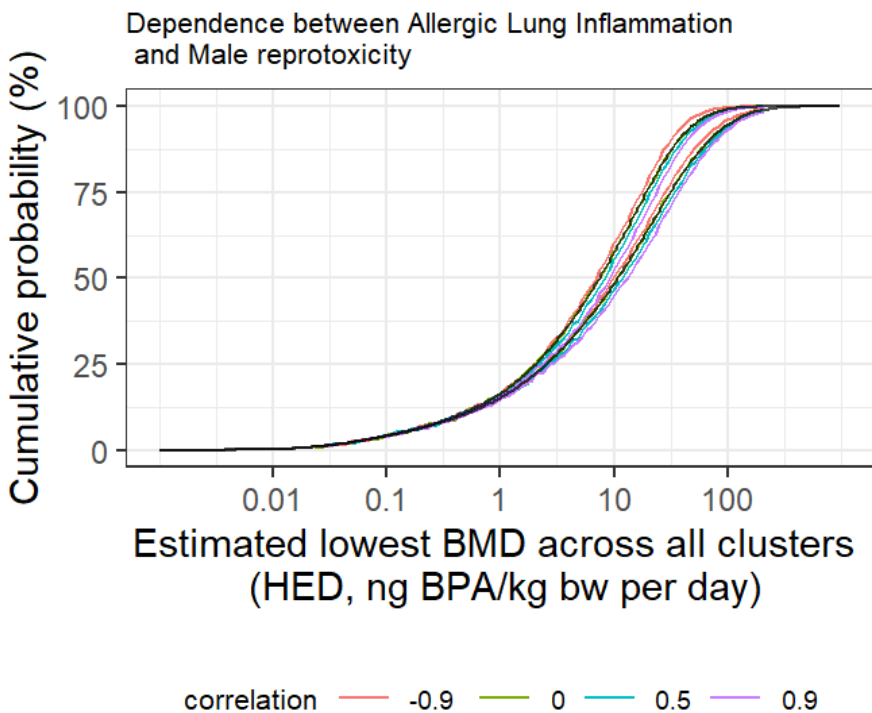
15577 **Figure D16:** Impact of omitting different clusters on the 15th percentile of the consensus cpf for the
 15578 estimated lowest BMD across clusters.



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The blue horizontal line marks the 15th percentile of cumulative probability.

Figure D17: Sensitivity analysis assessing the difference between using fitted parametric distributions or the histograms provided by the experts for Question 2.



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See text above Figure D14 for explanation of hypothetical dependencies.

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Figure D18: Sensitivity analysis assessing the impact of hypothetical dependencies between allergic lung inflammation and male reproductive toxicity.

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D.12. Additional uncertainty factor for deriving the TDI

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As the final step in the uncertainty analysis, the WG experts considered whether an additional UF is needed when deriving the TDI. To inform this discussion, tabulated percentiles of the cpf for the estimated lowest BMD across all clusters were displayed, based on the lower and upper bounds of the consensus cpf that resulted from the decisions made by the WG experts in the preceding steps (red dashed curves in right-hand graph of Figure D15). The ratios of the RP (BMDL) to each percentile of the lower and upper bounds of the consensus cpf were also displayed. These results are shown in Table D4.

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The WG experts noted from Table D3 that the RP of 0.93 ng BPA/kg bw per day is close to the 15th percentile of both the lower and upper bounds of the consensus cpf. This implies about 15% probability that the estimated lowest BMD for all clusters is lower than the RP and, therefore, 85% probability that the estimated lowest BMD for all clusters is above the RP.

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Accordingly, the ratio of the RP to the 15th percentile of both bounds of the cpf is close to 1. This implies about 85% probability that an additional UF of 1 (i.e. no additional UF) would be sufficient to cover all 21 of the clusters assessed in the HOCs for Immunotoxicity, Metabolic effects, Neurotoxicity and developmental neurotoxicity, Reproductive and developmental toxicity, and Carcinogenicity and mammary gland proliferative effects. The WG had concluded earlier (see above) that assessment of these clusters is

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15606 also protective for clusters in the HOC General toxicity, so they also do not require an additional UF. When
 15607 the calculations were repeated with the histograms provided by the experts rather than fitted parametric
 15608 distributions (as shown in Figure D17), the probability that the estimated lowest BMD for all clusters is
 15609 below the RP increased slightly, to 87%. The WG also noted that the large range of endpoints tested for
 15610 BPA makes the risk assessment for BPA more conservative than for most other chemicals, for which only
 15611 the standard endpoints are tested. Taking all these considerations together, the WG concluded that no
 15612 additional UF is needed.

15613 **Table D4:** Percentiles of the lower and upper bounds of the consensus cpf for the estimated lowest BMD
 15614 across all clusters (columns 2 and 3) and ratios of the RP of 0.93 ng BPA/kg bw per day to each
 15615 percentile (columns 4 and 5). The row shown in bold is the basis for the CEP Panel conclusion that an
 15616 additional UF is not needed. See text for details.

Percentile of consensus cpf	Lower bound for percentile (ng BPA/kg bw per day)	Upper bound for percentile (ng BPA/kg bw per day)	Ratio of reference point to lower bound	Ratio of reference point to upper bound
1%	0.028	0.028	33.8	32.7
2.5%	0.059	0.061	15.7	15.2
5%	0.135	0.141	6.9	6.6
10%	0.412	0.447	2.3	2.1
15%	0.881	1.002	1.1	0.9
20%	1.485	1.754	0.6	0.5
25%	2.182	2.673	0.4	0.3
30%	2.989	3.800	0.3	0.2
35%	3.931	5.171	0.2	0.2
40%	5.028	6.797	0.2	0.1
45%	6.293	8.705	0.1	0.1
50%	7.742	10.955	0.1	0.1
55%	9.406	13.591	0.1	0.1
60%	11.323	16.671	0.1	0.1
65%	13.501	20.438	0.1	0.0
70%	15.991	25.137	0.1	0.0
75%	19.054	31.067	0.0	0.0
80%	23.057	38.870	0.0	0.0
85%	28.472	49.854	0.0	0.0
90%	36.534	67.143	0.0	0.0
95%	51.747	101.729	0.0	0.0

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Appendix E – Summaries of genotoxicity studies

This Appendix reports summaries of genotoxicity studies on BPA identified in the literature (2013 - 2021) and studies considered in the Scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs (EFSA CEF Panel, 2015).

The evaluation of the studies on the main genetic endpoint (gene mutations, structural and numerical chromosomal alterations) is reported in tabular format in Annex L.

E.1. *In vitro* studies

E.1.1. Gene mutation

Masuda et al., 2005

The study evaluated the mutagenicity of BPA in Ames test in the presence or absence of S9-mix. BPA (Tokyo Kasei Kogyo Co., Ltd) was tested on *S. Typhimurium* strains TA98 and TA100 at the single dose of 0.1 µmole/plate (100 µL of 1 mM solution). No mutagenic effect was observed.

Tiwari et al., 2012

The study evaluated the mutagenicity of BPA in Ames test. BPA (purity 99%) was tested at concentrations from 6.25 to 200 µg/plate on different strains of *S. Typhimurium* (TA 98, TA 100 and TA 102). The mutagenic response was not observed in any of the tester strains at the various concentration of BPA in absence of S9 fractions. A slight increase in the numbers of revertants was observed in the presence of S9 fractions from the 6.25 - 25 µg/plate of BPA in each strain, but the increase was statistically significant only in strain TA 102 at 25 µg/plate.

Fic et al., 2013

In this study the mutagenic and genotoxic potential of eight BPA (purity >99%) structural analogues [BPF, BPAF, bisphenol Z (BPZ), BPS, bis(4-hydroxy-3-methylphenyl)propane (DMBPA), 4,4'-sulfonylbis(2-methylphenol) (DMBPS), [sulphonylbis(benzene-4,1-diyloxy)]diethanol (BP-1), and 4,4'-sulphanediylidiphenol (BP-2)] were investigated using the Ames and comet assay. None of these bisphenols were mutagenic in *Salmonella* Typhimurium strains TA98 and TA100 either in the presence or absence of external S9-mediated metabolic activation (Aroclor 1254-induced male rat liver). Potential genotoxicity of bisphenols was determined in the HepG2 human hepatoma cell line following 4-h and 24-h exposure to non-cytotoxic concentrations 0.1 µmol/L to 10 µmol/L. In the comet assay, BPA and its analogue BPS induced significant DNA damage only after the 24-h exposure, while analogues DMBPS, BP-1, and BP-2 induced a transient increase in DNA strand breaks observed only after the 4-h exposure. BPF, BPAF, BPZ, and DMBPA did not induce DNA damage.

Xin et al., 2015 [RefID 8150]

The study evaluated the cytotoxic, genotoxic and clastogenic activity of BPA (purity 99%)²² in CHO cells and its mutagenicity in the Ames test. The battery of assays applied in CHO cells included the MTT assay for the evaluation of cytotoxicity, and the comet, micronucleus and chromosome aberration tests. In the Ames test BPA (10-5000 µg/plate) was uniformly negative in all *Salmonella* Typhimurium strains (TA1535, TA97, TA98, TA100 and TA102), with and without metabolic activation. Exposure of CHO cells to four BPA doses (40, 80, 100 and 120 µM) for 12 and 24 h resulted in a significant decrease in cell viability (at 80 µM and above), which however remained above 50% in all cases; a concentration-related increase of DNA damage was observed in comet assay [increased Olive tail moment (OTM), tail length and % tail DNA, statistically significant at all doses] after 12 and 24 h exposure to BPA; after 24 h treatment, an increase in micronuclei (MN) (statistically significant at 100 and 120 µM) and structural chromosomal aberrations (chromatid breaks and chromosome fragments, statistically significant at 80 µM and above) was also observed.

Zemheri and Uguz, 2016 [RefID 9535]

²² Information on BPA purity provided by the study authors on 11 October 2021, upon EFSA request

The study evaluated the mutagenicity of BPA (Merck) in a limited Ames test, using two tester strains (TA98 and TA100) and four dose levels (0.1, 1, 10 and 100 µg/plate). The results were negative, with and without metabolic activation.

Balabanič et al., 2021 [RefID 224-G]

The study evaluated cytotoxic and genotoxic effects of some endocrine disrupting chemicals (EDCs), including BPA, which have been previously identified in effluents from two paper mills. BPA (Sigma-Aldrich) tested at concentrations of 1, 10, 100, 1000 µg/L with the bacterial SOS/umuC assay in *S. Typhimurium* TA1535/pSK1002 strain did not induce toxic nor genotoxic effects in the presence or absence of S9 metabolic activation. The compound was also assessed in HepG2 cells with MTT assay for cell viability and with comet assay at 1, 10, 100 and 1000 µg/L for 4 and 24 h. No significant reduction of the viability. A statistically significant concentration-dependent increase of DNA damage, expressed as percent of DNA in tail, was reported starting from 10 µg/L.

Hu X et al., 2021 [RefID 295-G]

This study shows that BPA (purity ≥ 99%) and styrene-7,8-oxide (SO) causes DNA damage and mutations in HEK293T human embryonic immortalized cells. A concentration-dependent increase in the formation of γH2AX foci was induced by a 24 h exposure to BPA (0.1, 1, 100 µM). Multiple clonally amplified cell populations, derived from HEK 293T cells treated with 100 µM BPA for 24 h, were subjected to whole genome sequencing (WGS). To identify mutations that were associated with BPA exposure, genomic changes occurring in BPA-treated cell populations were compared with those occurring in the parental cell line. The frequency of acquired single base substitutions (SBS), double base substitutions (DBS), and small insertions and deletions (InDel) were all increased in BPA-treated cell populations in comparison with untreated cell populations. Analyses of genome-wide point mutations and genomic rearrangements associated with BPA exposure indicate that a subset of SBS- and DBS substitutions occur near or at guanines. The authors conclude that these locations are consistent with BPA preference to form guanosine adducts. The occurrence of other mutational signatures suggests additional mutagenic events occurring at A:T base pairs (TA>CG transitions and TA>GC transversions). Analysis of data from 19 cancer cohorts suggest that tumours of digestive and urinary organs show a relatively high similarity in mutational profiles, with the burden of mutations increasing with age. The authors conclude that BPA (and SO) are relatively mild mutagens and other environmental agents might also potentially generate similar, complex mutational patterns in cancer genomes.

E.1.2. Chromosomal damage

Johnson and Parry, 2008

In this mechanistic study the aneugenicity of two known spindle poisons model compounds, namely rotenone and BPA, has been investigated following low dose-exposure to mammalian cells, using the cytokinesis blocked micronucleus assay (CBMA) and immunofluorescence methods to visualize modifications of the microtubule organizing centres (MTOCs) of the mitotic spindles. For induction of MN BPA (Sigma-Aldrich) was added over a range of narrowed low concentrations (1.5, 3.1, 6.2, 7.7, 9.2, 10.8, 12.3, 18.5, 24.6, and 37.0 µg/ml) to cultures of human (AHH-1) lymphoblastoid cell line for a complete cell cycle (22-26 h dependent upon any cell cycle delay) in the presence of cytochalasin-B. A minimum of five separate experiments were performed. A concentration-related and statistically significant increase of binucleate-micronucleated cells from 12.3 µg/mL was reported with a clear threshold for induction of MN (NOEL at 10.80 µg/mL and LOEL at 12.3 µg/mL). A NOEL and LOEL for percentage of binucleate cells was also observed at 9.2 µg/mL and 10.8 µg/mL BPA respectively. For mechanistic evaluation of the aneugenic effects of BPA, fluorescently labelled antibodies were used to visualize microtubules (α-tubulin) and MTOCs (γ-tubulin) in V79 culture. BPA in this case was added to V79 cells growing on sterile glass microscope slides placed in Petri dishes at concentrations 4.2, 4.9, 5.6, 7.0, 8.4, 9.8, 11.2 and 14 µg/mL for 20 h (i.e. one cell cycle for V79). Similarly for induction of aberrations in the mitotic machinery a NOEL was observed at 7.0 µg/mL and a LOEL at 8.4 µg/mL BPA in V79 cells. Aberrant mitotic divisions, in the form of multiple spindle poles were detected and it was suggested by the study authors to be the mechanism for the production of chromosome loss into MN.

Tayama et al., 2008

In this study, the authors evaluated the genotoxicity of some environmental estrogen-like compounds including BPA using sister chromatid exchanges (SCEs), CA and DNA strand breaks (comet) assays in CHO-K1 cell line *in vitro*. For CA and SCEs six concentrations of BPA (purity > 99%) ranging from 0.1 to 0.6 mM were added to CHO-K1 cells for 3 h. Following treatments cells were further incubated for 27 h in the presence of BrdU until preparation of slides for both SCE and CA from the same culture. For comet assay seven concentrations of BPA ranging from 0.1 to 0.7 mM were added to CHO-K1 for 1 h and following washes of test compound cells were processed for comet assay using a silver-staining method. The method applied for comet analysis was not clearly reported. Statistically significant increase for both SCE and chromosome aberrations were reported at the highest concentrations tested (0.4 and 0.5 mM and 0.5, 0.55 and 0.6 mM respectively). Significant increases of c-mitotic effects were detected at highest concentrations 0.3-0.6 mM. The positive results were observed only in presence of severe cytotoxicity. For comet assay significant increases of DNA breakage were only reported at the highest concentration (0.7 mM) for which the cytotoxicity was not adequately evaluated.

Ribeiro-Varandas et al., 2013 [RefID 6189]

The study investigated micronucleus formation in human umbilical vascular endothelial cells (HUVEC) and human colon adenocarcinoma (HT29) cell lines cultured in the presence of BPA (44 nM and 4.4 µM, i.e. 10 ng/mL and 1 µg/mL) for 24, 48 or 72 h. Effects on DNA stability and DNA damage repair were concurrently evaluated by means of the TUNEL assay and by the analysis of histone-H3 acetylation on lysine 56 (H3K56ac). At the concentrations applied, BPA did not affect cell viability and proliferation of both cell lines. The TUNEL assay did not detect DSBs or apoptotic cells associated with exposure to BPA. Micronucleus frequency was significantly increased in HUVEC cells (1.1% vs 1.6% and 2.2 % in control, low and high BPA dose, respectively), but not affected in HT29 cells (4.45% vs 4.6% and 4.4%). The transcriptional analysis of expression of genes encoding for proteins involved in chromosome segregation (CDCA8, SGOL2, Aurora A, α-tubulin and γ-tubulin), performed by quantitative real-time PCR, showed small (less than two-fold) increases in the expression of CDCA8 (both cell lines) and SGOL2 (HUVEC cells only). The immunofluorescence analysis of cytoskeleton organization of HUVEC cells with anti-α-tubulin and anti-γ-tubulin showed several aberrations, such as multipolar spindles and microtubule misalignment, associated with BPA exposure.

Šutiaková et al., 2014 [RefID 7026]

The study evaluated the genotoxic and cytotoxic effects of BPA (Sigma-Aldrich) on bovine peripheral lymphocytes *in vitro*. Lymphocyte cultures from two animals were exposed to four different concentrations of BPA (1×10^{-4} , 1×10^{-5} , 1×10^{-6} and 1×10^{-7} mol. L⁻¹) 24 h after stimulation by L-phytohemagglutinin, and incubated for total 72 h. Micronucleus frequency was determined using the cytokinesis block method, adding 6 µg/mL cytochalasin B at 44 h. A significant increase in the number of MN ($p = 0.018$) was observed at the highest concentration of BPA; at lower concentrations micronucleus frequency was not significantly different from vehicle (DMSO) control. The nuclear division index (NDI) was not affected by BPA treatment at any concentration level.

