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► **To cite this version:**

Davy Guignard, Véronique V. Gayrard-Troy, Marlène Z. Lacroix, Sylvie Puel, Nicole Picard-Hagen, et al.. Evidence for bisphenol A-induced disruption of maternal thyroid homeostasis in the pregnant ewe at low level representative of human exposure. *Chemosphere*, 2017, 182, pp.458-467. 10.1016/j.chemosphere.2017.05.028 . hal-01607369

HAL Id: hal-01607369

<https://hal.science/hal-01607369>

Submitted on 26 May 2020

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Accepted Manuscript

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PII: S0045-6535(17)30723-3

DOI: 10.1016/j.chemosphere.2017.05.028

Reference: CHEM 19243

To appear in: *Chemosphere*

Received Date: 10 January 2017

Revised Date: 28 April 2017

Accepted Date: 04 May 2017

Please cite this article as: Davy Guignard, Véronique Gayrard, Marlène Lacroix, Sylvie Puel, Nicole Picard-Hagen, Catherine Viguié, Evidence for bisphenol A-induced disruption of maternal thyroid homeostasis in the pregnant ewe at low level representative of human exposure, *Chemosphere* (2017), doi: 10.1016/j.chemosphere.2017.05.028

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1 **Evidence for bisphenol A-induced disruption of maternal thyroid homeostasis in the**
2 **pregnant ewe at low level representative of human exposure**

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Abstract

17
18 Many uncertainties remain regarding the potential of bisphenol A (BPA) as a thyroid disruptor
19 in mammals and the relevance of experimental data to humans. The relevance of the exposure
20 schemes used in experimental *in vivo* studies is also a major source of uncertainty when
21 analysing the risk of BPA exposure for human health. In this context, the goals of our study,
22 conducted in an ovine model relevant to human gestation and thyroid physiologies, were to: 1)
23 determine the equivalence of subcutaneous and dietary exposures and 2) determine if
24 environmentally relevant doses of BPA can alter gestational and newborn thyroid functions.
25 The difference between the two routes of exposure was mainly related to the overall BPA
26 exposure and much less to the peak serum concentrations. Interestingly, BPA-GLUC (the main
27 metabolite of BPA) internal exposure via both routes was almost identical. The decrease in
28 thyroid hormones concentration overtime was more accentuated in ewes treated with BPA,
29 particularly with the medium dose (50 µg/(kg.d); SC) for which the maximum BPA
30 concentrations were predicted to be within the 1-10 ng/mL range i.e. very similar to the highest
31 blood concentrations reported in humans. The balance between TT4 and rT3 varied differently
32 between the vehicle and the medium dose group. The mechanisms underlying those
33 modifications of maternal thyroid homeostasis remain to be determined. Our study did not
34 evidence significant modification of TSH secretion or binding to serum proteins but might
35 suggest an effect at the level of deiodinases.

Highlights

- 36
- 37 - Maximum BPA serum concentration is similar between subcutaneous and dietary routes
 - 38 -Bisphenol A alters the maternal thyroid status in pregnant ewe
 - 39 -Thyroid disruption is observed at BPA serum concentration relevant to human exposure
- 40

41 -BPA might disrupt the deiodinase activity in pregnant ewe

42 **Abbreviations:**

43 AUC, area under the curve; BPA, bisphenol A; BPA-GLUC, bisphenol A glucuronide; BPA-
44 SULF, bisphenol A sulfate; C_{max}, maximum concentration; GD, gestational day; LOQ, limit
45 of quantification; LOD, limit of detection; PK, pharmacokinetic; PND, post-natal day; rT₃,
46 reverse triiodothyronine; S.C., subcutaneous; TH, thyroid hormones; TSH, thyroid stimulating
47 hormone; TT₃, total triiodothyronine; TT₄, total thyroxine; fT₄, free thyroxine.

48

49 **Keywords:** bisphenol A; toxicokinetic; exposure; thyroid disruption; pregnancy; ovine model

50 **1. Introduction**

51 Bisphenol A (BPA), a major molecule used in the plastics industry, is now recognized to be an
52 endocrine disruptor that exerts deleterious effects on human health (Chapin et al., 2008; vom
53 Saal et al., 2007). Most investigations have focused on reproductive functions based on the
54 estrogenomimetic properties of this compound. However, accumulated evidence suggests that
55 BPA might have negative effects on other endocrine systems (Richter et al., 2007; Rubin, 2011)
56 including thyroid function (Zoeller, 2010).