Aghajanjpour-Mir et al., 2016 [RefID 57]

The study evaluated the clastogenic activity of BPA in the MCF-7 human breast cancer cell line and male and female human amniocytes. Cells were exposed 48 h to 0 (control), 0.4, 1, 4, 40 and 100 µg BPA/mL in the absence of exogenous metabolic activation. Giemsa staining was applied for the identification of structural chromosome aberrations in treated cells (200 per dose). Cytotoxicity was evaluated using the MTT assay. In a preliminary evaluation of cytotoxicity, the IC₅₀ of BPA was 100, 40 and 4 µg/mL in MCF-7 and ER-negative (male) and ER-positive (female) amniocytes, respectively. Following treatment with BPA a significant increase of cells with chromosome aberrations was observed at 1 µg/mL (and above) with all cell types, with no clear association with ER expression. The aberrations recorded were almost exclusively fragments in amniocytes (both female and male), and fragments and chromatid breaks and gaps in MCF-7 cells. The increase in cells with aberrations was not clearly concentration related and decreased at the highest doses, possible due to cytotoxicity that was not concurrently evaluated. A few numerical aberrations (both hypoploidy and hyperploidy) were also

recorded in amniocytes metaphases; in female amniocytes the decrease of cells with normal chromosome number reached statistical significance at the highest not toxic concentration (4 µg/mL).

Huang FM et al., 2018 [RefID 296-G]

The study reported positive results for induction of DNA strand breaks (evaluated by comet assay) and MN frequency in murine macrophage RAW264.7 cells. Cell cultures were treated at 0, 3, 10, 30, and 50 µM of BPA (Sigma-Aldrich) dissolved in DMSO for 24 h. Concentration-dependent increase of tail length, based on the analysis of 50 cells/slide, and of MN frequency by the evaluation of 1000 binucleated cells per concentration were observed. No positive controls were used. The genotoxic effects were observed starting from 10 µM and were associated with an increase of reactive oxygen species (ROS), measured by Dichlorofluorescein Diacetate Assay (DCFH-DA) and a decrease of antioxidant enzymes, including GPx, SOD and CAT. Concomitant phosphorylation of P53 and release of cyto C from mitochondria into cytosol were reported. A reduced expression of antiapoptotic proteins BCL2 and BCL-XL significant from 10 and 3 µM respectively and an increase of the expression of proapoptotic proteins BAX, BID, and BAD beginning at 10, 10 and 30 µM respectively were observed in a concentration-dependent manner. Increased level of the apoptosis-inducing factor (AIF) in the nucleus and a decrease in the mitochondria was detected. Expression of pro-caspase-3 and pro-caspase-9 is reduced by BPA in a concentration-dependent manner and PARP-1 cleavage was induced by BPA. Pre-treatment of the cell cultures with N-acetylcysteine (NAC), a cysteine precursor of the antioxidant glutathione, at the concentration of 10 µM for 30 min reduced BPA-induced cytotoxicity, apoptosis, and genotoxicity. The results of this study indicates that the toxic effects induced by BPA in macrophages was mainly through oxidative stress-associated mitochondrial apoptotic pathway.

Sonavane et al., 2018 [RefID 419-G]

The study investigated BPA's ability to confer resistance to the chemotherapeutic agent camptothecin (CPT). The study reports the results of an experimental study on the high-dose BPA (Sigma-Aldrich) co-exposure effects with the chemotherapeutic agent CPT in mouse embryonic fibroblasts (MEFs). A 24 h and a 48 h treatment of MEFs with a concentration of BPA (150 µM) did not result in an increase of DNA strand breaks detected by the comet assay and by the analysis of phosphorylated H2AX (γH2AX) and of chromosomal aberrations. The BPA treated cells showed a reduction in the nuclear volume associated with a DNA compaction detected by 3-D imaging using the nucleic acid fluorescent stain. This effect reduces the accessibility of DNA to topoisomerase-I (Top1). The mechanism of action of CPT is the formation of Top1 covalent complexes inducing DNA-protein cross-links, that result in single-strand and double-strand DNA breaks and chromosomal aberrations in treated cells. A co-exposure of MEFs with CPT and BPA for 24 and 48 h showed a significative reduction of Top1-DNA adducts decreasing the DNA strand breaks and chromosomal damage induced by CPT alone.

Santovito et al., 2018 [RefID 11220]

In this study the possible induction of chromosomal damage by BPA (Sigma-Aldrich) was tested in human peripheral blood lymphocytes cultures applying the CA assay and the micronucleus test (MN). Cell cultures were exposed to a range of concentrations from 0.01 to 0.20 µg/mL, (including the reference dose established by United States Environmental Protection Agency (US EPA) (0.05 µg/mL), the tolerable daily intake established by European Union (0.01 µg/mL) and the highest concentration of unconjugated BPA found in human serum (0.02 µg/mL)) for 24 h for the chromosomal aberration test and for 48 for the micronucleus test. A statistically significant increase of cells with structural chromosomal aberrations, with a prevalence of chromatid breaks, was reported starting from 0.05 µg/mL; no numerical aberration was observed. A concentration related increase in MN frequency was detected starting from 0.02 µg/mL in which a four-fold increase with respect to the control level was observed.

Ramos et al., 2019 [RefID 396-G]

The study evaluated the cytotoxic and genotoxic effects of BPA in human laryngeal carcinoma cells (Hep-2) and human lung fibroblasts (MRC-5). BPA (Sigma) was freshly diluted in ethanol and added to the culture media at the concentrations of 0.44 nM, 4.4 nM and 4.4 µM (0.1 ng/mL, 1 ng/mL, 1µg/mL) for 48 h. Cell viability after treatment was evaluated using the resazurin assay. DNA damage and oxidative DNA damage were assessed in cryopreserved cell samples by comet assay (100 cells per

slide), with and without the formamidopyrimidine-DNA glycosylase (Fpg). Mitotic abnormalities and MN were evaluated in DAPI stained cells (1000 cells per concentration). Treatments had no effect on cell viability in either cell lines. In comet assays with Hep-2 cells, DNA damage, measured as % DNA in the tail, was significantly increased at the lowest concentration (0.44 nM) and decreased at 4.4 nM and 4.4 μM; oxidative DNA damage (in presence of Fpg) was significantly decreased at all concentrations. In MRC-5 cells, there was no effect on DNA damage and an increase in oxidative DNA damage at the two highest concentrations. The microscopic analysis of DAPI stained cells showed a significant increased mitotic index (percent of cells undergoing mitotic division) in MRC-5 cells at all BPA concentrations and in Hep-2 cells at the highest concentration. The percentage of MN were slightly (up to 2.5-fold) but significantly increased in both cell lines at the two highest concentrations.

Di Pietro et al., 2020 [RefID 258-G]

The study investigated the effects of BPA exposure on cell proliferation, cell cycle progression and DNA damage in human peripheral blood mononuclear cells (PBMC) and the BPA-induced neurotoxicity in rats exposed to environmental relevant doses of BPA during development. Human PBMC from five unrelated healthy donors (adult males and females) were cultured and treated with BPA (Merck) from 5 nM to 200 μM. The treatment with BPA of unstimulated resting PBMC did not affect cell proliferation (determined by the colorimetric MTT) at all the concentrations tested except for 200 μM for which a marked inhibition of cell proliferation was observed at 24 and 48 h after the treatment. By contrast, in PHA-stimulated cells, BPA caused a pronounced increase of cell growth starting from 10 nM to 100 nM and a concentration-dependent decrease of cell proliferation from 25 to 200 μM. The cell cycle was analyzed by flow cytometry. BPA at 50 nM increased the percentage of cells in S phase of the cell cycle at 24 h and this effect was higher at 48 h with an increase of about 17% of cells in the S phase compared with the control. At 100 μM, BPA induced a significant increase of the percentage of cells in the G0/G1 phase, suggesting that BPA affected cell growth in a non-monotonic way. BPA-treatment at 25, 50 and 100 nM for 48 h induced a significant increase ($p < 0.001$) of both the percentage of aberrant cells (about 20% at 100 nM) and structural aberrations (about 27% at 100 nM) including chromatid and chromosome breaks, rings and fragments. BPA also increased significantly the percentage of highly fragmented metaphases (shattered cells). In PHA-stimulated PBMC treated with BPA (50 nM) for 24 h, γH2AX was significantly increased in CD3+ T lymphocytes and was also detected in a higher proportion of CD8+ T lymphocytes than the CD4+ T lymphocytes and a slight percentage of γH2AX was reported among the B cells. The treatment of PHA-stimulated PBMC with BPA (50 nM) induced p21/Waf1 and PARP1 protein expressions approximately within the same time interval. These findings suggest that BPA could affect the p53-p21/Waf1 checkpoint and PARP1 levels resulting in DNA damage repair defects. BPA (50 nM) for 24 h modulated the expression of ER-α and ER-β in both sexes inducing or inhibiting its expression in males and in females with effects similar to the variations induced by pharmacological concentrations of E2 (100 nM). The study investigated also the BPA-induced neurotoxicity in terms of DNA damage. After the coupling period, three females/group received BPA (0.1 mg/L), or vehicle (ethanol 0.1 mL/L) in the drinking water during gestation, lactation and weaning of their offspring. Five females and three males pups from BPA-exposed mothers and five females and three males newborns from vehicle-treated dams were then sacrificed at PND 17. BPA was shown to induce γH2AX phosphorylation in cells possessing immune function in the CNS, such as microglia and astrocytes of rat hippocampus. In BPA-exposed rats a marked decreasing trend of ERα expression was found therefore proposing a role for this receptor in the effects induced by BPA.

Yu et al., 2020 [RefID 475-G]

In this study, induction of MN and double-strand DNA breaks by BPA, BPF, and BPS were investigated in Chinese hamster V79-derived cell lines expressing various human CYP enzymes and a human hepatoma (C3A) (metabolism-proficient) cell line. In a first step a prediction of BPA, BPF, and BPS as potential substrates for several human CYP enzymes, which are commonly involved in the metabolic activation of compounds, was conducted by molecular docking. The results of the analysis showed a similar affinity of the compound with all the enzymes tested: CYP1A1, 1A2, 1B1, 2B6, 2E1, and 3A4. BPA (99.6% analytical purity) tested at 40, 80 and 160 μM for 9 h, followed by 15 h of recovery induced a concentration related increase of MN frequency in V79-hCYP1A1. In V79-hCYP1B1 cells MN were observed only at the two highest concentrations. No induction of MN was reported in V79-Mz, V79-hCYP1A2, V79-hCYP2E1, or V79-hCYP3A4-hOR cells. A consistency with the results of the molecular

docking was observed only for CYP1A1 and CYP1B1. Following extended exposure (two cell cycles, i.e., 24 h in V79-derived cells) without recovery period, BPA reduced the cell viability at 80 μM in V79-Mz and V79-hCYP1A1 cells; at lower concentrations, the cell growth was even enhanced (compared with the control). BPA induced MN in V79-hCYP1A1 (24 h treatment) and C3A cells (72 h treatment) from 5 and 2.5 to 80 μM in a concentration-dependent manner. The increase of MN was completely blocked by 1-aminobenzotriazole (1-ABT CYP inhibitor) and by 7-hydroxyflavone (7-HF selective CYP1A1 inhibitor). Co-exposure of C3A cells to pentachlorophenol (sulfotransferase 1 inhibitor) or ketoconazole (UDP-glucuronosyltransferase 1A inhibitor) potentiated MN induction with thresholds lowered to 0.6 μM . The use of Centromere Protein B-staining immunofluorescent assay indicated clastogenic effects. BPA tested for 9 h from 10 to 160 μM in V79-Mz, V79-hCYP1A1, and C3A cells induced a concentration-dependent increase of γH2AX foci which were blocked in presence of 1-ABT and 7-HF.

Özgur et al., 2021 [RefID 631-G]

In this study BPA was tested in a chromosomal aberration assay in cultured human peripheral blood lymphocytes. Cells were exposed for 24 h to BPA, dissolved in DMSO, at the final concentrations of 5, 10 and 20 $\mu\text{g}/\text{mL}$. No data on chromosome aberrations are reported as, under the conditions of this study, BPA (source and purity not defined) was completely cytotoxic.

E.1.3. DNA damage (by comet assay)

Iso et al., 2006

In this study the effects of BPA and 17 β -estradiol (E2) on DNA damage was analysed in ER-positive MCF-7 cells by comet assay. One thousand higher concentrations of BPA (Wako Pure Chemicals Industries, Ltd.) were needed to induce the same levels of effects of E2. Levels of γH2AX foci measured by immunofluorescence microscopy were increased after treatment with E2 or BPA. Foci of γH2AX co-localized with the Bloom helicase, an enzyme involved in the repair of DSBs. In comparison with MCF-7 cells, DNA damage was not as severe in the ER-negative MDA-MB-231 cells. In addition, the ER antagonist ICI182780 blocked E2 and BPA genotoxic effects on MCF-7 cells. These results together suggest that BPA causes genotoxicity ER dependently in the same way as E2.

Xin et al., 2014 [RefID 8147]

The aim of this study was to assess how BPA can influence the function of pancreatic islets. To measure DNA damage, rat INS-1 insulinoma cells were exposed to different concentrations of BPA (Sigma-Aldrich, 99% purity) (0, 25, 50, 100 μM for 24 h) and analysed by the single-cell gel electrophoresis (comet assay). To investigate the possible mechanism of DNA damage induced by BPA, p53 and p-Chk2 levels were also analysed by western blotting together with measurements of intracellular ROS and glutathione (GSH). The results show that BPA caused an increase in DNA strand-breaks at 50 and 100 μM (as measured by tail moment, tail length and tail DNA %). The authors state that these experimental conditions did not cause any significant toxicity (90% survival; no data provided). Pre-treatment with NAC decreased to half the number of DNA strand breaks induced at the highest dose. A significant increase in intracellular ROS, which was decreased by NAC pre-treatment, was also observed. A significant reduction in the level of GSH levels was observed at all BPA concentrations. Finally, expression of DNA damage-associated proteins (p53 and p-Chk2) was significantly increased by BPA exposure (all concentrations).

Chen et al., 2016 [RefID 1130]

The study investigated the cytotoxic and genotoxic effects induced by BPA alone and in combination with cadmium (Cd) *in vitro* in mouse embryonic fibroblast cell line (NIH3T3). The treatment of the cell cultures with BPA (Sigma-Aldrich) at 2, 10 and 50 μM was shown to induce, only at the highest concentration tested, a decrease in the cell viability and an increase of the oxidative damage as reactive oxygen species (ROS), measured by DCFH-DA and as 8-OHdG. Significant increase of DNA strand breaks was also detected as tail DNA% and tail moment by comet assay. Higher number of γH2AX foci detected through the use of immunofluorescence and increased γH2AX expression evaluated by western blot in BPA treated cells are indicative of DNA double strand breaks. In addition, 50 μM BPA treatment did significantly decrease the percentage of cells in G1 phase and increased the percentage of cells in G2 phase but not in S phase. Pre-treatment of cells with Cd was observed to aggravate BPA-

induced cytotoxicity, and increase ROS production, DNA damage, G2 phase arrest, total TUNEL positive cells and cleaved-PARP expression levels.

Porreca et al., 2016 [RefID 5892]

This study reports on the thyroid-disrupting activity of low-dose BPA (Sigma-Aldrich) on the rat immortalized FRTL-5 thyrocytes cell line using microarray experiments. Exposures to 10^{-9} M BPA for 1 and 3 days resulted in increased ROS production. Exposures for 3 and 7 days lead to gene expression changes with transcriptome alterations being quantitatively and qualitatively dependent on the duration of exposure. Cell survival (decreased), cell death (increased), cell cycle (decreased), and cancer (increased) were among the most significant functions predicted deregulated. The response involved many genes belonging to specific pathways mainly related to cell death/proliferation and DNA repair (genes involved in "DNA replication, recombination and repair" including "checkpoint control" functions). Although gene expression levels are only slightly altered, a long-term exposure to BPA (28 days, 10^{-9} M) impaired the cellular response to other stressors (mainly COP9 signalosome). A delayed reduction of UV-C-induced DNA damage was identified by comet assay. This was due to the impairment of p21-Tp53 axis, with BPA-treated cells being more prone to cell death (increased apoptosis and decreased proliferation following UVC damage). The authors propose a possible mechanism by which BPA does not cause direct genetic damage but may exert an indirect genotoxic activity.

Lei et al., 2017 [RefID 3960]

In this study changes in cytotoxicity, genotoxicity, intracellular ROS formation, and Ca^{2+} levels induced by BPA were evaluated in MCF-7 cells in comparison with other BPs. The range of BPA (98% purity, Tokyo Chemical Industry) concentrations tested was 0.01-100 μM (24 h exposure). An increase in cell viability was observed at 1 μM , while a significant drop in cell survival (to approximately 30%) was observed at higher concentrations (10–100 μM). In parallel, a significantly increase in LDH release was observed (10–100 μM). BPA (1–25 μM) significantly increased ROS levels in a concentration-dependent manner. At 50 μM however BPA caused death of over 90% cells. BPA exposure resulted in a significantly increase in DNA-damaging effect on MCF-7 cells in the range 10–50 μM . In addition BPA at 0.0001–1 μM significantly increased intracellular Ca^{2+} level. Finally, the estrogenic and thyroidal hormone effects induced by BPA were also evaluated using the yeast two-hybrid bioassay.

Li XH et al., 2017 [RefID 4176]

The study investigated the cytotoxic effects and oxidative stress induced by BPA (Sigma-Aldrich) alone and in combination with dibutyl phthalate (DBP) or cadmium (Cd) *in vitro* in HepG2 cells. The cell cultures were exposed for a period of 6 h to a range of concentrations of the single substances ensuring a cell viability above 50%. BPA tested from 10^{-8} to 10^{-4} mol/L for 6 hours induced a concentration dependent increase of reactive oxygen species (ROS), measured by DCFH-DA, and malondialdehyde (MDA) level and a decreased activity of SOD. An increase of DNA strand breaks (up to eight-fold with respect to the control value) applying the comet assay, was detected after BPA treatment at 10^{-8} , 10^{-7} , 10^{-6} mol/L for 24 h without a clear concentration response. The co-exposure treatments (BPA and DBP or BPA and Cd) showed higher ROS and MDA levels and lower SOD activity than the mono-exposure treatments. The combined treatments with BPA and Cd had stronger DNA damage effect.

Mokra et al., 2017 [RefID 5170]

The study reported concentration-related induction of DNA single and double strand breaks (detected with alkaline and neutral comet assay) by BPA (Sigma-Aldrich) and its analogues, BPS, BPF and BPAF in human peripheral blood mononuclear cells (PBMC) treated in the concentrations ranging from 0.01 to 10 $\mu\text{g}/\text{mL}$ after 1 and 4 h treatment. No significant decrease of cell viability, evaluated using calcein-AM/PI stains, was observed at the concentrations tested for DNA damage. After 1 h incubation, BPA caused statistically significant increase in DNA strand breaks at 0.1 mg/mL. The highest effects were induced by BPA and BPAF, which produced single strand breaks starting from 0.01 $\mu\text{g}/\text{mL}$, while BPS caused the lowest effect at 10 $\mu\text{g}/\text{mL}$ after 4 h of exposure. Statistically significant increases of DNA double strand breaks were induced by BPA at concentrations of 1 $\mu\text{g}/\text{mL}$ and 10 $\mu\text{g}/\text{mL}$ after 1 h incubation and at 0.1 $\mu\text{g}/\text{mL}$ and 1 $\mu\text{g}/\text{mL}$ after 4 h incubation. The strongest effect was observed with BPAF. DNA repair was also evaluated at different times (30, 60 and 120 min) after the treatment with

BPA at 10 µg/mL. A significant decrease of the DNA damage was observed at 60 min, but the repair was not complete after 120 min.

Durovcova et al., 2018 [RefID 262-G]

The study evaluated by alkaline comet assay the induction of DNA strand breaks by BPA (Sigma-Aldrich) in cultures of peripheral blood lymphocytes at concentrations of 0.001, 0.1, 2.5 mM. The results show a statistically significant increase only at the first two concentrations tested. The presence of oxidative DNA damage was also shown using modified comet assay with Fpg enzyme: the extent of DNA single strand breaks is higher at the lower concentration. The study reports also a protective effect of BPA on plasmid DNA against iron ions induced single-strand DNA breaks as showed by a test measuring the electrophoretic mobility of pDNA topoisomers (DNA-topology assay).

George and Rupasinghe, 2018 [RefID 277-G]

This study investigated the relative toxicity of BPA (Sigma-Aldrich) and BPS on human bronchial epithelial cells (BEAS-2B). The tested endpoints included cytotoxicity, induction of ROS, DNA fragmentation, γH2AX foci and DNA tail damage. To evaluate mechanism of cell death the DDR and activation of caspase-3 were also investigated. In all the assays a single concentration and a single time of exposure were used (200 µM BPA for 24 h). According to the authors this concentration caused 50% of cell viability loss (IC₅₀). However, the data reported indicate high levels of toxicity (90%), with all the results being unreliable at this level of toxicity.

Goncalves et al., 2018 [RefID 11104]

The aim of this study was to evaluate the effects of BPA (Sigma-Aldrich) on the growth, viability, and testosterone production of TM3 murine Leydig cells after exposure to BPA for 24 or 48 h (1, 10, 100 µM). BPA reduced testosterone production, cell viability and cell growth in a concentration-dependent manner. The highest tested concentration of BPA (100 µM) increased cellular death, as indicated by an increased sub-G1 phase population and a larger number of cells labelled with Hoechst 3342. This concentration of BPA also decreased the number of metabolically active mitochondria as revealed by rhodamine staining. No DNA strand breaks evaluated by alkaline comet assay was detected in the cells after 3 h of BPA exposure at any concentration tested. The authors conclude that BPA is toxic to Leydig TM3 cells and impairs their steroidogenic function.

Mokra et al., 2018 [RefID 364-G]

The study reported that BPA (Sigma-Aldrich) and its analogues, BPS, BPF and BPAF caused oxidative DNA damage to purine and pyrimidines in human peripheral blood mononuclear cells (PBMC) treated at concentrations of 0.01, 0.1 and 1 µg/mL for 4 h and 0.001, 0.01 and 0.1 µg/mL for 48 h. BPA was dissolved in ethanol. No significant decrease of cell viability, evaluated using calcein-AM/PI stains, was observed at the concentrations tested. DNA damage was detected with alkaline comet assay coupled with repair enzyme endonuclease III (Nth) and 8-oxoguanine DNA glycosylase (hOGG1). Statistically significant and concentration related oxidative damage to purines (from 0.01 µg/mL) and to pyrimidines (from 0.1 µg/mL) was reported after 4 h treatment. After 48 h treatment significant damage to purine was observed from 0.001 µg/mL and to pyrimidines from 0.01 µg/mL. Statistically significant differences for DNA damage between 4 h and 48 h exposure at the highest concentrations tested (0.01 and 0.1 µg/mL)

Yuan et al., 2019 [RefID 478-G]

In this study, markers of oxidative stress and DNA damage were evaluated in Marc-145 rhesus monkey embryo renal epithelial cells exposed to BPA (Sigma-Aldrich, purity > 99%) in the range 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵ and 10⁻⁶ M (24 hr exposure). The results showed that BPA induced a concentration-dependent decrease in cell viability (from 20% at the lowest concentration up to almost 80% at the highest concentration), in SOD activity and GSH level. Concomitant concentration-dependent increases in apoptosis, lactate dehydrogenase (LDH) activity, ROS and thiobarbituric acid reactive substances content were observed. BPA also induced a concentration-dependent increase in DNA strand breaks by comet assay in the range of concentrations measured (10⁻³ -to 10⁻⁶ M).

Kose et al., 2020 [RefID 325-G]

This study investigated the relative toxicity, potential oxidative stress and genotoxicity induced by BPA (>99% purity), BPS and BPF on the RWPE-1 non-tumorigenic prostatic cell line. RWPE-1 cells were incubated with BPA at concentrations of 50–600 μM for 24 h exposure. The IC_{20} and IC_{50} values, concentrations that causes 20 and 50% of cell viability loss, after a 24 exposure to BPA were 45 and 113.7 μM . BPA induced significant decreases in the activities of glutathione peroxidase (GPx1) and SOD, an increase in glutathione reductase and total GSH and a decrease in total antioxidant capacity. At a single concentration (IC_{20}), BPA produced significantly higher levels of DNA damage vs the control both in the standard (2.5-fold increase) and Fpg-modified comet assays. No changes in the mRNA levels of p53 and the OGG1, Ape-1, DNA polymerase β , base excision repair (BER) proteins were induced by BPA. The single exception was a small decrease in the expression levels of MYH expression.

E.2. Other studies

E.2.1. DNA adducts

De Flora et al., 2011

The ability of BPA to form DNA adducts was investigated in two human prostatic cell lines: PNT1a non tumorigenic epithelial cells and PC3 cells androgen-independent prostate cancer cells originated from bone metastasis of prostatic carcinoma. PNT1a and PC3 cells were treated with BPA (Sigma-Aldrich), dissolved in ethanol at a concentration corresponding to the IC_{50} (200 μM for PNT1a and 250 μM for PC3) for 24 h. PNT1a cells were also treated at a concentration of 1 nM, for 2 months. Significant levels of DNA adducts were detected by ^{32}P -postlabeling technique in prostate cell lines treated with high-concentration of BPA for 24 h (4.2-fold increase over controls) in PNT1a cells and a 2.7-fold increase over controls in PC3 cells) and in a lower extent in PNT1a cells treated at low-concentration for 2 months.

E.2.2. DNA repair

Yin et al., 2014 [RefID 8519]

The study analyzed gene expression profiling in ER-negative human embryonic kidney cells (HEK293) following 48 h exposure to 10^{-6} M BPA. The comparison of gene expression profiles of BPA (no information on purity or the supplier company) treated and control (DMSO) samples identified 15 differentially expressed genes after BPA treatment, eight upregulated and seven downregulated (while more than 300 genes are differentially expressed in ER-positive cells). A computer-assisted functional analysis indicated the BPA exposure perturbed many unrelated metabolic pathways. BAX, a proapoptotic regulator, was downregulated by BPA treatment, suggesting that low-dose BPA may reduce apoptosis of HEK293 cells. Differentially expressed genes of BPA exposure include upregulation of ERCC5 encoding a DNA endonuclease involved in nucleotide-excision repair. Using an electrochemical method based on the preferential electrochemical oxidation of chemically damaged DNA, no DNA damage was identified in HEK293 cells treated with 10^{-6} M BPA for 48 h, while a peak indicating DNA damage was observed in the ER-positive MCF-7 cell line.

Gassman et al., 2015 [RefID 2214]

In this study, DNA damaging effects of BPA were shown to be induced indirectly *in vitro* in mouse fibroblasts through the generation of ROS. A statistically significant increase of intracellular ROS production, measured by DCFH-DA, was observed in mouse fibroblasts (Ku70-deficient cell line) treated *in vitro* with BPA (Sigma-Aldrich) at 150 μM for 1 h. Treatment with BPA increased also the levels of DNA modified bases (5-OH-Cyt and ThyGly) consistent with the increase in ROS. Ku70 cell line, deficient in strand break repair by non-homologous end joining (NHEJ), a back-up repair pathway for BER, was used to investigate the effect of BPA on the BER response to oxidative stress. Ku70-deficient cells were very sensitive to the oxidative damaging agents: treatment with KBrO_3 induced a significant dose-response reduction of cell viability. Co-treatment with BPA partially reversed the KBrO_3 -induced cytotoxicity in these cells, associated with the increase in oxidatively induced DNA base lesions, suggesting that BPA may prevent initiation of repair of oxidized base lesions. Examination of γH2AX foci revealed also a significant reduction in DNA damage signaling induced by BPA confirming the possible inhibition of BER repair.

Tran et al., 2020 [RefID 438-G]

The aim of the study was to investigate the effects of BPA in PBMC by using freshly isolated T cell subpopulations of healthy donors for short and long-term BPA treatment. Long term cultures of CD4+ or CD8+ T lymphocytes isolated from blood of male donors were established. Cell cultures were treated with BPA (purity >99%) from 0.3 to 30 nM for 24 h. A non-monotonic telomerase enzyme inhibition in CD8+T-cells, but not in CD4+T-cells of male donors was observed. The maximum inhibition (30% compared with control) was seen at 0.3 nM BPA. At higher concentrations the effect subsequently weakened. An impact of BPA on DNA repair capacity of CD8+T-cells upon exposure to oxidative stress (treatment with hydrogen peroxide) was also reported. A decreased DNA repair (maximum 45% inhibition at 0.3 nM), measured by comet assay, after BPA treatment compared with the control was observed. DNA repair gene pathway analysis was carried out by using a human DNA repair RT2 Profiler PCR array: no regulation on the expression of 84 DNA repair genes upon BPA exposure could be observed. Long-term treatment (after 35 days of culture) with BPA significantly reduced cell proliferation only at concentration <30 nM. The maximum inhibition was seen at 3 nM, with a reduction of 25% in comparison with the solvent control. Long-term treatment (49 days of culture) of CD8+T cells with BPA impaired T cell response upon antigen stimulation, as shown by decrease of telomere length and mitochondrial DNA (mtDNA) copy number by 35% and 25%, respectively. At a 10-fold higher or lower concentration, the effect was marginally evident for both parameters. A strong inhibitory effect of intracellular anti-viral interferon- γ expression was evident on day 42 of BPA exposure, compared with the solvent control.

E.2.3. Spindle stability**Campen et al., 2018 [RefID 240-G]**

The aim of the study was to compare the effects of *in vitro* exposure to either BPA (Sigma-Aldrich) or BPS on meiotic progression, spindle morphology and chromosome alignment in the bovine oocyte. Bovine ovaries were sourced from an abattoir. Groups of 5–20 cumulus–oocyte complexes (COCs) extracted from the bovine ovaries were treated with BPA or BPS at 10 concentrations between 1 fM and 50 μ M and underwent to *in vitro* maturation for 24 h, then the oocytes were extracted. For BPA experiments, a total of 939 oocytes were analyzed for meiotic stage (including 250 vehicle-only control oocytes), of which a total of 767 were at metaphase II (MII) (including 211 MII oocytes in the control) and were included for analysis of spindle and chromosome configuration. Immunocytochemistry was used to label the chromatin, actin and microtubules in the fixed oocytes. The meiotic stage was assessed using immunofluorescence, and the MII oocytes were further assessed for spindle morphology and chromosome alignment (in all MII oocytes regardless of spindle morphology). No difference in the proportion of bovine oocytes that reached MII was observed for BPA treatment. Significant effect on spindle morphology ($p < 0.0001$) was induced by BPA treatment at very low concentration (1 fM). Fewer oocytes with bipolar spindles were seen following exposure to BPA at concentrations of 1 fM, 10 fM, 100 fM, 10 pM, 1 nM, 10 nM, 100 nM and 50 μ M, compared with the control. There was no effect of BPA on spindle morphology at concentrations of 1 or 100 pM. Increased chromosome misalignments were observed at BPA concentrations of 10 fM, 10 nM and 50 μ M of BPA, no effect was detected at any other concentration. The study presents limitations: in the ovaries the effects were evaluated in a specific period of development (namely, the 24 h window of oocyte maturation), without considering potential prior historical exposures *in vivo*.

Kim et al., 2019 [RefID 319-G]

In vitro effects of BPA (Sigma-Aldrich) on mitotic progression were examined in HeLa cells exposed to 100 nM BPA for 5 h. Proteins involved in mitotic processes were detected by western blot, live cell imaging, and immunofluorescence staining. Under the applied treatment conditions, BPA was shown to disturb spindle microtubule attachment to the kinetochore, with the concomitant activation of spindle assembly checkpoint (SAC). Spindle attachment failure was attributed to BPA interference with proper localization of microtubule associated proteins, such as HURP to the proximal ends of spindle microtubules, Kif2a to the minus ends of spindle microtubules, and TPX2 on the mitotic spindle. BPA also caused centriole overduplication, with the formation of multipolar spindle.

Yang et al., 2020 [RefID 469-G]

The effect(s) of exposure to BPA (Sigma-Aldrich) on assembled spindle stability in ovulated oocytes were studied. Mature M II oocytes, recovered from the oviducts of superovulated B6D2F1 mice, were cultured for 4 h in the presence of increasing concentrations (5, 25, and 50 µg/mL) of BPA. After treatment oocytes were analyzed by immunofluorescence and live cell imaging to investigate the effect of BPA on spindle dynamics. BPA disrupted spindle organization in a dose-dependent manner, resulting in significantly shorter spindles with unfocused poles and chromosomes congressed in an abnormally elongated metaphase-like configuration, with increased erroneous kinetochore-microtubule interactions.

E.2.4. γH2AX**Audebert et al., 2011**

In this study the authors investigated the capability of established human cell lines, ACHN (human kidney adenocarcinoma cells), HepG2 (human hepatocellular carcinoma cells) and LS174T (human epithelial colorectal adenocarcinoma cells) to biotransform BPA and BPF. The potential genotoxicity of BPA and BPF was assessed by the examination of γH2AX foci. BPA (radiochemical purity: 95%, specific activity: 3.6 GBq/mmol) was purchased from Moravек Biochemicals and Radiochemicals (Brea, CA) was shown to be metabolized by HepG2 and LS174T cell lines. Intestinal cells showed stronger biotransformation capabilities than liver cells, in terms of production of the glucuronide- and the sulphate-conjugates (phase II metabolites). Conversely, ACHN cell line was not able to metabolize BPA. Relevant metabolites were separated and quantified by radio-HPLC. Following treatment with BPA (Sigma-Aldrich, purity >99%) at concentration-levels of 1, 5, 10, 50 and 100 µM for 24 h no increases of γH2AX were observed at any concentration tested in HepG2 and in LS174T cell lines. A concentration related increase of γH2AX was observed in ACHN cells.