57 Although sometimes controversial, the results of numerous epidemiological studies in humans
58 indicate that BPA exposure might be associated with diverse degrees of thyroid disruption in
59 adults, children and new born. Epidemiological data obtained in a cohort of men from infertile
60 couples (Meeker et al., 2010) and in a larger cohort of healthy individuals (NHANES cohort;
61 Meeker and Ferguson, 2011) suggest that BPA exposure might be associated with some degrees
62 of thyroid disruption characterized by an inverse relationship between urinary BPA
63 concentrations and total thyroxine (TT4) concentrations. In another cohort of adults, urinary
64 BPA concentration was, respectively, positively and negatively correlated with free T3 and
65 TSH serum concentrations (Wang et al., 2013). In a large cohort of Thai adults, BPA serum
66 concentrations were shown to be negatively correlated with free T4 (fT4) concentrations in men
67 but not in women (Sriphrapradang et al., 2013). In a cohort of 9 to 11 year old Chinese children,
68 the BPA urinary concentration in morning spot sampling was negatively correlated with thyroid
69 gland volume (Wang et al., 2015) and this correlation did not seem to depend upon the iodine
70 supply and/or iodine status of the children.

71 As far as gestational exposure is concerned, data are very scarce in human. However, there is
72 some evidence that infant thyroid function might be altered in relation to maternal exposure to
73 BPA. In a cohort of pregnant women and their infants (CHAMACOS study), urinary BPA in

74 the mother was shown to be negatively correlated with maternal TT4 blood concentration when
75 the urine BPA and TT4 blood samples were collected close together in time (Chevrier et al.,
76 2013). As a result, the average maternal urinary BPA was related to increased odds of low TT4
77 in the mother. An inverse relationship between maternal urinary BPA and neonatal blood TSH
78 was evidenced in newborn boys. In the HOME study, 26-week maternal urinary BPA
79 concentrations were inversely correlated with serum TSH concentrations in newborn girls but
80 not in boys (Romano et al., 2015).

81 Very few experimental studies have been done on the effect of BPA on thyroid function in
82 mammals. In one study, the physiological increase in circulating TT4 that generally occurs
83 around post-natal day 15 (PND15) was amplified in rats born to and fed by mothers treated with
84 an oral BPA dose of 1 mg/(kg.d) from gestational day (GD) 6 until weaning (Zoeller et al.,
85 2005). In another study in pregnant rats (Xu et al., 2007), BPA oral treatment of 0.1 mg/L in
86 drinking water (approximately 10 µg/(kg.d)) led to a transitory decrease in fT4 concentration
87 in mothers during the early postpartum period (PND0 to PND7), while their male offspring
88 showed an increase in fT4 at PND7 followed by a decrease at PND21.

89 The relevance to humans of thyroid regulation in rodents is nevertheless controversial due to
90 the different timing of thyroid function ontogenesis and the lack of expression of thyroxine
91 binding globulin (TBG), the high affinity specific binding protein in adult rats (Fisher et al.,
92 1976). This serum protein is considered a key player in the maintenance of thyroid homeostasis
93 (Hard, 1998; Wu and Farrelly, 2006). Ovine model is considered as a more relevant model to
94 human than rodents from the thyroid regulation standpoint. Indeed, in this species as in human,
95 TBG is the major serum carrier protein of thyroid hormones. Furthermore, ovine and human
96 species are both considered as precocious species for the ontogenesis of the thyroid system
97 because fetal thyroid axis is almost mature at birth. In sheep, thyroid gland can accumulate
98 iodine and synthesize T4 around the 70th day of gestation, the neuroendocrine control of the

99 thyroid axis starts to develop as soon as the 50th day of gestation (Fisher et al., 1976) and TH
100 receptor expression is observable around 80 days of gestation in the brain and the liver (Polk et
101 al., 1989). T3 levels in blood are much lower in the fetus than in the mother and start to rise
102 significantly only on the very late stages of pregnancy in both human (Fisher et al., 2002) and
103 sheep (Fisher et al., 1994).

104 In one of our studies in pregnant ewes, BPA exposure was associated with hypothyroxinemia
105 (about 30% decrease in TT4) in both the mothers and newborn lambs after subcutaneous
106 exposure of the ewes to 5 mg/(kg.d) throughout gestation (Viguié et al., 2013).

107 Evidence that BPA can be a potential thyroid disruptor has also arisen from studies in
108 mammalian cells and in amphibian or fish larvae models. The results of these studies converge
109 to suggest that BPA can exert an antagonistic effect on TH-dependent mechanisms (Fini et al.,
110 2007; Goto et al., 2006; Heimeier et al., 2009; Iwamuro et al., 2003; Kaneko et al., 2008; Sun
111 et al., 2009; Terrien et al., 2011; Yang and Chan, 2015) and that several pathways involved in
112 TH economy can be affected by BPA. Hepatic regulation of thyroid hormone homeostasis can
113 also be affected by BPA as shown in a zebrafish liver cell line in which BPA (45-180 μ M)
114 decreased the expression of several genes involved in thyroid homeostasis i.e., deiodinases I
115 and III, sulfotransferases 1,2 and 3, UGT2A1 and thyroid receptor β (Yang and Chan, 2015).
116 In addition, the antagonistic effect of BPA could be mediated, at least in cell models, by the
117 increased recruitment of co-repressors of TH nuclear receptors from the N-CoR family
118 (Moriyama et al., 2002; Sheng et al., 2012).

119 Overall, it is apparent that uncertainties remain regarding the potential of BPA as a thyroid
120 disruptor in mammals and the relevance of experimental data to humans. Another major source
121 of uncertainty on BPA risk analysis for human health based on experimental data obtained in
122 animals is related to the level of internal exposure, particularly with regard to the route of

123 administration. Indeed, in many good quality experimental studies, animals were exposed via
124 parenteral or gastric gavage routes to ensure better control of the dose administered although
125 none of these routes is totally representative of the acknowledged main route of human exposure
126 i.e. via food contamination (Guignard et al., 2016; Taylor et al., 2011; Vom Saal et al., 2014).
127 Thus, the relevance of the route of administration is a critical issue that every scientist needs to
128 bear in mind when performing toxicological evaluations of small lipophilic molecules which,
129 alike BPA, are subjected to a high hepatic first pass effect.

130 In this context, the goals of our study conducted in an ovine model relevant to human gestation
131 and thyroid physiologies were to 1) determine the equivalence of subcutaneous and dietary
132 exposures and 2) determine if environmentally relevant doses of BPA can alter gestational and
133 newborn thyroid functions.

134

135 **2. Materials and Methods**

136 **2.1. Chemicals**

137 Bisphenol A 99% purity (Sigma-Aldrich, Saint-Quentin Fallavier, France) was dissolved in
138 corn oil / absolute ethanol (6/1: V/V). For experiment 1, a single BPA solution (100 mg/mL)
139 was prepared and used for both routes of administration. The volume administered to the
140 animals was adjusted to the bodyweight recorded the day before the administrations. For the
141 dietary route, BPA solution was spilled onto the pellet ration (on average 6 mL of solution on
142 75g of pellets). The subcutaneous (S.C.) injection of BPA was performed on the distal part of
143 the neck (on average 3 mL/ewe). For experiment 2, a vehicle and three different BPA solutions
144 (0.01 – 1 and 100 mg/mL) were prepared so that the injected volume adjusted to individual
145 bodyweight was the same in all groups. The solutions were renewed monthly and were stored
146 at ambient temperature in sealed amber glass bottles for the entire duration of their use. The

147 vehicle solution was prepared and stored in a different room. All material used for the solution
148 preparation, sample processing and assays was either made of glass or of BPA-free plastics.

149

150 2.2. Animal husbandry

151 All animal procedures were carried out in accordance with the accepted standards of humane
152 animal care under agreement 311155515 for animal experimentation from the French Ministry
153 of Agriculture and validated by the local ethical committee for animal experimentation (project
154 number: 2016 0323 10542323) and by the French Ministry of Higher Education and Research
155 (project number: 00890.01) for experiments 2 and 1, respectively. The study was performed on
156 adult Lacaune ewes in the sheep research facility at the National Veterinary School of Toulouse,
157 France. The ewes were kept under natural photoperiodic and temperature conditions, with free
158 access to water, hay *ad libitum* and plant based pellets (Brebilac® RAGT, Rodez, France). The
159 absence of BPA contamination of water, hay and pellets was checked by assaying BPA in 100
160 mg samples of each matrix homogenized in 1 mL of acetonitrile. BPA was not detected in any
161 of the samples (LOD 10 ng/g of food).

162 In experiment 1, animals were penned individually the day before the administrations and for
163 24h following administrations. For experiment 2, all animals in a same group were kept together
164 and no direct contact was allowed between animals from different groups, with separate food
165 and water supplies provided for each group.

166

167 2.3. Experimental design

168 Experiment 1: relative bioavailability via subcutaneous and dietary routes of administration

169 This experiment was designed to evaluate the equivalence, in terms of internal exposure to
170 BPA, of the S.C. route of administration used for convenience in this experiment and the dietary
171 one thought to be the main route of exposure in human populations. It was conducted on 4 ewes
172 (3-5 year old; 50-73 kg) and was divided into 2 periods conducted at least 13 days apart. The
173 first period was devoted to the S.C. route of administration (5 mg/kg of body weight). During
174 the second period, the same ewes received BPA via the diet (10 mg/kg of body weight). Before
175 the dietary period, ewes were fasted overnight to facilitate intake of the BPA-treated pellets.
176 The ewes ingested the pellets within 2 – 9 minutes. Free access to hay and water was maintained
177 after the administrations. The different doses for the different routes were based on previous
178 studies and were chosen to produce BPA concentrations above our assay limit of quantification,
179 for at least 8-10 hours after the administrations. Use of different doses for the two routes was
180 possible because there is evidence that the parameters of BPA pharmacokinetics in several
181 species including sheep are linear functions of the dose (Collet et al., 2010; Taylor et al., 2011;
182 Tominaga et al., 2006) over a dose range of 5 to 10 mg/kg. Results regarding the dietary route
183 have been described in detail in a previous publication (Guignard et al., 2016).