Pfeifer et al., 2015 [RefID 5815]

The objective of this study was to investigate the effects of low-dose BPA (Sigma-Aldrich) in mammary gland cells. The human cell lines used in the study are the ERα-negative immortalized benign and normal breast epithelial cell lines (MCF10A and 184A1, respectively) and the ERα-positive MCF7 and MDA-MB-231 cell lines originate from human breast epithelial adenocarcinomas. Low concentrations BPA (10 and 100 nM) induced double strand breaks (DSBs) as measured by γH2AX foci in all cell lines. Both MCF10A and MCF7 cells had also a greater number of ATM-pS1981-positive nuclei after 24 h treatment compared with the control. Low-concentration BPA significantly increased the level of c-Myc protein and other cell-cycle regulatory proteins (cyclin D1, cyclin E and E2F1) and induced proliferation in parallel in ERα-negative 184A1 mammary cells. Silencing c-Myc reduced BPA-mediated increase of γH2AX suggesting that c-Myc plays an essential role in BPA-induced DNA damage.

The increased level of DNA double strand breaks induced by BPA exposure in 184A1 cells was also confirmed in a neutral comet assay and was found to be reduced by c-Myc silencing. Similarly, silencing c-Myc abolished BPA-mediated ROS production, which was localized to mitochondria. The authors concluded that low-concentration BPA exerted a c-Myc-dependent genotoxic and mitogenic effects on ERα-negative mammary cells (results reported as tail moment only and a single BPA concentration analysed).

Ganesan and Keating, 2016 [RefID 2141]

The study investigated ovarian DNA damage induced by BPA exposure using an *in vitro* model system. At PND 4, F344 rat ovaries were cultured in medium containing 1% DMSO ± BPA (from Sigma-Aldrich, 440 µM) for 1-4 days. Increased expression of γH2AX foci was observed by immunofluorescence staining in the ovaries evaluated (three ovaries/treatment/time point) at days 1, 2 and 4 of treatment, with a time-related increasing trend. Increased abundance of phosphorylated H2AX and ataxia-telangiectasia mutated (ATM), markers of DNA double-strand breaks, were also detected by western blotting in total ovarian protein homogenates. Expression of DNA repair genes (ATM, Prkdc, Xrcc6, Brca1, Mre11a, Rad50, and Smc1a) assessed by qRT-PCR was also increased ($p < 0.05$) 1 and 2 days after BPA exposure (the 4 day time point was not analysed). Overall, the results of this study indicate that the *in vitro* exposure to BPA can induce DNA double strand breaks that activate a DDR in ovarian cells.

Quesnot et al., 2016 [RefID 6033]

The study authors applied an automated *in situ* γ H2AX detection system in metabolically competent HepaRG cells to evaluate the genotoxicity of 10 environmental contaminants, including BPA, after single or long-term repeated *in vitro* exposure (1, 7 or 14 days). Cytotoxicity was determined using the MTT assay. BPA (Sigma-Aldrich) was tested at four concentrations (20, 40, 50 and 60 μ g/mL), selected to avoid excessive toxicity (i.e. with viability > 60%). A significant increase in the H2AX positive cell index (fold increase over control) was observed after 24h at 40 μ g/mL and above, but not after 7 days or 14 days treatments. The incidence of Hoechst-stained MN was also quantified in HepaRG cells exposed to BPA for 1 or 7 days before mitogenic stimulation by EGF for 3 days. Under the test conditions, no significant increase of MN was observed in BPA treated cells, while positive results were obtained in parallel experiments with known genotoxic contaminants (benzo(a)pyrene, AFB1, DMBA).

Liang SX et al., 2017 [RefID 4246]

The testicular toxicity of BPA (99% purity) was compared with that of BPS, BPAF, and tetrabromobisphenol A (TBBPA) on the C18-4 spermatogonial cell line. The authors developed and validated an automated multi-parametric high-content analysis to study nuclear morphology, DNA content, cell cycle progression, DNA synthesis, cytoskeleton integrity, and DDRs induced by these compounds. The only marker of DNA damage analysed was the formation of γ H2AX foci. BPA showed a small increase at a single dose of 50 μ g/mL after 48 and 72h exposure (doses tested: 0.1, 1, 5, 10, 25, 50 μ g/mL). The study presents several limitations in the reported methodology.

Mahemuti et al., 2018 [RefID 11171]

The aim of this study was to investigate the key molecular pathways involved in the developmental effects of BPA on human fetal lung and their potential implications in the link between pre-natal exposure to BPA and increased sensitivity to childhood respiratory diseases. Global gene expression profiles and pathway analysis was performed in cultured HFLF exposed to non-cytotoxic concentrations of BPA (0.01, 1 and 100 μ M BPA for 24 h, 99% purity, Sigma-Aldrich). Molecular pathways and gene networks were affected by 100, but not 0.01 and 1 μ M BPA. These changes were confirmed at both gene and protein levels. The pathways affected by BPA included the cell cycle control of chromosome replication and a decreased DDR. BPA increased DNA DSBs as shown by phosphorylation of H2AX and activated ATM signaling (increased phosphorylation of p53). This resulted in increased cell cycle arrest at G1 phase, senescence and autophagy, and decreased cell proliferation in HFLF. Finally, BPA increased cellular ROS level and activated Nrf2-regulated stress response and xenobiotic detoxification pathways. The authors suggest that pre-natal exposure to BPA may affect fetal lung development and maturation, thereby affecting susceptibility to childhood respiratory diseases.

Kim et al., 2018b [RefID 11137]

BPA (> 99% purity, Sigma-Aldrich) promoted cell proliferation in undifferentiated and differentiated human hepatocyte cell lines (HepG2 and NKNT-3, respectively) at submicromolar concentrations (0.3-5 μ M for 24 h). The proliferative effects of BPA disappeared at concentrations higher than 5 μ M (cell viability decreased at concentrations higher than 10 μ M). Exposure to BPA in the submicromolar range induced DNA damage in both cell lines as shown by a dose-dependent increase in phosphorylation of histone H2AX (γ H2AX), p53 activation and induction of cyclin B1. Increased levels of γ H2AX were also observed in liver tissue of juvenile rats (PND 9) orally exposed to a relatively low dose of BPA (0.5 mg/kg for 90 days). At a higher BPA dose (250 mg/kg) no increase in hepatocyte proliferation or cyclin B1 was observed. BPA promoted ROS generation as measured by DCF-DA-enhanced fluorescence in HepG2 cells. Increased levels of ROS were suggested to play a role in BPA-induced proliferation and DNA damage as shown by the partial reversion of both processes upon pre-treatment with NAC.

Hercog et al., 2019 [RefID 287-G]

With the aim of comparing the toxicological profiles of possibly safer analogues of BPA, the authors investigated the cytotoxic/genotoxic effects of BPS, BPF and BPAF and their mixtures in human hepatocellular carcinoma HepG2 cells. Single exposure to BPA (99% analytical purity, Sigma-Aldrich) did not induce any significant changes in cell viability at the tested concentrations (2.5, 5, 10, 20 μ g/mL for 24 or 72 h). Induction of a significant increase in DNA double strand breaks, as determined by

γ H2AX assay, was observed only at the highest dose (20 μ g/mL for 72 h). BPA (tested at the 10 μ g/mL concentration) induced changes in the expression of some genes involved in the xenobiotic metabolism (*CYP1A1*, *UGT1A1*, but not *GST1*), response to oxidative stress (*GCLC* but not *GPX1*, *GSR*, *SOD1*, *CAT*), while no changes were observed in any of the genes involved in the DDR (*TP53*, *MDM2*, *CDKN1A*, *GADD45A*, *CHK1*, *ERCCA*). Similar results were obtained when cells were exposed to BPA as a single compound or in mixtures with its analogues at concentrations relevant for human exposure (10 ng/mL). The relevance of these changes is of uncertain biological significance.

Hercog et al., 2020 [RefID 288-G]

In a follow-up study by Hercog et al. (2020) the genotoxic effects induced by co-exposure of the cyanotoxin cylindrospermopsin (CYN)(0.5 μ g/mL) and BPA (Sigma-Aldrich), BPS and BPF(10 μ g/mL, 24 and 72 h exposure) were investigated on HepG2 cells using the same techniques and experimental conditions of Hercog et al. (2019). The results obtained with BPA confirm the previously published observations, but the relevance of these changes remains of uncertain biological significance.

Yin et al., 2020 [RefID 474-G]

The scope of the study was developing a novel *in vitro* three-dimensional testicular cell co-culture mouse model that enables the classification of reproductive toxic substances. BPA (99%, Sigma-Aldrich) as well as BPS, TBBPA, and BPAF were used as model compounds. A concentration-dependent increase in BPA toxicity was found in the range 2.5 - 400 μ M following 24, 48 and 72 h exposures. The large variations in the number of γ H2AX foci observed at 72 h make the relevance of these results questionable. No increase in γ H2AX used as marker of DNA damage was found up to a dose of 100 mM (70% cell viability).

Nair et al., 2020 [RefID 367-G]

The effects of BPA (Sigma-Aldrich) as a single agent, or in combination with 4-tert-octylphenol (OP) and hexabromocyclododecane (HBCD), were studied in the HME1 mammary epithelial cells and in the MCF7 breast cancer cell line. Following a 2-month exposure to a low non-toxic BPA concentration (0.0043 nM), increased levels of DNA damage were evidenced by upregulation in both cell lines of phosphorylated DNA damage markers (γ -H2AX, pCHK1, pCHK2, p-P53). Disruption of the cell cycle was observed both after short exposures (24 h and 48 h, G2/M arrest) as well as after the 2-month exposure treatment (G1 and S phase increases). BPA increased cellular invasiveness through collagen. Methylation changes were investigated by Methylation Specific Multiplex-Ligation Dependent Probe Amplification (MS-MLPA) using a panel of 24 tumour suppressor genes (all hypomethylated) and identified hypermethylation of *TIMP3*, *CHFR*, *ESR1*, *IGSF4* in MCF7 cells and *CDH13* and *GSTP1* genes in HME1 cells. Finally, BPA induced phosphorylation of six protein kinases in HME1 cells (EGFR, CREB, STAT6, c-Jun, STAT3, HSP60) and increased levels of several other proteins involved in potential oncogenic pathways (HSP27, AMPK α 1, FAK, p53, GSK-3 α/β , and P70S6).

Yuan et al., 2021 [RefID 477-G]

This study investigated the combinatorial toxicity of BPA (\geq 99.8% purity), decabrominated diphenyl ether and acrylamide to HepG2 cells. Increased number of γ H2AX foci were induced in HepG2 by a 24h exposure to a single BPA dose that induced 25% toxicity. The majority of the data (ROS measurements, Ca²⁺ flux, DNA damage, Caspase-3 and decreased mitochondrial membrane potential) refers to additive/synergistic effects induced by varying combinations of contaminants. The authors conclude that BPA induced an increase in γ H2AX fluorescence and in the number of γ H2AX foci/nucleus. However, this conclusion is not fully supported by the data presented.

Escarda Castro et al., 2021 [RefID 266-G]

The ability of BPA to induce genotoxic and epigenetic changes was investigated before and during cardiomyocyte differentiation in H9c2 rat myoblasts exposed to 10 and 30 μ M BPA (92% and 73% of cell viability, respectively). Exposure to BPA (no information on purity or the supplier company) before differentiation repressed the expression of the *Hand2* and *Gata4* heart transcription factors and three genes belonging to the myosin heavy chain family (*Myh1*, *Myh3*, and *Myh8*), whereas exposure after the 5 days of differentiation reduced the expression of cardiac-specific *Tnnt2*, *Myom2*, *Sln*, and *Atp2a1* genes. BPA did not induce ROS and did not increase DNA 8-oxodG levels (as measured by

immunostaining) in either myoblasts or cardiomyocytes. After BPA exposure the percentage of DNA repair foci formed by co-localization of the γ H2AX and 53BP1 proteins increased in a concentration-dependent manner in myoblasts (from 44% in the control group to 61% and 86% at 10 and 30 μ M BPA, respectively), with no increase in MN. Repair foci also increased in cardiomyocytes (from 45% in the control group to 59% and 72% at 10 and 30 μ M BPA, respectively). A small increase (up to 13%) in MN was also reported only in cardiomyocytes treated with 10 μ M BPA. A decrease in the epigenetic markers H3K9ac and H3K27ac was also reported. The authors concluded from these *in vitro* data that BPA interferes with the process of cardiomyocyte differentiation. However, the reliability and significance of the data on BPA-induced DNA damage is questioned by several negative factors (high background levels of DNA repair foci, lack of information on methods for micronucleus assays and the small increase of MN over high background).

E.2.5. DNA oxidative damage

Barbonetti et al., 2016 [RefID 419]

With the aim of studying whether *in vitro* exposure to BPA affected human sperm integrity, the authors investigated the induction of pro-oxidative/apoptotic mitochondrial dysfunction. The decrease in the mitochondrial membrane potential of motile sperm observed following a 4 h exposure to 300 μ M BPA (Sigma-Aldrich) was associated with increased mitochondrial generation of superoxide anion, caspase-9 and caspase-3 activation and motility decrement. Loss in sperm vitality associated with a complete sperm immobilization was observed following a 20h exposure to 300 μ M BPA. This was accompanied by a significant increase in the formation of DNA 8-hydroxy-2'-deoxyguanosine. The significance of the increase levels of oxidative DNA damage in these extreme experimental conditions (no viability and no motility) is questionable.

Budiawan et al., 2018 [RefID 757-G]

The formation of the oxidized base 8-oxodG was analyzed by reacting dG with BPA with the addition of Fenton's reagent. DNA adduct 8-oxodG was analyzed by reverse phase HPLC with a UV/vis light detector at 254 nm. The results indicate that BPA acts as prooxidant because of the increase yield of 8-oxodG. The concentration of DNA 8-oxodG increases with increasing pH, temperature and length of incubation time. In the presence of BPA and Fenton's reagent the highest DNA 8-oxodG levels (42.258 ppb) are detected at pH 7.4, 60°C and 12 h incubation time (these are very low levels of DNA adduct formation). The relevance of this short report published in a conference proceedings is limited.

E.2.6. Effects on meiotic cells

Karmakar et al., 2017 [RefID 3388]

The objective of this *in vitro* study was to investigate the proliferation, survivability and apoptotic rate of mouse testicular germ cells (CD-1 and C57-GFP) exposed to BPA (information on purity no reported) and to examine the differential expression of germ cell markers in these cultured cells. Cell viability and proliferation were not affected by low BPA concentrations (0.01, 0.1, 1, and 10 μ M). Germ cell self-renewal and expression of differentiation related marker proteins were also found to be unchanged at these concentrations. In contrast, a significant reduction in survival was observed at 100 μ M (increased apoptosis). When *in vitro* treated BPA germ cells were transplanted into recipient testes, fewer colonies were observed at high BPA concentrations (10 and 100 μ M). A significant frequency of recombination failure during meiosis (production of "crossover-less" synaptonemal complex in pachytene spermatocytes) was observed in 10 μ M BPA-exposed germ cell transplanted recipient. Experiment on continuous BPA-exposed and 100 μ M BPA-recovered germ cells suggested that spermatogonial stem cells have a larger potential to survive in adverse environment. Finally, in a comparative proteomic analysis several differentially expressed cellular proteins were identified after BPA treatment of germ cell in culture.

E.2.7. Epigenetics

De Felice et al., 2015 [RefID 1462]

The aim of this study was to investigate whether a) BPA exposure was associated to deregulated expression of microRNAs and b) these epigenetic effects induced alterations in gene expression able to persist throughout a lifetime. Placenta samples from pregnant women living in a polluted area (40 patients subjected to therapeutic abortion for fetal malformation) and placenta samples from a control group of women living in a non-polluted area (40 pregnant women with a healthy pregnancy) were used for genome-wide miRNA expression profiling using microarray technology. This approach allowed identification of miRNAs that were aberrantly expressed in placentas from malformed fetuses. Twelve miRNAs were upregulated and six miRNAs were downregulated in placenta samples from malformed fetuses. BPA was absent in the control group, while it was detected only in patients subjected to therapeutic abortion. High levels of BPA were associated with a significantly increased miR-146a expression. The authors conclude that miR-146a, which correlates with BPA accumulation in the placenta, is a measure of fetal exposure related to fetal malformations. Finally, the use of bioinformatics tools allowed prediction of the target genes of miR-146a and exploring their functions including the downstream pathways. Several pathways involved in immune/ inflammatory, cancer and neural diseases were identified.

Karaman et al., 2019 [RefID 269-G]

The aim of this study was to investigate the epigenetic regulation induced by *in vitro* exposure to BPA to reveal a possible role on the progression of prostate cancer. Changes in gene expression of chromatin modifying enzymes, promoter methylation of tumour suppressor genes and histone modifications were studied in the PC-3 human prostate carcinoma cell line. A significant decrease in global levels of 5-methylcytosine and an increase in 5-hydroxymethylcytosine were observed following exposure to 10 μ M of BPA (99% purity, Sigma-Aldrich) for 96 h (in these experimental conditions no changes in cell proliferation were observed). A significant increase in DNA methylation of the *p16* promoter region, with a concomitant decrease in *p16* gene expression, was observed (1 and 10 μ M), while no changes were found for other genes involved in cell cycle control (*Cyclin D2* and *Rassf1*). Significant changes were observed in global histone modifications (H3K9ac, H3K9me3, H3K27me3, and H4K20me3) after 48 and 96 h exposures to BPA (0.1, 1 and 10 μ M). Analysis of the promoter methylation status of 94 tumour suppressor genes identified significant changes in *BCR*, *GSTP1*, *LOX*, *NEUROG1*, *PDLIM4*, *PTGS2*, *PYCARD*, *TIMP3*, *TSC2* and *ZMYDN10* (including the *MGMT* DNA repair protein). The authors conclude that epigenetic signatures such as DNA methylation and histone modifications could be proposed as molecular biomarkers of BPA-induced prostate cancer progression.