184 Serial jugular blood samples were collected before all administrations and 0.5 – 1 – 2 – 4 – 6 –
185 8 - 10 – 24 h after the S.C. administration or 0.03 – 0.08 – 0.16 – 0.25 – 0.5 – 1 – 2 – 4 – 6 - 8
186 – 10 – 26 h after the dietary exposure. For the dietary administration, time 0 was the time when
187 the ewes had finished ingesting the pellets. The blood samples were centrifuged at 3000g at
188 4°C for 20min, then the serum was decanted and stored in propylene tubes at -20°C until
189 assayed.

190 Experiment 2: Effect of environmentally relevant doses of BPA on maternal and fetal thyroid
191 status.

192 This experiment was conducted on 22 pregnant ewes (1-3 year old; 55-103 kg) artificially
193 inseminated on the same day after hormonal synchronization of estrus. A vitamin and
194 iodine/selenium-enriched mineral supplement (Alimal gestante® Alliance Elevage, Souvigny,
195 France) was provided to cover the specific requirements of gestation. The ewes were weighed
196 twice a month. The amount of pellets was regularly adjusted to pregnancy requirements and
197 changes in body weight. The ewes were randomly allocated to 4 treatment groups balanced for
198 TT4 concentration, body weight and age. From GD28 to GD132-134, each group received
199 different daily doses of BPA by S.C. administration i.e., low dose group 0.5 µg/(kg.d) (n=6);
200 medium dose group 50 µg/(kg.d) (n=5); high dose 5000 µg/(kg.d) (n=5) and vehicle group
201 (n=6). The administered volume was regularly adjusted to the most recently recorded body
202 weight.

203 Maternal blood samples were collected twice a week during the treatment period, just before
204 the next BPA administration. The first maternal blood sample was taken on GD28 before the
205 first BPA or vehicle administration. On GD62, serial blood samples were collected from
206 animals in the high dose treatment group (0 - 1.5 - 4 - 8 and 10 h post administration).

207 At GD133-135, i.e. about two weeks before the expected term, the fetuses were delivered by
208 cesarean section under rachianesthesia and local anesthesia of the muscle wall (5 mL of
209 lidocaine in the sacrolombar space and 10 mL along the incision line on the abdominal wall;
210 Lurocaine®, Vetoquinol SA, Lure, France) to avoid spontaneous deliveries. Although not
211 totally mature at this age, thyroid function is already well established at this stage in the sheep
212 as in human.

213 Maternal and fetal jugular blood, cord blood and amniotic fluid were collected at delivery after
214 which the ewes and fetuses were immediately euthanized by intravenous overdose of sodium
215 pentobarbital (Doléthal®, Vetoquinol France, 60 mg/kg of pentobarbital i.v.).

216

217 2.4. BPA, BPA-GLUC and BPA-SULF assay

218 BPA, BPA-GLUC and BPA-SULF (BPA sulfate) concentrations were simultaneously
219 measured in the serum samples collected during experiment 1, in the amniotic fluid, cord blood
220 and maternal and fetal blood samples collected at delivery, and in the serial samples collected
221 from the high dose group in experiment 2, using a previously described UPLC/MS/MS method
222 (Lacroix et al., 2011). The validated LOQs were 1 ng/mL for BPA and 5 ng/mL for BPA-
223 GLUC/S. The mean intra- and inter-day coefficients of variation for three concentration levels
224 of BPA and BPA-GLUC were lower than 15%.

225

226

227 2.5. Pharmacokinetic analyses

228 All pharmacokinetic analyses were performed with the Phoenix® software, version 6.4
229 (Pharsight Corporation, Cary, NC, USA).

230 Experiment 1:

231 Serum concentration–time profiles of BPA and BPA-GLUC were first analyzed using a non-
232 compartmental approach. The areas under the BPA and BPA-GLUC serum concentrations
233 curves (BPA and BPA-GLUC AUC_{last}) were calculated using the linear trapezoidal rule from
234 dosing time to the last quantifiable (i.e. >LOQ) serum concentration sampling time. The AUC
235 from t=0 to infinity was obtained by adding to AUC_{last} the area extrapolated from the last
236 observation to infinity obtained by dividing the last observed quantifiable serum concentration
237 by the slope of the terminal phase estimated by linear regression, using the best fit option of
238 Phoenix®. As previously explained (Gayrard et al., 2013; Guignard et al., 2016), to avoid any
239 confusion due to high concentration resulting from direct drainage of the mouth by the jugular