E.3. *In vivo* studies

E.3.1. Chromosomal damage

Masuda et al., 2005

The study investigated the systemic genotoxicity elicited in mice from BPA treated with nitrite under acidic conditions to simulate the stomach environment. BPA, purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), was dissolved in DMSO and co-incubated for 1 h at 37°C in buffer (1:100 v:v) containing 100 mM sodium nitrite at pH 3.0-5.0. The reaction mixture was extracted with ethyl acetate, evaporated to dryness, and the residue dissolved in DMSO for the mutagenicity tests: Ames test (reported under *in vitro studies*) and micronucleus test in mouse peripheral blood reticulocytes. For the micronucleus test, samples of nitrite-treated BPA dissolved in DMSO were administered by gastric intubation to male ICR mice (five per experimental group) at a dose of 228 mg/kg body weight (bw); untreated BPA dissolved in DMSO (228 mg/kg bw) served as control. Five microliters of peripheral blood were collected from a tail blood vessel at 24, 48 and 72 h after sample administration. Blood samples were stained on acridine orange and at least 1000 RNA-containing erythrocytes were observed by fluorescence microscopy for micronucleus evaluation. Results obtained indicated that BPA under the condition of the study did not induce a detectable increase of micronucleated reticulocytes in treated mice. Conversely, nitrite-treated BPA significantly increased the frequency of MNRETs compared with vehicle controls and untreated BPA at 48 and 72 h after oral administration.

Naik and Vijayalaxmi, 2009

This study evaluated potential genotoxic effects of BPA by induction of chromosomal aberrations and MN in bone marrow cells of Swiss albino mice. To assess for potential interference of BPA with mitotic spindle apparatus, induction of c-mitoses was also performed. BPA (Loba Chemie, Mumbai, India) was administered orally in a 2% acacia gum suspension at dose-levels of 10, 50 and 100 mg/kg bw to groups of three male and three female mice, as single acute dose. Cumulative dose-level experiments were also performed at the lowest (10 mg/kg bw) dose-level for five consecutive days. In single treatment schedule, sampling of bone marrow was performed at 6, 24, 48 and 72 h from beginning of treatment for both micronucleus and chromosome aberration assays. In cumulative treatment schedule, bone marrow was sampled in both assays 24 h after the last administration of BPA. For induction of c-mitoses, the same dose levels used for micronucleus and chromosome aberration assays were applied as single dose and sampling of bone marrow was performed at 2, 6, 12, 24, 48 and 72 h. Results showed that no significant increases of chromosomal aberrations or MN were induced at any dose-level and sampling time used. Conversely, significant increases in the frequencies of gaps were observed in all dose-levels assayed at the 48 and 72 h sampling time and at the two higher dose-levels (50 and 100 mg/kg bw) at the 24 h sampling time. The significant increases of achromatic lesions (gaps) are not considered relevant for clastogenicity. In addition, BPA also induced c-mitotic effects through increases of mitotic indices and decrease in anaphase for both higher dose-levels at 24, 48 and 72 h sampling times.

De Flora et al., 2011

The study evaluated the MN frequency in bone marrow cells and DNA damage by the alkaline comet assay in peripheral blood lymphocytes, in male Sprague-Dawley rats treated with BPA (Sigma-Aldrich) via drinking water for a calculated daily intake of 200 mg/kg bw for 10 consecutive days and sacrificed at the end of treatment. Eight rats were used for each experimental group (control and BPA treated animals). No increase of MN frequency was reported in the bone marrow. No increase of DNA damage, expressed as tail moment, was observed in peripheral lymphocytes.

Tiwari et al., 2012

This study was aimed to assess potential genotoxic effects of BPA (Sigma-Aldrich) in rats (five males and five females per group) following oral administration of test compound once a day for 6 consecutive days at dose-levels of 2.4 µg, 10 µg, 5 mg and 50 mg/kg bw by measuring induction of MN and structural chromosome aberrations in bone marrow cells and primary DNA damage in blood lymphocytes using single cell gel electrophoresis (comet assay). Furthermore, plasma concentrations of 8-hydroxydeoxyguanosine (8-OHdG), lipid peroxidation and glutathione activity were evaluated to assess potential induction of oxidative DNA damage. Results obtained for genotoxicity endpoints show marked dose-related increases of both MN and structural chromosome aberrations in bone marrow cells of male and female rats exposed to BPA. The observed increases achieved statistical significance at dose-levels as low as 10 µg/kg bw per day. Similarly, primary DNA damage evaluated by comet assay, in isolated peripheral blood lymphocytes showed marked and dose-related increases that were statistically significant at dose-levels as low as 10 µg/kg bw per day. Significant increase in plasma concentration of 8-OHdG was detected only at 50 mg/kg bw. A dose-related increase of malonaldehyde and decrease of glutathione were observed in liver.

Gajowik et al., 2013 [RefID 2120]

The study investigated the effects of 2-week exposure to BPA, either alone or in combination with X-rays, on the induction of genotoxic effects in different tissues of female mice. Outbred female mice were treated with BPA (no information on purity) at 5, 10, or 20 mg/kg in drinking water for 2 weeks. The statistically significant increase in DNA damage, evaluated by comet assay was observed in lung only at 5 and 10 mg/kg. Negative results were reported in all the other organs analyzed in the same animals (spleen, kidneys, liver and bone marrow). Increase of MN frequency was observed in peripheral blood reticulocytes, but not in the bone marrow after 2 weeks of exposure. No increase was detected after 1 week of treatment. No clear results were reported for the combined exposure BPA and X-rays for both the biomarkers.

Srivastava and Gupta, 2016 [RefID 9611]

The genotoxic effects of repeated oral exposure to BPA in adult male Wistar rats was evaluated. Groups of 10 animals were administered with BPA (Sigma-Aldrich) dissolved in olive oil at three dose levels (5 µg, 50 µg and 100 µg/100 g bw), with and without vitamin E (4 mg/100 g bw), once a day for 90 days and sacrificed on day 91. The positive control group was injected with mitomycin C (3 µg/g bw) 48 h before sacrifice. Bone marrow was collected and micronucleus frequency determined in polychromatic and normochromatic erythrocytes in bone marrow smears stained with May-Gruenwald and Giemsa. A slight increase of MN in polychromatic (three-fold) and normochromatic (two-fold) erythrocytes was observed comparing the overall incidence of MN in vehicle controls and high dose BPA in PCE (4/2362 vs 12/2456) and NCE (5/9635 vs 11/9712). No statistical analysis was performed. The effect was not observed when animals were co-administered with BPA + vitamin E. No deviation of PCE/NCE ratio was observed.

Fawzy et al., 2018 [RefID 270-G]

The study was conducted to evaluate the protective action of pumpkin seed oil (PSO) against adverse effects induced by BPA. BPA (Sigma-Aldrich) was administered orally to male Swiss albino mice at 50 mg/kg bw once a day for 28 days. PSO was administered at 1 mL/kg bw either before, with or after treatment of BPA, for 28 days. Seven groups of animals (n = 10) were treated: group 1 (control); group 2 (vehicle); group 3 (PSO); group 4 (BPA); group 5 (PSO before BPA); group 6 (PSO with BPA) and group 7 (PSO after BPA). DNA damage was evaluated by comet assay in liver and testes. Fifty randomly selected nuclei per experimental group were analysed. MN frequencies were evaluated in bone marrow. Two thousand polychromatic erythrocytes (PCE) were scored per animal. A significant (p<0.05) increase of tail DNA % in liver and testes of BPA-treated group with respect to controls (19.93 ± 0.68 vs 13.15 ± 0.22 and 23.56 ± 0.45 vs 15.00 ± 0.50) was observed. A significant increase of MNPCEs (66.40 ± 9.94 vs 10.40 ± 2.96) and a decrease in the ratio of PCE/NCE were also detected. The histopathological examination revealed hepatocyte vacuolar degeneration with many necrotic cells. A defective spermatogenesis was also observed characterized by severe necrosis and loss of the spermatogonial layers with multiple spermatid giant cells formation in most of the seminiferous tubules and a congestion of the interstitial blood vessels. The treatment with PSO reduced the genotoxic effects induced by BPA. PSO before BPA treatment was the best regimen in the alleviation of the adverse effects.

Panpatil et al., 2020 [RefID 379-G]

The study evaluated the protective action of turmeric acid on the genotoxic effects of BPA in Wistar rats. Six groups of six animals were administered with BPA (Sigma-Aldrich) at 0, 50 and 100 µg/kg by oral gavage for a period of 4 weeks: three groups were fed with a normal diet, the others with a diet containing 3% turmeric. At the end of the experiment the animals were sacrificed. Urine was collected 24 h before the sacrifice. 8-OHdG was measured in urine using an ELISA kit. DNA damage by comet assay was evaluated in blood, liver and kidney: 50 cells per slide were counted twice. Micronucleus assay was applied in bone marrow: 2000 PCE were evaluated. A weak but statistically significant and dose related increase of tail length was observed in liver. In kidney an increase of DNA damage was observed only at the dose of 50 µg/kg. A dose related increase of 8-OHdG in urine and of the concentration of MDA in blood serum was observed. A dose related increase of MNPCE was reported associated with a low decrease of the PCE/NCE ratio. A significant decrease of the genotoxic effects was observed in animal fed with diet with turmeric.

E.3.2. DNA damage on somatic cells

Ulutaş et al., 2011

The authors aimed to assess potential genotoxicity of BPA (purity > 99%) in peripheral blood nucleated cells of rats by comet assay. Groups of six rats were dosed orally for 4 weeks at dose-levels of 125 and 250 mg/kg bw. Control group animals (5 animals) were administered orally with corn oil for four weeks. At the end of treatment peripheral blood cells were collected via cardiac puncture and stored at 4°C until preparation of slides for comet assay. Authors showed significant increases of both tail length and tail moment for BPA only at the highest dose-level (250 mg/kg bw per day) used.

Dobrzyńska and Radzikowska, 2013

This study investigated the effects of BPA (no information on purity) alone or in combination with X-rays on the sperm and induction of DNA strand breaks in somatic and germ cells of mice. Male Pzh:SFIS mice received BPA orally in drinking water for 2 weeks. Levels in drinking water were designed to achieve BPA intakes of 0, 5, 10, 20 or 40 mg/kg bw per day. Two additional groups received either 5 or 10 mg BPA/kg bw per day via drinking water in combination with daily radiation doses of 0.05 Gy or 0.10 Gy of X-rays. For comet assay animals were sacrificed 24 h after the last treatment and DNA tail moment was used to assess the levels of DNA breakage induced in cells isolated from liver, spleen, bone marrow, lungs, and kidneys. Results obtained indicate that BPA induced statistically significant increases of DNA tail moment in bone marrow, spleen, kidney and lung cells at any of the dose-levels assayed. No DNA breakage was detected in liver cells.

Zhou YX et al., 2017 [RefID 9083]

The study investigated the neurotoxicity of low-dose exposure to BPA in a mouse model, examining brain cell damage and the effects of learning and memory ability after 8 weeks exposure to BPA at 0.5, 50 and 5000 µg/kg bw (daily dose, by gavage). The comet assay was used to detect brain cell damage. At the end of treatment 11 mice per group were sacrificed and brain processed for comet assay. Forty cells from each brain were analyzed. Based on tail DNA percentage, the damage level was divided into five grades, from 0 (undamaged) to 4 (maximum damage). The results obtained indicated that with increasing exposure concentrations the fraction of damaged cells (all types) increased significantly from 23.0% in the control group to 47.3%, 66.6% and 72.5% in the low-, medium and high exposed groups, respectively. Also, the severity of DNA damage, expressed as arbitrary units (AUs), increased with AUs of 0.28 in the control to AUs of 0.59, 0.96 and 1.28 in the low-, medium and high-exposed groups, respectively.

Abdel-Rahman et al., 2018 [RefID 199-G]

The study evaluated the protective action of lycopene (LYC), an antioxidant agent, on the toxic effects of BPA (Sigma-Aldrich). Four groups of seven Wistar rats were treated daily for 30 days via gavage: the first group (controls) received corn oil, the second group was given lycopene at a dose of 10 mg/kg bw, the third group was given BPA at 10 mg/kg bw, the fourth group was administered both BPA and LYC at the 10 mg/kg. Rats were sacrificed immediately after the last administration. Liver was frozen at -80 °C. Single-cell suspensions for comet assay were prepared from frozen livers. No positive controls were used. The comet method applied was not reported. A significant ($p < 0.05$) increase of tail DNA % in liver of BPA-treated group with respect to controls (25.05 vs 6.68) was observed. Higher activities ($p < 0.05$) of liver enzymes (serum ALT, alkaline phosphatase (ALP) and GGT and lower levels of total protein and albumin than control rats were detected in serum. Antioxidant enzymes (GPx, SOD and CYP450 activities) significantly ($p < 0.05$) decreased while MDA level significantly increased in liver of BPA treated animals. Caspase-3 protein in liver of BPA-treated rats is overexpressed. Histopathological analyses showed deleterious hepatic changes ranging from hepatocytes' vacuolization and eccentric nuclei to focal necrosis and fibrosis. LYC administration reduced the cytotoxic effects of BPA on hepatic tissue, through improving the liver function biomarkers and oxidant-antioxidant state as well as DNA damage around the control values.

Kazmi et al., 2018 [RefID 315-G]

The study evaluated the protective role of *Quercus dilatate* extracts against BPA (no information on purity) induced hepatotoxicity. Ten groups of SD rats (7 animals/group) were considered, including untreated control group and a group receiving the vehicle. The distilled water-acetone (QDDAE) and methanol-ethyl acetate (QDMEtE) extracts were administered in high (300 mg/kg bw) or low (150 mg/kg bw) doses to SD rats, intraperitoneally injected with BPA (25 mg/kg bw). A group of rats was treated only with BPA. Rats were sacrificed after 4 weeks of treatment and blood and liver were collected. The comet method applied was not sufficiently detailed. An increase of DNA strand breaks in hepatocytes was reported for animals treated with BPA alone. However, the results reported using the different parameters (tail length, % of DNA in tail, tail moment) are not consistent. The % of DNA in tail is 28.35 ± 1.2 in BPA treated animals vs 0.01 ± 0.005 in controls. The value of % of DNA in tail in controls is extremely low with respect to the data reported in the scientific literature. Significant reduction in haemoglobin level, red blood cells and platelet count, whereas elevated levels of white blood cells and erythrocyte sedimentation rate (ESR) were observed in the BPA treated group.

Administration of BPA significantly ($p < 0.05$) decreased the endogenous antioxidant enzyme (CAT, GPx, superoxide dismutase (SOD) and GSH) levels compared with control group. In addition, in the BPA treated group, H_2O_2 , nitrite and TBARS levels in the hepatic tissue were found to be higher when compared with controls. Histopathological examination of BPA treated animals revealed intense hepatic cytoplasm inflammation, centrilobular necrosis, cellular hypertrophy, fatty degeneration, vacuolization, steatosis and distortion of portal vein. A dose dependent hepatoprotective activity was exhibited by both the extracts of *Quercus dilatata* in different extent for the parameters analysed.

Elhamalawy et al., 2018 [RefID 510-G]

The study was aimed to evaluate the protective effects of sesame oil (SO) against BPA-induced hepatotoxicity in mice. Groups of male Swiss albino mice ($n = 10$) were given BPA (from Sigma-Aldrich, dissolved in ethanol and diluted in corn oil) by gavage at 50 mg/kg bw, with/without SO (1 ml/kg bw), once a day for 28 successive days. SO was administered through three regimens (before, with or after treatment with BPA). Liver DNA damage was evaluated by alkaline comet assay in 50 nuclei per experimental group. Mean tail length, tail moment and % tail DNA were significantly increased ($p < 0.05$) in liver of BPA treated mice. The effect was partially alleviated by SO administration at all the three regimens.

Sharma et al., 2018 [RefID 662-G]

The *in vivo* genotoxic potential of BPA in mouse organs was investigated using the alkaline comet assay. Male CD-1 mice (5 per group) were administered by gavage with BPA (Sigma-Aldrich) suspensions in corn oil prepared by ultrasonication at three dose levels (125, 250 and 500 mg/kg bw), twice 24 h apart. Ethyl methane sulphonate, given once by gavage at 300 mg/kg bw, served as positive control. Animals were sacrificed 3 h after the last treatment and DNA damage investigated by a commercial kit for comet assay in liver, kidney, testes, urinary bladder, colon and lungs cells. For each mouse, 200 cells were analysed (100 per gel) using an automatic comet assay scoring imaging system. Median values for each tissue from each animal were used, and the mean of the median values was evaluated in a statistical analysis. The results of comet assay did not show BPA related effects in any tissue, except for the testes, in which an increased level of DNA strand breaks ($p < 0.01$ compared with control group) was observed at the lowest dose; however, no dose response relationship was observed as the effects at the medium and highest doses were at the same level as the control group. A modified alkaline comet assay was conducted on human sperm cells treated with BPA 0, 1, 1.5, 2 and 3 $\mu\text{mol/L}$ for 1h. BPA 3 $\mu\text{mol/L}$ reduced cell viability to 60%, therefore it was the highest concentration tested. Ethyl methanesulfonate (EMS) was used as positive control. In total, 600 cells were scored for each concentration. No increase in % tail DNA was observed compared with the negative control.