240 veins, the data obtained during the first 10 minutes after final pellet ingestion were not taken
 241 into account in the calculation of BPA AUC_{inf} . The digestive absorption of BPA being close to
 242 100% (Doerge et al., 2010), the molar ratio AUC_{inf} BPA-GLUC / AUC_{inf} BPA could be used
 243 as an index of BPA metabolism. The relative bioavailability of BPA by dietary and
 244 subcutaneous routes was compared using the following equation:

$$245 \quad \text{Relative bioavailability} = \frac{BPA \ AUC_{inf} \ diet}{BPA \ AUC_{inf} \ S.C.} \times \frac{BPA \ dose \ S.C.}{BPA \ dose \ diet}$$

246 The time courses of serum BPA concentrations obtained in the 4 ewes after the S.C.
 247 administration were fitted to a triexponential equation, corresponding to an extravascular
 248 administration, to generate the pharmacokinetic (PK) parameters required to predict the time
 249 course of BPA blood concentrations for the lowest doses in experiment 2:

$$250 \quad C(t) = A.e^{-\alpha.t} + B.e^{-\beta.t} + C.e^{-K01.t}$$

252 Experiment 2:

253 Given the known BPA PK parameters and our assay LOQ, the maternal serum concentrations
 254 of BPA were likely to be non-quantifiable after repeated S.C. administrations of BPA at 0.5 or
 255 50 $\mu\text{g}/(\text{kg}.\text{d})$. Time-concentration profiles of BPA were therefore simulated, using the PK
 256 parameters and the model of experiment 1, to estimate the animal internal exposure. BPA
 257 disposition was assumed to be linear overtime, as no accumulation or increase in the
 258 metabolism of BPA or BPA-GLUC had been observed with repeated administrations (Collet et
 259 al., 2010; Viguié et al., 2013) and because gestation does not affect BPA metabolic rate in the
 260 ewe (Corbel et al., 2013). The goodness-of-fit was determined using data obtained from the
 261 serial samples collected from ewes receiving the highest dose at GD62.

262

263 2.6. TH and TSH assays

264 Total and free T4 and total T3 concentrations were measured in one every two samples
265 collected twice a week in ewes from GD28 to GD128 and from fetal jugular samples collected
266 at delivery using a RIA kit (T4 MAb RIA kit, MP Biomedicals, Illkirch, France). The LOQ of
267 the RIA assays (lowest value with an intra-assay CV lower than 20% and an accuracy above
268 90%) was validated at 10 ng/mL and 0.4 ng/mL for total T4 and T3, respectively. For free T4,
269 a limit of sensitivity was set at the lowest concentration of the standard curve providing that
270 the intra-assay CV was below 20% (3.2 pg/mL). The mean intra- and inter-assay CVs for three
271 QC pools were systematically below 10%.

272

273 The balance between T4 activation into T3 and its inactivation to rT3, through different
274 deiodinase activities, particularly within the placenta, has a key role in ensuring adequate TH
275 supply to the fetus to meet its needs. We therefore used a previously described UPLC/MS/MS
276 assay (Roques et al., 2012) to simultaneously determine TT4 and rT3 concentrations in the
277 cord blood samples and in maternal samples collected after TT4 modification had been
278 established, at GD 41 – 44 – 48 – 51 – 55 – 58 – 72 – 86 – 100 – 114 and 128. The LOD for
279 each assay run was defined as the calculated concentration corresponding to a peak area at a
280 given elution time, equal to three fold the area obtained by solvent injection. These LOD were
281 2.8, 0.5 and 0.15 ng/mL for TT4, rT3 and TT3, respectively. Mean intra- and inter-assay CVs
282 for three QC pools were systematically below 15% and accuracy was between 88 and 108%.
283 To determine the consistency between RIA and UPLC/MS assays, we assayed TT4 in a pool
284 of 43 fetal sheep blood samples from another experiment using both methods. We found a
285 mean inter-method coefficient of variation of 5.2% for concentrations ranging from 53 to 138
286 ng/ml.

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287

288 TSH serum concentration was measured in maternal samples collected at GD 34 – 65 - 97 and
289 132 using an ELISA kit (Sheep Ultrasensitive TSH ELISA kit®, Blue Gene, Shanghai, China)
290 following the manufacturer's instructions. The lowest measurable concentration was 1 μ UI/ml.

291

292 2.7. Statistical analyses

293 Statistical analyses were performed with Systat® (Systat Software Inc., San Jose
294 California,USA).

295 For experiment 1, mean AUC and Cmax for both BPA and BPA-GLUC concentrations were
296 compared using paired T-tests.

297 For experiment 2, the effect of the treatment on the time course of maternal TT4, fT4, TT3 and
298 TSH was analyzed by using a two-way repeated-measures ANOVA, with time, treatment and
299 their interaction as fixed factors and animals nested in the treatment as a random effect factor.
300 A Bonferroni test was performed to identify the dose that individually would give a different
301 serum TH profile to the one observed in the control group.

302 The effect of the treatment on hormone concentrations measured in fetal and maternal blood at
303 delivery was analyzed with a one-way ANOVA.

304

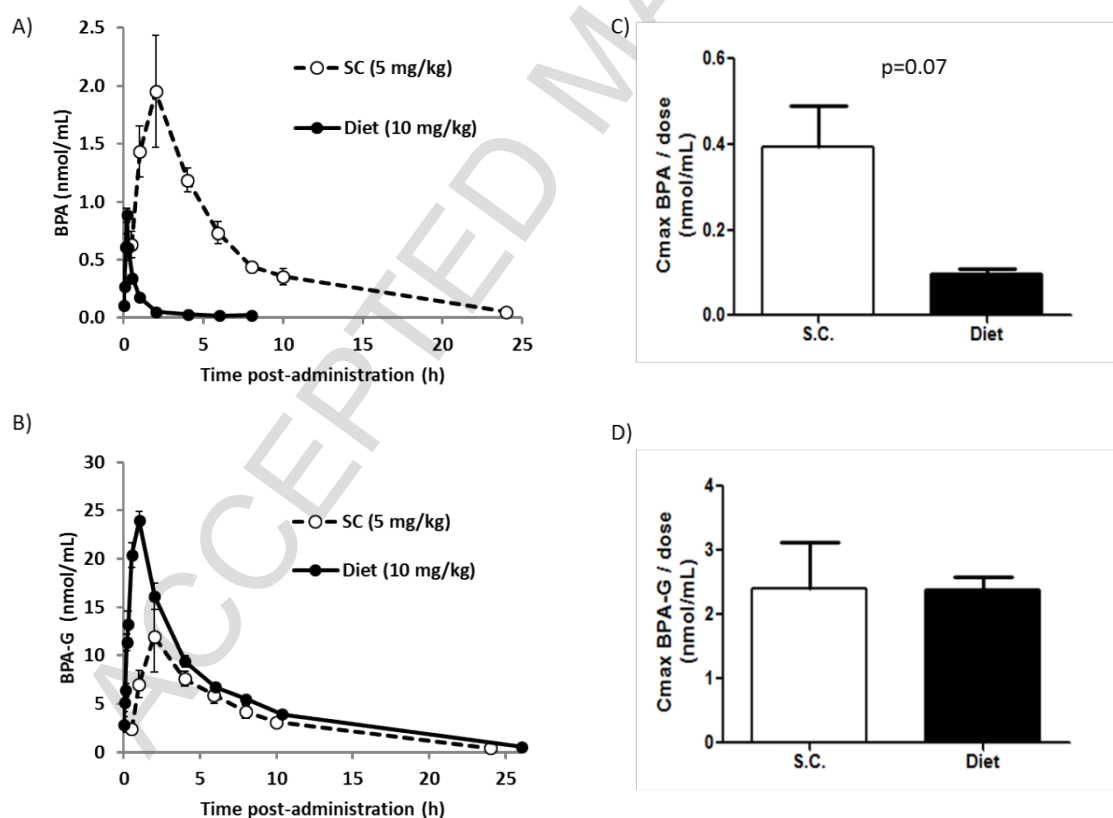
305 3. Results

306 3.1. Relative bioavailability by subcutaneous and dietary routes of administration 307 (Experiment 1)

308 Fig. 1 (A-B) shows the mean (\pm S.E.M.) serum BPA and BPA-GLUC concentration-time
309 profiles following S.C. (5 mg/kg) or dietary (10 mg/kg) administration of BPA. The

310 concentrations were not normalized by the dose. The concentration-time profiles varied greatly
 311 between the two routes. With the dietary route, the maximum serum concentration (C_{\max}) was
 312 attained very quickly (at 0.12 h for 2 ewes and 0.20 h for 2 others) and BPA concentrations
 313 decreased quickly to reach the assay LOQ within 8 h post-administration. For the S.C. route,
 314 C_{\max} was obtained later (after 2 h for 3 ewes and 1 h for one ewe) and the BPA concentrations
 315 decreased more slowly. The mean normalized by the dose BPA C_{\max} for the S.C. route was only
 316 4.6 ± 1.5 fold higher than for the dietary route ($p=0.07$; Fig. 1 C). By contrast to BPA, the
 317 BPA-GLUC concentration time course for the two routes of exposure was very similar and the
 318 maximum concentration attained with both routes, when normalized by the administered dose,
 319 was about 2.4 nmol/mL (Fig. 1 D).