Amin, 2019 [RefID 216-G]

The aim of the study was to evaluate the cardiotoxicity of BPA and to assess copeptin as a cardiotoxic diagnostic and prognostic biomarker. Three groups of Wistar rats were treated subcutaneously (SC) once daily (6 days/week) for 4 weeks: group I, naive group received regular diet and water; group II, vehicle group administered corn oil; and group III received BPA (Sigma-Aldrich) daily (30 mg/kg bw per day SC). Rats were sacrificed immediately after the last administration: blood samples were collected for estimating serum copeptin levels and the hearts were subjected to histological, immunohistochemical, and electron microscopic examination. Heart cells were isolated and processed for comet assay. A statistically significant increase of % of DNA in tail was detected in BPA treated animals with respect to controls (6.88 vs 1.67). BPA induced a significant increase in mean values of serum copeptin level and histopathological changes with dilated congested blood vessels and extensive collagen fiber deposition in the myocardium. Light microscopic examination revealed focal disruption of cardiomyocytes with some nuclear changes, such as karyolysis and pyknosis and sarcoplasmic vacuolization. The mitochondria appeared swollen and deranged with different sizes and shapes.

Majid et al., 2019 [RefID 354-G]

The study evaluated the protective role of sweet potato (*Ipomoea batatas* L. Lam.) against BPA-induced testicular toxicity. Sixteen groups of seven Male SD rats were established, including controls, animals treated with the vehicle, with ethyl acetate and methanol extracts from tuber and aerial part of *Ipomoea batatas*, with BPA (Merck KGaA) and with BPA and different extracts of *Ipomea batatas*. The BPA group

received 50 mg/kg bw dissolved in 10% DMSO, injected intraperitoneal on alternate days for 21 days. The rats were sacrificed 24 h after the last treatment. Comet assay was applied to evaluate the DNA damage. An average 50–100 cells were analysed in each sample for comet parameters (head length, comet length, tail moment, tail length, and amount of DNA in head) of gonadal cell's nuclei. A statistically significant increase of % DNA in tail (3 folds with respect to the control value) was reported in the group of rats treated with BPA. Endogenous antioxidant enzymes were measured in supernatant from the testicular homogenates: BPA decreased the levels of peroxidases (POD), CAT, SOD. BPA induced also gonadotoxicity measured as size and weight of testes and epididymis, concentration and quality of sperms. The treatment with extracts of *Ipomea batatas* significantly reduced the gonadotoxicity induced by BPA, the DNA damage and restored the levels of antioxidant enzymes.

Mohammed et al., 2020 [RefID 363-G]

The study evaluated the protective role of ginger extract (GE) against BPA-induced toxic effects on thyroid. Four groups of 20 male albino rats were treated orally with BPA (Sigma–Aldrich), GE or both once a day for 35 days as follow: Control group: 0.1 ml/rat of corn oil; BPA group: 200 mg/kg bw per day (1/20 of the oral LD50); GE group: ginger extract 250 mg/kg bw; BPA + GE group: ginger extract followed by BPA after 1 h with the same doses as the other groups. The animals were sacrificed 24 h after the last administration. DNA damage was evaluated by comet assay. A statistically significant increase of DNA damage expressed as tail % DNA, tail length and tail moment were shown in thyroid follicular cells of animals treated with BPA. A concurrent increase of MDA and a decrease of GSH, and SOD were also observed. Adverse effects on the thyroid gland were reported with a significant decrease in serum levels of T₃ and T₄ accompanied by a significantly increase in serum TSH level. A decrease of Nrf-2 mRNA relative expression and protein concentration and of HO-1 mRNA expression in the BPA-induced thyroid injured rats were also described. The histopathological analysis revealed an alteration of the thyroid gland follicles most of which containing scanty colloid secretion and some others atrophied. The treatment with GE significantly reduced the genotoxic damage and the alteration of thyroid hormones regulating genes.

Zhang et al., 2020 [RefID 485-G]

The study investigated the long-term neurotoxicity of maternal exposure to BPA in mouse offspring. Pregnant mice (F0) were orally dosed with BPA (from Sigma-Aldrich) dissolved in tea oil at 0.5, 50, 5000 µg/kg bw per day until weaning. Then, the first generation (F1) of mice were used to generate the F2. DNA damage in brain cells was evaluated by comet assay in eight male and eight female mice from both F1 and F2. DNA damage, expressed as AU, was slightly (less than 2-fold) increased in the F1 male mice at the lowest dose and in females at the intermediate dose. No effect of BPA exposure was observed in the F2 mice.

E.3.3. DNA damage on germ cells

Ullah et al., 2019 [RefID 443-G]

The study evaluated the effect of subchronic exposure to BPA (Santa Cruz Biotechnology) and the analogues bisphenol B, F and S, on DNA integrity of rat spermatozoa. Sprague-Dawley rats (7 per group) received daily administrations of bisphenols by gavage in 0.1% ethanol solution at 5, 25 and 50 mg/kg bw per day. Animals were sacrificed on day 29th and DNA damage in spermatozoa from the cauda epididymis evaluated using a modified neutral comet assay scoring 100 cells per animal. Both tail moment and % tail DNA were significantly ($p < 0.05$) increased in the BPA 50 mg/kg bw per day group compared with vehicle controls, while no significant difference with controls was observed in the BPA 5 and 25 mg/kg bw per day groups. Comet assay was also performed on rats sperm cells treated *in vitro* with BPA 0, 1, 10, and 100 µg/L for 2h. A statistically significant increase in % tail DNA was observed at the highest concentration tested. Under the same treatment conditions statistically significant increases in SOD, TBARS and total ROS were observed at BPA 100 µg/L.

Zhang S et al., 2019 [RefID 486-G]

The study investigated the effects of maternal exposure to BPA on testicular development in offspring males. Pregnant Kunming mice were randomly divided into seven groups with 20 mice in each group. One group served as control, the others received BPA (Sigma, USA) in drinking water at 0.05, 0.5, 5,

10, 20, and 50 mg/kg bw per day, for 40 days from gestation day 0 to lactation day 21. F1 male mice were sacrificed at weaning (PND21). Testicular DNA damage was evaluated by comet assay and expressed as OTM. The results obtained showed that the testicular germ cell damages of group D, group E, group F and group G (5, 10, 20 and 50 mg/kg bw per day, respectively) were significantly higher than that of the control group ($p < 0.05$). No experimental data are shown.

Pan et al., 2020 [RefID 377-G]

The study evaluated the effect of BPA on DNA integrity and protamination of mouse sperm cell. Newborn male mice were subcutaneously injected with BPA (from Sigma-Aldrich, 0.1 and 5 mg/kg bw, $n = 15$) or corn oil (control group, $n = 20$) daily from PND 1 until 35. At PND 70, epididymis caudal spermatozoa and testes were collected. A flow cytometry sperm chromatin structure assay (SCSA) was performed to evaluate DNA fragmentation of sperm cells. DNA fragmentation was expressed as DNA Fragmentation Index (DFI), calculated as the ratio between sperm cells with strands DNA. Apoptosis of spermatogenic cells (spermatogonia, spermatocytes, round spermatids, and elongated spermatids) was determined by the TUNEL method. Exposure to BPA was associated with a significant ($p < 0.01$) and dose-related increase of the DFI in sperm cells and apoptosis in spermatogenic cells ($p < 0.05$).

Sahu et al., 2020 [RefID 405-G]

The study investigated BPA toxicity in sperm cells of juvenile Sprague-Dawley rats. BPA (Sigma-Aldrich) was administered by gavage daily for 5 days per week for 8 consecutive weeks to 4 rat groups (7 animals/group, of which only 4 used for genotoxicity assessment): i) Control (normal feed and water); ii) BPA (100 mg/kg bw per day); iii) control fed with a zinc deficient diet (ZDD); IV) BPA + ZDD. Sperm DNA damage was evaluated by the comet and Halo assays, apoptosis in testes cells was quantified by TUNEL assay, and testicular levels of 8-OHdG were determined by immunohistochemistry. All comet assay parameters (tail length, OTM and % DNA in the tail) and the nuclear diffusion factor in the halo assay, were slightly but not significantly increased in testes cells of BPA treated rats compared with controls. When associated with a ZDD, BPA exposure resulted in a significant ($p < 0.05$) effect in both assays. TUNEL-positive cells and percent of 8-OHdG positive areas in testicular tissue were also slightly but non significantly increased in BPA treated rats fed with standard diet, while the effect was statistically significant in rats receiving a ZDD.

Zahra et al., 2020 [RefID 481-G]

The study aimed to evaluate the protective potential of methanol extract of the Apocynaceae plant *Vincetoxicum arnottianum* (VAM) on BPA induced testicular toxicity in male SD rat. BPA (source and purity not specified) diluted in 10% DMSO was injected intraperitoneally at 25 mg/kg on alternate days for 30 days, with or without the oral co-administration of VAM extracts (150 and 300 mg/kg bw) to groups of seven rats. Sperm DNA damage was evaluated by comet and DNA ladder assays. Subchronic administration of BPA was associated with a significant ($p < 0.01$) increase of all comet parameters compared with vehicle controls. The effect was attenuated by co-administration of VAM extract in a dose-related manner. Electrophoresis on agarose gel showed extensive DNA fragmentation in testes of BPA treated rats.

Tiwari and Vanage, 2013

This study investigated the induction by BPA of dominant lethal mutations in the different stages of spermatogenesis in the rat. Furthermore, the induction of DNA damage by BPA in epididymal sperm was investigated. Holtzman male rats (7 per group) were treated by oral gavage with BPA (Sigma Chemical Co.) dissolved in ethyl alcohol and diluted in sesame oil, at dose-levels of 10 μ g/kg bw and 5 mg/kg bw once a day for 6 consecutive days. Negative controls were treated with vehicle. Each treated male was mated with two females per week over a period of eight weeks. The mated females were then sacrificed on the day 15th of their gestation and uterine content examined. DNA damage in epididymal sperm was evaluated by alkaline comet assay in sperm samples from treated males (4 animals per group) sacrificed after completion of the mating phase. In the dominant lethal study, a significant decrease in total implants/female and live implants/female, with a concurrent significant increase in the number of resorbed embryos per female, was observed during the fourth week and sixth week in females mated with males treated with 5 mg BPA/kg bw, suggesting the induction of

post-implantation loss due to dominant lethal mutations in mid-spermatids and spermatocytes. No significant change was observed in the pre-implantation and post-implantation losses in pregnant female mated with males exposed to 10 µg/kg bw of BPA. In the comet assay with epididymal sperm, a significant increase in comet parameters (tail length, tail moment and % tail DNA) was observed in rats treated with 5 mg/kg bw compared with control.

E.3.4. Effects on meiosis

Pacchierotti et al., 2008

The study evaluated the potential aneugenic effects of BPA on mouse male and female germ cells and bone marrow cells following acute, subacute or subchronic oral exposure. For experiments with acute and subacute exposure, female C57BL/6 mice were treated by gavage with BPA (from Sigma-Aldrich) dissolved in corn oil once with 0.2 and 20 mg/kg bw, or with seven daily administrations of 0.04 mg/kg bw. In subchronic experiments, mice received BPA in drinking water at 0.5 mg/L for 7 weeks. The dose levels tested for subacute effects in bone marrow and male germ cells were 0.002, 0.02 and 0.2 mg/kg bw for 6 days. For the assessment of aneugenicity in female germ cells, M II oocytes were harvested 17 h after induced superovulation, and cytogenetically analyzed after C-banding. The percentages of metaphase I-arrested oocytes, polyploid oocytes and oocytes that had undergone Premature Centromere Separation (PCS) or Premature Anaphase II (PA) were calculated. To evaluate the aneugenic effects of BPA upon the second meiotic division, zygote metaphases were prepared from superovulated females mated with untreated C57Bl/6 males. Zygote metaphases were prepared, C-banded and cytogenetically analyzed for the occurrence of polyploidy and hyperploidy. Experiments on male germ cells were performed with 102/ElxC3H/EI)F1 males. Epididymal sperms were collected and hybridized with fluorochrome-labelled DNA probes for chromosomes 8, X and Y and 10,000 sperm per animal were analyzed to evaluate the incidence of hyperhaploid (X88, Y88, XY8) and diploid (XY88, XX88, YY88) sperm cells. Micronucleus test was performed with four groups of five (102/ElxC3H/EI)F1 male mice treated with 0, 0.002, 0.02 or 0.2 mg/kg BPA by gavage on 2 consecutive days and sacrificed 24 h after the second administration. In total, 2000 PCE from two slides were scored per animal for the presence of MN. No significant induction of hyperploidy or polyploidy was observed in oocytes and zygotes at any treatment condition. The only detectable effect was a significant increase of M II oocytes with prematurely separated chromatids after chronic exposure; this effect, however, had no consequence upon the fidelity of chromosome segregation, as demonstrated by the normal chromosome constitution of zygotes under the same exposure condition. Similarly, with male mice no induction of hyperploidy and polyploidy was shown in epididymal sperm after six daily oral BPA doses, and no induction of MN in PCE.

Liu C et al., 2014 [RefID 4378]

The study authors exposed 9-week-old male Wistar rats to BPA (Sigma-Aldrich) by gavage at 20 µg/kg bw per day for 60 consecutive days to coincide with one cycle of spermatogenesis. At the end of treatment testes were removed for the analysis of the staging of the seminiferous epithelium and microscopic analysis of meiotic chromosomal spreads. The formation of DNA double strand breaks in meiotic cells was assessed by immunofluorescence staining with anti-γH2AX antibodies. Immunostaining for synaptonemal complex protein 3 (SCP3), ATM kinase and pCHK1 was also performed on the spread nuclei. The assessment of the relative frequency of the 14 stages of the cycle of the seminiferous epithelium showed significant increases in stage VII and decreases in stages VIII and XIV, indicating an impairment of spermatogenesis following BPA exposure. Meiotic recombination, initiated by programmed double-strand breaks (DSBs) and marked by γH2AX expression, was also impaired in BPA-exposed rats. BPA exposure resulted in meiotic DSBs accumulation in pachytene spermatocytes. These cells exhibited chromosomal abnormalities, including asynapsis, end-to-end associations and interrupted regions of SCP3 staining, and increased activation of ATM. The authors concluded that BPA exposure at an environmentally relevant dose (20 µg/kg bw per day) induced meiotic abnormalities in adult male rats, with inhibition of spermiation and disruption of spermatogenesis. BPA exposure resulted in the delayed initiation of meiosis in the early meiotic stage and chromosomal abnormalities and meiotic DSBs accumulation in the late meiotic stage, which subsequently activated the ATM DNA damage checkpoint kinase.

Vrooman et al., 2015 [RefID 7521]

The aim of the study was to test the effect of estrogenic chemicals on meiotic chromosome dynamics during neonatal development when the first cells initiate meiosis. Outbred CD-1 and inbred C57BL/6J (B6) newborn male mice were given single oral doses of 20 or 500 ng/g per day BPA (supplied by NIEHS), ethinyl estradiol (EE) as positive control 0.25 ng/g per day or vehicle from 1–12 days post-partum (dpp). In total, six to 12 males (one to three males per litter from at least three litters) for each exposure group at 20 dpp at 12 weeks or 1 year of age (CD-1 only) were analyzed to assess the effects on synapsis and recombination. Immunostaining surface spread preparations of meiotic cells with antibodies for SYCP3 (a component of the synaptonemal complex) and MLH1 (DNA mismatch repair protein that localizes to the large majority of sites of meiotic exchange) was applied to evaluate the synapsis and recombination foci. Perturbations in synapsis were not observed in either strain, but MLH1 foci were significantly reduced in BPA and EE exposed CD-1 males. Mean MLH1 counts for juvenile CD-1 males were 22.27 ± 0.12 , 21.50 ± 0.10 , 21.87 ± 0.10 , and 20.85 ± 0.10 for placebo, 20 ng BPA, 500 ng BPA, and 0.25 ng EE-exposed, respectively ($p < 0.0001$). No differences were detected in B6 males with any exposure. Similar reductions were observed in CD-1 males at 12 weeks and at 1 year. Insensitivity of B6 males to estrogen was considered as a reflection of genetic differences. The study shows that a brief exposure of newborn male mice to exogenous estrogen reduces the meiotic recombination increasing the incidence of meiotic errors and affecting permanently the spermatogenesis in the adult. The effect induced by BPA is similar to the positive control, no dose-response was observed for the BPA doses tested.