320

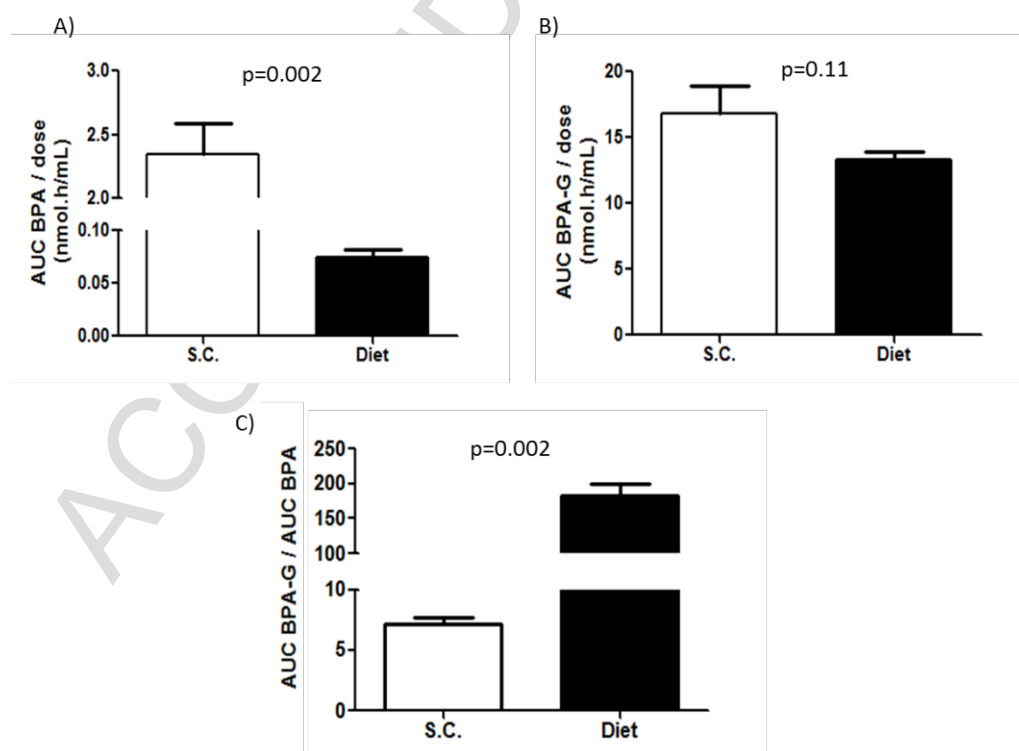


321

322 **Fig. 1:** A-B) mean (\pm S.E.M.) serum BPA (A) and BPA-GLUC (B) concentration-time profiles
 323 obtained for the same ewes ($n=4$) after subcutaneous (5 mg/kg) and dietary (10 mg/kg)

324 administrations. Blood samples were collected 0.5 – 1 – 2 – 4 – 6 – 8 – 10 and 24 h after
 325 subcutaneous administration and 0.03 – 0.08 – 0.16 – 0.25 – 0.5 – 1 – 2 – 4 – 6 – 8 – 10 and 24
 326 h after dietary administration. The serum concentrations were not normalized by the dose. C-
 327 D) Mean (\pm S.E.M.) maximum concentration of BPA (C) and BPA-GLUC (D) normalized by
 328 the BPA dose obtained after subcutaneous and dietary administrations.

329
 330 Administration by S.C. route led to a higher overall internal exposure to BPA characterized by
 331 a much higher normalized by the dose AUC_{inf} ($p=0.002$; Fig.2 A). The calculated relative
 332 bioavailability of BPA for the dietary as compared to S.C. route was $3.3 \pm 0.3 \%$. The AUC_{inf}
 333 of BPA-GLUC was very similar for both routes of administration ($p=0.11$; Fig. 2 B). The ratio
 334 of BPA-GLUC to BPA AUC_{inf} was about 25 fold higher for the dietary route than for the S.C.
 335 route ($p=0.002$; Fig. 2 C). This can be explained by the extensive hepatic first-pass effect that
 336 immediately transforms BPA into BPA-GLUC after BPA intestinal absorption.



337

338 **Fig. 2:** A-B) mean (\pm S.E.M.) AUC_{inf} of BPA (A) and BPA-GLUC (B) concentrations
339 normalized by the BPA dose obtained after subcutaneous (5 mg/kg) and dietary (10 mg/kg)
340 administrations in the same ewes (n=4). The BPA concentrations obtained during the first 10
341 minutes after the end of food intake were not taken into account in the AUC estimation. C)
342 Mean (\pm S.E.M.) ratio of the BPA to the BPA-GLUC molar dose-normalized AUC_{inf} after
343 subcutaneous and dietary administrations.

344
345 Overall, the maximum serum concentration of BPA obtained after subcutaneous administration
346 was not dramatically higher than after dietary administration. However, BPA high level
347 exposure was more prolonged after administration by S.C. route than by dietary route.
348 Interestingly, the internal exposure to BPA-GLUC after both routes of administration was
349 almost identical.