Zhang MQ et al., 2017 [RefID 8813]

The study investigated the protective role of melatonin against the adverse effects induced by BPA on the female reproductive system in mice. Three groups of female ICR mice were used: a control group, a BPA group treated daily with oral doses of BPA (supplier company and purity not reported) at 100 $\mu\text{g}/\text{kg}$ bw and a group treated with BPA+melatonin (administered daily at 15 or 30 mg/kg bw) for 7 days preceding oocyte collection and analysis. The number of animals in each group was not reported. Oocytes were immunostained with anti- α -tubulin-FITC antibody to observe the spindle morphologies and counterstained with Hoechst to visualize the chromosome alignment. The treatment with BPA was shown to disrupt normal spindle assembly (70% of oocytes had disrupted spindles) chromosome alignment (80% of oocytes exhibited misaligned chromosomes). Metaphase I oocytes were briefly chilled to induce the depolymerization of microtubules that are not attached to kinetochores and then immunostained with CREST to detect kinetochores, with anti- α -tubulin-FITC antibody to visualize the microtubules and counterstained with Hoechst to observe the chromosomes. An increased frequency of kinetochores without attachment by microtubules was observed in BPA-exposed oocytes ($76.1 \pm 6.1\%$ $n = 99$ vs $19.7 \pm 1.8\%$, $n = 92$). A statistically significant higher frequency of aneuploid oocytes in M II was also observed in BPA-exposed oocytes ($42.6 \pm 1.8\%$, $n = 94$ vs $3.7 \pm 3.7\%$, $n = 82$). In BPA-exposed oocytes, the fluorescence intensity of ROS was increased compared with controls (153.8 ± 7.7 , $n = 138$ vs 70.9 ± 3.1 , $n = 96$). An increase of early apoptosis was shown by the annexin-V signal. In addition, BPA-exposed eggs had significantly reduced fertilization rates ($41.1 \pm 1.5\%$, $n = 65$ vs $87.2 \pm 0.9\%$, $n = 72$). The melatonin administration significantly improved the oocyte quality via reduction of ROS levels and inhibition of apoptosis.

Horan et al., 2018 [RefID 291-G]²³

In a comparative study on the meiotic effects in mice of BPA and its analogues, oral doses of 20 ng/g BPA (purity > 99%)²⁴ or placebo (vehicle only) control were administered on 14 and 15 days post coitum (dpc) to coincide with the time of meiotic entry in the fetal ovary. Variation in meiotic recombination was measured by the number of MLH1 foci in pachytene stage meiocytes. By comparison with unexposed female fetuses, BPA induced a significant increase in mean MLH1 counts ($p < 0.01$), indicating increased levels of meiotic recombination in developing oocytes as a result of maternal BPA

²³ Information on BPA purity provided by the study authors on 4 November 2021, upon EFSA request

exposure. According to the study authors, the subtle changes in meiotic recombination induced are compatible with continued oocyte survival, but increase the frequency of aneuploid eggs and embryos produced by the adult female. In contrast, in male neonatal exposure to BPA causes a permanent reduction in recombination levels spermatocytes, with an increase in the frequency of spermatocytes with at least one synaptonemal complex lacking an MLH1 focus. Low recombination rates were also considered deleterious because spermatocytes with homologues that failed to undergo recombination faced death due to the actions of the spindle assembly checkpoint mechanism that causes arrest of cells with unpartnered chromosomes at metaphase. Further data are presented in this work on the effects on male meiotic recombination in the progeny of 129S1/SvlimJ males (3 animals) inadvertently exposed to BPA released by PC cages, showing that the reduction of meiotic recombination rates persists for several generation (up to F3).

E.4. Other studies

E.4.1. DNA adducts

Izzotti et al., 2009

The study evaluated the formation of DNA adducts in female CD-1 mice receiving BPA (from Sigma Chemical Co.) in their drinking water (200 mg/kg bw) for 8 consecutive days by the ³²P postlabelling method. Results obtained indicated that administration of BPA, under the experimental conditions of the study, resulted in the formation of bulky DNA adducts (two major DNA adduct) in the liver (3.4 fold increase over control level) as well as in the target mammary cells (4.7 fold increase over control level). The level of DNA adducts in the liver of BPA-treated mice, as evaluated in three replicate analyses, was $5.88 \pm 0.29/10^8$ nucleotides (means \pm SE of the data obtained in five mice) vs. 1.71 ± 0.14 in controls ($p < 0.001$). The level of DNA adducts in the pooled mammary cells of BPA-treated mice was $4.97 \pm 0.61/10^8$ nucleotides (means \pm SE of three replicate analyses) vs. 1.05 ± 0.13 in controls ($p < 0.001$). DNA adducts were not chemically characterized, but the authors noted that DNA adducts formed in liver and mammary cells had the same chromatographic mobility as those formed *in vitro* by the reaction of BPA with calf thymus DNA in the presence of S9 mix .

E.4.2. γ H2AX

Chianese et al., 2018 [RefID 249-G]

The study investigated the effects of chronic exposure to the low dose of BPA, from foetal period to sexual maturation, on post-natal testis development. Wistar rats were used. BPA (Sigma Aldrich) at 0.1mg/L BPA was orally administered to dams or weaned offspring *via* drinking water. The daily dose of 10 μ g/kg bw was calculated based on daily drinking consumption (the BPA dose used was lower than or within the reference limit for humans, currently considered "safe" by ESFA and by EPA). To analyze the possible effects of BPA on the first round of spermatogenesis, the male newborns were sacrificed at 17 PND (late infantile), 45 PND (pubertal), or 60 PND (young adult), randomly choosing a total of five animals/treatment/time point from different litters. Serum levels of BPA did not differ between exposed and control groups. The cytoarchitecture of the seminiferous epithelium was impaired in BPA-exposed animals due to low expression and scattered localization of connexin 43 (Cx43) and zonula occludens 1 (ZO-1), well-known markers of the BTB. DNA breaks were detected by immunofluorescence for γ H2AX in spermatocytes and in round spermatids. A statistically significant increase of the expression rate of the crossover-associated protein *Mlh1*, a marker of DNA mismatch repair system and of *Rad51*, which encodes DNA repair protein (detected by quantitative real-time RT-PCR) was observed in all BPA-exposed rats. Massive and diffuse ROS production was observed in the testis of BPA-exposed animals. CAT and SOD significantly decreased at both 45 and 60 PND in BPA-exposed animals, compared with controls. Oxidative stress at DNA level induced by BPA was determined by 8-OHdG immunostaining. Immunofluorescence for 8-OHdG revealed diffused DNA damage in the germinal epithelium of treated animals. TUNEL signal was detected in the germinal compartment at basal levels and in Sertoli cells in BPA-exposed animals at 45 and 60 PND. A significantly higher fraction of apoptotic cells/tubule was detected in BPA-exposed animals. A different pattern of *sirt1* mRNA expression was observed in BPA-treated rats with respect to the controls during the first round of spermatogenesis at

17, 45 and 60 PND with highest expression levels observed at 45 PND and lowest expression rate observed at 60 PND. SIRT1 has a recognized role in spermatogenesis in the regulation of the hypothalamus-pituitary-gonad (HPG) axis. *SIRT1* impairment may affect the progression of spermatogenesis towards late meiotic and post-meiotic stages, and the maturation of spermatozoa.

Yang et al., 2021 [RefID 471-G]

The aim of this study was to investigate the mechanisms underlying BPA-aggravated atherosclerosis. Four-week-old male *Ldlr*^{-/-} C57BL/6 mice were administered 250 mg/L BPA (Sigma-Aldrich) via drinking water for 30 weeks with or without a western diet and/or resveratrol (RESV) for 12 weeks. The results indicate that chronic BPA exposure significantly aggravated atherosclerosis, enhanced the production of inflammatory cytokines and promoted macrophage infiltration into plaque areas. Peritoneal macrophages isolated from BPA-exposed mice exhibited a more pro-inflammatory phenotype in response to cholesterol crystal treatment than those from control mice. In comparison with the control group no change in the levels of DNA breaks (as measured by tail moment or tail DNA % in comet assays) was found in macrophages isolated from BPA-treated mice (data are not reported). The authors conclude that the DNA repair capacity of BPA-exposed macrophages, as measured by the kinetics of reduction in the number of DNA breaks following methyl methanesulfonate treatment, are reduced in comparison to the control group. However, the significance of a delay in DNA strand joining is uncertain and no quantitative evaluation of the data is presented. The overall significance of these contradictory results is uncertain.

E.4.3. DNA oxidative damage

Esplugas et al., 2018 [RefID 267-G]

The aim of the study is to investigate the renal and hepatic effects induced by a co-exposure to low doses of ionizing radiation and BPA. Sixty male mice (C57BL/6J) were randomly assigned to six experimental groups (n = 10) at PND 10 and received a single subcutaneous dose of 0.9% saline solution, BPA (Sigma-Aldrich) (25 µg/kg bw), cesium at two different doses or combined BPA and cesium. Urine (24 h) and blood were collected after 2 months. No increase of 8-OHdG in liver and kidney was observed in BPA treated mice. Following exposure to BPA the mRNA expression of CYP1A2 in liver decreased in respect to that in the control group. Limitations: only a single subcutaneous dose was applied; the analyses were carried out after 2 months. The study has a low relevance for the genotoxicity evaluation.

Franklyn et al., 2020 [RefID 521-G]

The study investigated the oxidative damage induced *in vivo* in rats by the subchronic administration of BPA, alone or in combination with chromium (VI), through the determination of 8-OHdG adducts in urine. Groups of five young adult rats received water (10 ml/kg bw per day) and served as controls, or BPA (from Sigma-Aldrich) (dissolved in DMSO and water at 2 mg/kg bw per day) or BPA (4 mg/kg bw per day) together with potassium dichromate (1.28 µg/kg bw per day) by gavage for 28 consecutive days. Urine was collected every week and the formation of 8-OHdG analyzed using LC-MS/MS with reverse-phase chromatography. The formation of 8-OHdG was detected in urine samples from BPA treated rats from week 1 onwards, with a slight increasing trend. The lowest and highest 8-OHdG concentrations were equal to 27.597 ng/mL and 31.683 ng/mL, respectively. 8-OHdG level in urine of control animals (only shown in a graph) was apparently zero. 8-OHdG levels in urine of rats treated with BPA and CrVI were higher than in the BPA group only at week 4. The authors concluded that CrVI had a synergistic effect on the formation of 8-OHdG by BPA, but the lack of a groups treated with Cr VI alone, and the different BPA levels administered without (2 mg/kg bw per day) and with Cr VI (4 mg/kg bw per day) prevent a firm conclusion on this aspect. This study did not follow a validated test procedure for which an OECD TG is available. No positive control was used. The study has a low relevance for the genotoxicity assessment due to the experimental limitations.

E.5. Epidemiological studies

Lv YS et al., 2017 [RefID 4702]

The study investigated the contribution of BPA exposure via dermal contact route to human body burden and the relationship between BPA exposure level and oxidative DNA damage. Six male students living at the university were recruited and required to simulate the cashiers' work in handling the thermal receipts for 8 h/day for a period of 5 days. The volunteers were provided the same foods and drinks during the experimental periods to control the variations of BPA exposure levels from dietary and dust ingestion. Urine were collected for 5 days before the simulation, during the experimental period, and for 2 days after the simulation. BPA and 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentrations were determined by high performance liquid chromatography/tandem spectrometer (LC/MS/MS). BPA concentrations was shown to increase four times during the simulation period compared with those before the experimental period. A similar trend was observed also for 8-OHdG. No significant decrease of both the parameters was reported in the post-experimental period. It was estimated that the contribution ratio of BPA via dermal contact to urinary BPA was 70.9%. BPA levels for individuals showed a similar trend with a large interindividual variability. A weak correlation, although statistically significant, was observed between urinary BPA and urinary levels of 8-OHdG ($R^2=0.237$, $p < 0.001$). The study presents several limitations such as the low number of the subjects, the short post-experimental period, and does not allow any clear conclusion to be drawn.

Huang YF et al., 2017b [RefID 2926]

In this study the association between pre-natal exposure to nonylphenol (NP) and BPA and inflammation biomarkers was investigated in 241 mother-fetus pairs. Third-trimester urinary NP and BPA levels, and urinary biomarkers of oxidative/nitrative stress, were simultaneously measured in 233 urine samples. The biomarkers included products of oxidatively and nitratively damaged DNA (8-hydroxy-2'-deoxyguanosine (8-OHdG) and 8-nitroguanine (8-NO₂Gua)), as well as products of lipid peroxidation (8-isoprostaglandin F_{2a} (8-isoPF_{2a}) and 4-hydroxy-2-nonenal-mercapturic acid (HNE-MA)), analyzed by HPLC electrospray ionization (ESI)-MS/MS. Inflammatory biomarkers (IL-6, TNF- α and C reactive protein) were analysed in maternal and umbilical cord plasma samples by immunoassays, and the antioxidant GPx by a commercial kit. Maternal urinary NP and BPA levels were detectable in 99.2% and 82.2%, respectively. The geometric mean of the NP and BPA levels were 3.99 and 2.24 $\mu\text{g/g}$ creatinine, respectively. After controlling for covariates, NP was significantly associated with increases in 8-NO₂Gua and 8-OHdG and decreases in TNF- α in the pregnant women. BPA was significantly associated with increased maternal 8-isoPF_{2a} levels and decreased maternal and cord blood GPx levels. This study did not follow an internationally agreed guideline, not available for the effects evaluates, but it was adequately performed and reported.

Radwan et al., 2018 [RefID 390-G]

The study examined the associations between urinary BPA concentration and male fertility in 315 men under 45 years of age with normal sperm concentration, attending a male reproductive health clinic for diagnostic purposes. Participants provided urine and semen samples on the same day of their clinic visit. BPA urinary concentrations were measured using gas chromatography coupled with tandem mass spectrometry. Semen parameters (sperm concentration, motility and morphology) were analyzed by standard procedures. Sperm DNA damage was assessed by a flow cytometry based sperm chromatin structure assay (SCSA). Sperm aneuploidy was evaluated by multicolour FISH analysis using DNA probes specific for chromosomes 13, 18, 21, X, Y; based on the information on six types of chromosome disomies, total disomy rates were calculated. A multiple linear regression analysis identified a positive association between the urinary concentrations of BPA in the 25th to -50th percentile and total sperm sex chromosome disomy ($p = 0.004$), but not with the higher quartiles (50th to -75th and > 75th). When modelled as continuous variable, urinary BPA concentration was associated with increased sperm sex chromosome disomy ($p = 0.01$). Such statistically significant relation was not noticed in case of sperm total chromosome disomy. Urinary concentration of BPA was also associated with increased percentage of immature sperms ($p = 0.018$) and decrease sperm motility ($p = 0.03$). No statistically significant association was observed between urinary BPA and sperm DFI. This study did not follow an internationally agreed guideline, not available for the effects evaluates, but it is adequately planned and reported. As acknowledged by the study authors the findings reported need to be confirmed in future studies. The lack of association between sperm disomy and BPA concentration in the higher quartiles is noticed.

Omran et al., 2018 [RefID 373-G]

The aim of the study is to evaluate a possible role of BPA exposure to the infertility in men. This case-control study involved 50 infertile patients and 50 matched controls. Sperm concentration, morphology, and motility were evaluated to estimate the semen quality. Urinary BPA levels were measured by high-performance liquid chromatography. Sperm DNA damage was determined by alkaline comet assay. Oxidative stress (total antioxidant activity and MDA levels) was also determined. BPA concentrations were similar in urine samples from all infertile patients and fertile controls, with median values of 24.2 µg/L and 20.9 µg/L, respectively. BPA levels were negatively associated with semen quality and antioxidant levels. The parameters of comet assay were statistically significant higher in infertile patients with respect to the controls (mean tail DNA % was approximately three-folds). The correlations between BPA levels and the comet parameter were analysed: weak but statistically significant correlations were reported for tail DNA % in the control group and in the total cases (Spearman's correlation coefficient: 0.500 (p value < 0.001) and 0.199 (p value < 0.05), respectively). The study presents some limitations: number of subjects recruited, the lack of the analysis of possible confounding factors.

Rocha et al., 2018 [RefID 402-G]

In this study, concentrations of 40 EDCs, including BPA, were determined in urine samples collected from 300 Brazilian children of ages 6–14 years. Oxidative DNA damage was evaluated from the urinary concentrations of 8-hydroxy-2'-deoxyguanosine (8-OHdG). BPA was the major compound found in 98% of the samples analyzed at concentrations ranging from < LOD to 35.9 ng/mL with a geometric mean of 1.74 ng/mL. Statistically significant correlation based on conventional univariate statistics was found between log-transformed urinary concentrations of 8OHdG and BPA. The study has low relevance for risk assessment due to the limitations in identifying the association in a scenario with multiple exposures.

Kiwitt-Cárdenas et al., 2021 [RefID 321-G]

In this cross-sectional study the association between urinary BPA concentrations and human sperm DNA fragmentation was investigated. The study was conducted with 158 healthy university students (18–23 years) that provided urine and semen samples on a single day. Urinary BPA concentrations were measured by dispersive liquid–liquid microextraction and ultrahigh performance liquid chromatography with tandem mass spectrometry detection. Sperm DNA fragmentation was analyzed by a Sperm Chromatin Dispersion (SCD) test, which measures halos of DNA loop dispersion after sequential incubation in acid and lysis solution under fluorescence microscopy, using a commercial kit. A SDF (sperm DNA fragmentation) index was calculated as the percentage of sperm with fragmented DNA divided by the total sperm analyzed (300 for each sample). No association was found between urinary BPA concentrations and SDF index in the total study population. A significant positive association with urinary BPA levels was only observed in the subgroup of men with SDF index > 30% (59 subjects). This study used a non-standards method to evaluate sperm DNA fragmentation that is not validated for regulatory purpose.

E.5.1. Polymorphisms in DNA repair genes

Kim and Hong, 2020 [RefID 564-G]

This study investigated the possible role of nine polymorphisms in three DNA repair genes (poly(ADP-ribose) polymerase family member 4 (*PARP4*); X-ray repair cross complementing 3 (*XRCC3*); RAD51 recombinase (*RAD51*)), on the relationship between BPA exposure and liver abnormalities in an elderly population. In total, 502 older adults without liver disease or renal failure aged 60 or over were included in the study. Urinary BPA, malonaldehyde (MDA) and cotinine levels (to monitor tobacco exposure) were measured. The serum levels of ALT, AST, and γ-GTP enzymes were used to detect liver abnormalities. LDL cholesterol levels were also measured. A significant association between BPA levels and liver abnormality was found only in elders with the *PARP4* G-C-G haplotype, *XRCC3* G-A-G haplotype, or *RAD51* T-A A haplotype (OR = 2.16 for *PARP4*; OR = 1.57 for *XRCC3*; OR = 1.43 for *RAD51*). Particularly, *PARP4* and *XRCC3* showed significant interactions with BPA exposure in relation to liver abnormality (p < 0.05 for both genes). These results suggest that *PARP4*, *XRCC3*, and *RAD51*

gene polymorphisms may have modification effects on the relationship between BPA exposure and liver abnormality.

DRAFT

Appendix F – Benchmark dose analysis from which the reference point was selected (Luo et al., 2016 [RefID 4679])

F.1. Data Description

Th17 cell frequency in the spleen in offspring mice (%) was analysed. In the paper, the data are represented in a graph and the actual numbers were provided to EFSA by T. Shen. Based on the provided information, EFSA calculated the standard deviation (SD). The group, specified by sex and day of measurement, was used as a covariate. The 10/11 animals within each group are coming from different litters, supporting the independence assumption for which no litter effect was considered in the analysis. The data were treated as continuous data since the number of cells in the denominator were not available to consider the data as a quantal response. Both controls (blanc and vehicle) were not significantly different (t-test, $p=0.89, 0.94, 0.74, 0.82$ for male PND21, female PND21, male PND42, female PND42, respectively) and used in the analysis.

Table F1: Data used for analysis.

Dose ($\mu\text{g}/\text{kg}$ bw per day)	Mean	SD	n	group
0.000	1.336	0.360	10	female PND21
0.000	1.348	0.310	10	female PND21
0.475	1.625	0.343	10	female PND21
4.750	2.200	0.663	10	female PND21
47.500	3.336	0.637	11	female PND21
0.000	1.222	0.233	10	female PND42
0.000	1.252	0.326	10	female PND42
0.475	1.541	0.325	10	female PND42
4.750	1.819	0.556	10	female PND42
47.500	2.477	0.477	11	female PND42
0.000	1.347	0.369	10	male PND21
0.000	1.327	0.282	10	male PND21
0.475	1.554	0.242	10	male PND21
4.750	1.774	0.501	10	male PND21
47.500	2.405	0.532	11	male PND21
0.000	1.194	0.235	10	male PND42
0.000	1.234	0.287	10	male PND42
0.475	1.426	0.278	10	male PND42
4.750	1.646	0.477	10	male PND42
47.500	2.226	0.526	11	male PND42

F.2. Selection of the BMR

An adverse outcome pathway for BPA leading to allergic responses that can be modelled to establish a BMD is currently not available. What can be stated is that T helper cells are key players in the immune-inflammatory chain of molecular events leading to amplification or suppression of specific immune elements, orienting the immune response towards effective resolution or chronic disease, and, according to an equilibrium in which these same cells and through the production of specific cytokines, restrict each other's own activity. Functionally, T helper 17 cells play a role in host defence against extracellular pathogens by mediating the recruitment of inflammatory cells to infected tissues. Aberrant regulation of Th17 cells plays a significant role in the pathogenesis of multiple inflammatory and autoimmune disorders. The most notable role of IL-17 produced by Th17 cells is its involvement in inducing and mediating proinflammatory responses, associated with allergic responses. IL-17 induces the production of many other cytokines (such as IL-6, G-CSF, GM-CSF, IL-1 β , TGF- β , TNF- α), chemokines (including IL-8, GRO- α , and MCP-1), and prostaglandins (e.g., PGE2) from many cell types (fibroblasts, endothelial cells, epithelial cells, keratinocytes, and macrophages). As such, numerous

studies have shown that Th17 cells and their cytokines are also associated with the development of asthma (Doe et al., 2010). IL-17A is considered an important cytokine to induce the inflammatory response asthma. In the pathogenesis of asthma, Th17/IL-17A can induce the accumulation of inflammatory cells in the airway and participate in the process of asthma. In addition, the activation of Th17 cells and the secretion of IL-17 can increase the immune response of Th2 cells, thereby aggravating the severity of allergic asthma.

BPA exposure led to a dose-related increment of Th17 cells in mice. This effect was consistent with effects on cellular immunity based on Th17 cells and associated cytokines (IL-17, IL-21 and IL-23), as well as with effects of BPA in the cluster of allergic lung inflammation.

When using the benchmark approach, for dose-response analysis, a BMR needs to be selected. The EFSA guidance (EFSA Scientific Committee, 2017a) recommends a BMR of 5% as default. However, deviating from this default is possible, based on toxicological or statistical considerations. For Th17 cells, there is currently insufficient information available on the normal variability of this measure, either in the mouse strain used in the study, or other strains. In humans, a study published in 2016 reported a retrospective analysis on lymphocyte subpopulations, analysed over few years in an outpatient laboratory in Northeast Italy (Sorrenti et al., 2016) to provide reference ranges. In Caucasian patients (mean age 42 ± 8.5 years), mean values \pm SD of Th17 in peripheral blood are 221.6 ± 90.2 cells/ μ L ($10.5 \pm 4.4\%$). Registered cases of lymphocyte associated diseases (immunodeficiencies and lymphoproliferative disorders) were excluded from the study, as well as samples with values of total erythrocytes, total leukocytes, total lymphocytes, and major lymphocyte populations (T cells, Th, Tc and B lymphocytes) outside the normal range according to guidelines.

Furthermore, the CEP Panel notes that the increment of Th17 cells is an intermediate endpoint, and some reserve capacity will exist. While considering that in the human population, for individuals a 20% increase may not necessarily imply an adverse condition for that person, given the pivotal role of Th17 cells in lung allergy, the CEP Panel considered that if the population at large showed a 20% increment in Th17 cells, individuals that are in the higher segment of the normal range, will be put out of the normal range, and as a consequence numbers of lung allergy cases would be expected to go up.

In conclusion, while the effect of BPA exposure on Th17 cells is clear, considering the standard deviation in the outcomes of the animal study (see the Table in Section 2.1.1 of Annex I), the CEP Panel considered 20% would be in line with the variability noted in the animal study and the wider normality range in humans, and considered it as adverse and took it as the BMR.

F.3. Results

Table F2: Fitted Models.

model	converged	loglik	npar	AIC
full model	yes	18.60	21	4.80
full-v	yes	18.65	24	10.70
null model	yes	-80.77	2	165.54
null model-a	yes	-76.25	5	162.50
Expon. M3-	yes	1.97	4	4.06
Expon. M3-a	yes	12.43	7	-10.86
Expon. M3-ab	yes	18.12	10	-16.24
Expon. M5-a	yes	12.43	8	-8.86
Expon. M5-ab	yes	18.12	11	-14.24
Hill m3-a	yes	12.43	7	-10.86
Hill m3-ab	yes	18.12	10	-16.24
Hill m5-a	yes	12.42	8	-8.84
Hill m5-ab	yes	18.12	11	-14.24
Inv.Expon. m3-a	yes	12.28	7	-10.56
Inv.Expon. m3-ab	yes	17.88	10	-15.76
Inv.Expon. m5-a	yes	12.21	8	-8.42
Inv.Expon. m5-ab	yes	17.74	11	-13.48
LN m3-a	yes	12.37	7	-10.74

LN m3-ab	yes	18.03	10	-16.06
LN m5-a	yes	12.33	8	-8.66
LN m5-ab	yes	17.97	11	-13.94

Table F3: Estimated Model Parameters.**EXP**

estimate for var- : 0.04902
 estimate for a-female PND21 : 1.291
 estimate for a-female PND42 : 1.22
 estimate for a-male PND21 : 1.299
 estimate for a-male PND42 : 1.187
 estimate for CED-female PND21 : 0.1863
 estimate for CED-female PND42 : 0.4994
 estimate for CED-male PND21 : 0.9104
 estimate for CED-male PND42 : 0.8481
 estimate for d- : 0.2959

HILL

estimate for var- : 0.04902
 estimate for a-female PND21 : 1.291
 estimate for a-female PND42 : 1.22
 estimate for a-male PND21 : 1.299
 estimate for a-male PND42 : 1.187
 estimate for CED-female PND21 : 0.1867
 estimate for CED-female PND42 : 0.5023
 estimate for CED-male PND21 : 0.917
 estimate for CED-male PND42 : 0.8541
 estimate for d- : 0.2977

INVEXP

estimate for var- : 0.04913
 estimate for a-female PND21 : 1.287
 estimate for a-female PND42 : 1.222
 estimate for a-male PND21 : 1.306
 estimate for a-male PND42 : 1.193
 estimate for CED-female PND21 : 0.2047
 estimate for CED-female PND42 : 0.6054
 estimate for CED-male PND21 : 1.177
 estimate for CED-male PND42 : 1.089
 estimate for d- : 0.0651

LOGN

estimate for var- : 0.04907
 estimate for a-female PND21 : 1.289
 estimate for a-female PND42 : 1.221
 estimate for a-male PND21 : 1.303
 estimate for a-male PND42 : 1.19
 estimate for CED-female PND21 : 0.1977
 estimate for CED-female PND42 : 0.5625
 estimate for CED-male PND21 : 1.063
 estimate for CED-male PND42 : 0.9869
 estimate for d- : 0.1117

Table F4: Weights for Model Averaging.

EXP	HILL	INVEXP	LOGN
0.27	0.27	0.21	0.25

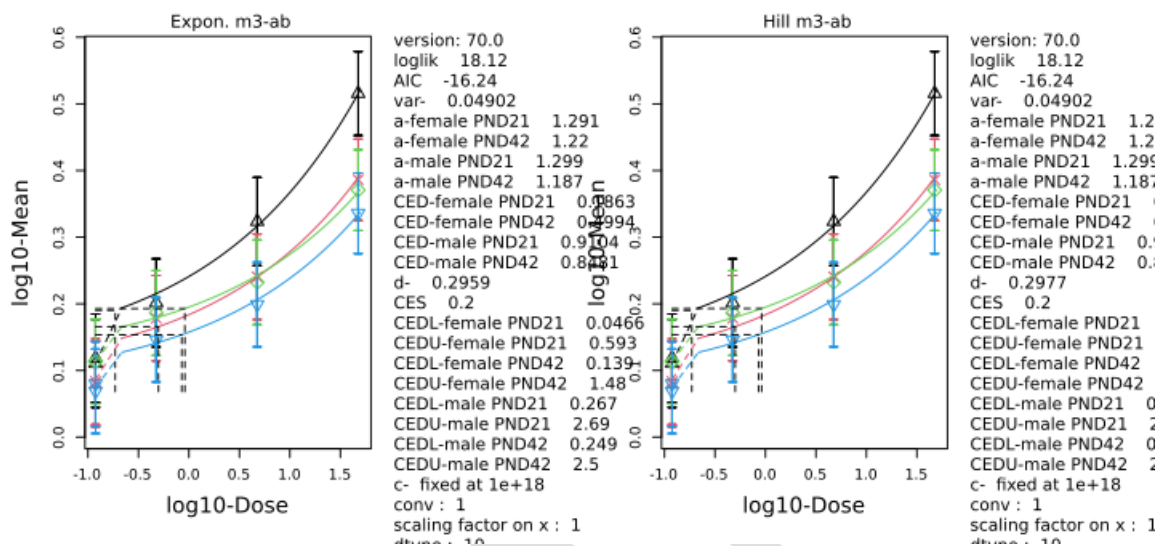
Table F5: Final BMD Values.

Subgroup	BMDL ($\mu\text{g}/\text{kg bw per day}$)	BMDU ($\mu\text{g}/\text{kg bw per day}$)
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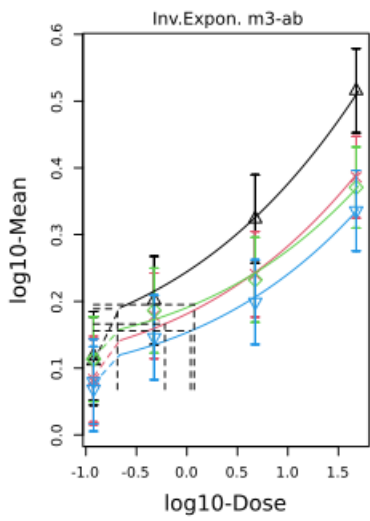
female PND21	0.06	0.74
female PND42	0.17	1.79
male PND21	0.30	3.39
male PND42	0.35	3.38

Confidence intervals for the BMD are based on 1000 bootstrap data sets.

Visualisation

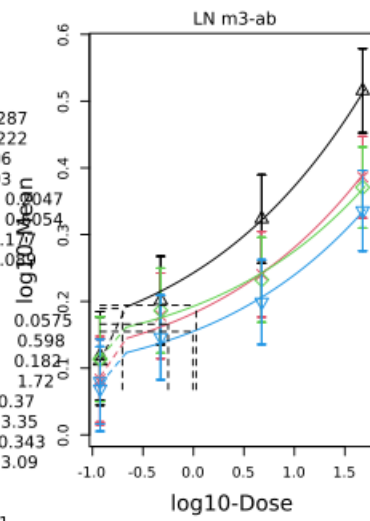


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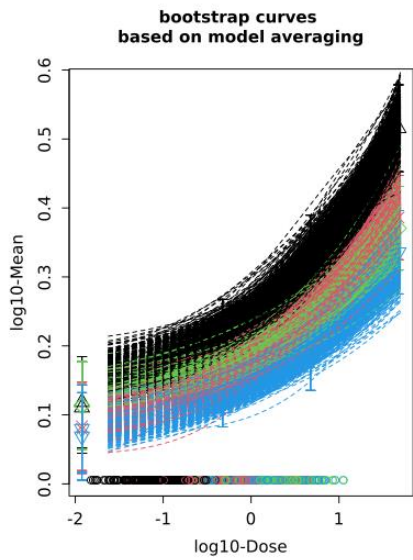
```

version: 70.0
loglik 17.88
AIC -15.76
var- 0.04913
a-female PND21 1.287
a-female PND42 1.222
a-male PND21 1.306
a-male PND42 1.193
CED-female PND21 0.0047
CED-female PND42 0.0054
CED-male PND21 1.175
CED-male PND42 1.018
d- 0.0651
CES 0.2
CEDL-female PND21 0.0575
CEDU-female PND21 0.598
CEDL-female PND42 0.182
CEDU-female PND42 1.72
CEDL-male PND21 0.37
CEDU-male PND21 3.35
CEDL-male PND42 0.343
CEDU-male PND42 3.09
c- fixed at 1e+18
conv : 1
scaling factor on x : 1
dtype : 10
    
```



```

version: 70.0
loglik 18.03
AIC -16.06
var- 0.04907
a-female PND21 1.2
a-female PND42 1.2
a-male PND21 1.306
a-male PND42 1.193
CED-female PND21 0.0047
CED-female PND42 0.0054
CED-male PND21 1.175
CED-male PND42 1.018
d- 0.1117
CES 0.2
CEDL-female PND21 0.0575
CEDU-female PND21 0.598
CEDL-female PND42 0.182
CEDU-female PND42 1.72
CEDL-male PND21 0.37
CEDU-male PND21 3.35
CEDL-male PND42 0.343
CEDU-male PND42 3.09
c- fixed at 1e+18
conv : 1
scaling factor on x : 1
dtype : 10
    
```



```

version: 70.0
model averaging results
dtype 10
selected all
dose scaling: 1
conf level: 0.9
number of runs: 1000
CES 0.2
BMD CI
0.056 0.742
0.17 1.79
0.3 3.39
0.35 3.38
    
```

Annexes

- Annex A – Revised protocol on BPA hazard assessment**
- Annex B – Appraisal of internal validity of epidemiological studies**
- Annex C – Data extraction of epidemiological studies**
- Annex D – Weight of evidence from epidemiological studies**
- Annex E – Appraisal of internal and external validity of animal studies**
- Annex F – List of animal studies with endpoints appraised and relevant**
- Annex G – Data extraction of studies reporting relevant endpoints in animal studies**
- Annex H – Weight of evidence from animal studies**
- Annex I – Benchmark dose analysis**
- Annex J – Uncertainty analysis - blank excel templates including definitions**
- Annex K – Uncertainty analysis - additional results and calculation methods**
- Annex L – Weight of evidence on Genotoxicity**
- Annex M – Uncertainty analysis Genotoxicity - blank excel templates**