350
351 3.2. Maternal and fetal monitoring of BPA and BPA-GLUC internal exposure (Experiment
352 2)

353 Table 1 describes the mean BPA, BPA-GLUC and BPA-SULF concentrations measured in the
354 maternal and fetal jugular blood, cord blood and amniotic fluid collected at delivery for the
355 medium and high BPA dose groups. Concentrations were below the assay LOQ in all samples
356 from the control group. BPA concentrations were quantifiable in all collected blood samples
357 only for animals in the highest dose group and in the amniotic fluid from the medium and high
358 dose groups. It was quantifiable in cord blood samples from 4/10 and 5/10 lambs from the low
359 and medium dose groups, respectively. BPA-GLUC concentrations could be systematically
360 measured in fetal jugular blood, cord blood and amniotic fluid samples from the medium and
361 high dose groups and in a few fetal jugular (5/10), cord blood (3/10) and amniotic fluid (3/7)

362 samples from the low dose group. Overall, BPA-GLUC concentrations in the fetal compartment
363 were much higher than the BPA fetal concentrations or the BPA-GLUC maternal
364 concentrations. BPA was detectable in the maternal blood only for the highest dose group. BPA-
365 GLUC in the maternal blood was detectable in a few samples of the low and medium doses
366 groups and was systematically quantified in all the ewes from the high dose group. When
367 quantifiable, BPA maternal concentrations were very similar to the amniotic ones, while for
368 BPA-GLUC amniotic fluid concentrations were much higher than the maternal ones and within
369 the same range of the fetal ones.

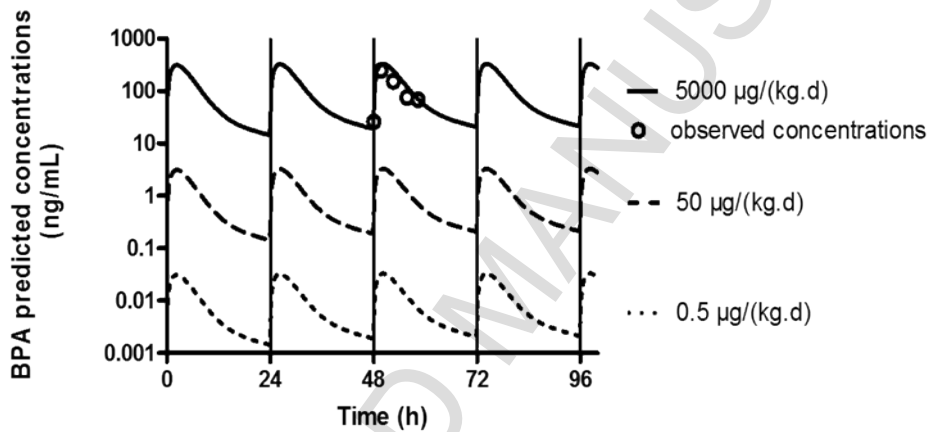
370 BPA-SULF could be measured in samples from the fetal compartment (jugular fetal and cord
371 blood) in all samples from the medium and high dose groups. For the amniotic fluid and
372 maternal samples, it was rarely measurable in samples from the medium dose group. In the fetal
373 and cord blood samples from the high dose group, BPA-SULF was detected at levels about 50
374 to 80 fold higher than BPA and about 20 fold lower than BPA-GLUC. BPA-SULF
375 concentration in amniotic fluid were about 10 fold lower than that in the fetus while they were
376 about 10 fold higher than that of the maternal compartment.

377 **Table 1:** mean (\pm SEM) BPA and BPA-GLUC/S concentrations (nmol/mL) measured in the
 378 maternal and fetal jugular blood, cord blood and amniotic fluid after treatment of pregnant ewes
 379 with BPA from GD 28 to 132-134. LOQ were validated at 1 ng/mL and 5 ng/ml for BPA and
 380 BPA-GLUC/S, respectively (4.4, 12.4 and 16.2nmol/L for BPA, BPA-GLUC and BPA-SULF,
 381 respectively). Mean concentration were noted as "<LOQ" when the concentrations in more than
 382 half the samples were below LOQ. When BPA and/or its metabolites were measurable in at
 383 least half the samples, the non-measurable values were replaced by the LOQ/2 for the mean
 384 calculation.

| | | Cord blood | | | Fetal blood | | | Amniotic fluid | | | Maternal blood | | |
|---|--------|------------|----------|----------|-------------|----------|----------|----------------|----------|----------|----------------|----------|----------|
| | | BPA | BPA-GLUC | BPA-SULF | BPA | BPA-GLUC | BPA-SULF | BPA | BPA-GLUC | BPA-SULF | BPA | BPA-GLUC | BPA-SULF |
| Medium dose 50 $\mu\text{g}/(\text{kg}\cdot\text{d})$ 0.2 $\mu\text{mol}/(\text{kg}\cdot\text{d})$ | Mean | 0.007 | 1.180 | 0.046 | <LOQ | 1.09 | 0.040 | 0.012 | 0.284 | <LOQ | <LOQ | 0.022 | <LOQ |
| | S.E.M. | 0.002 | 0.077 | 0.01 | | 0.058 | 0.006 | 0.005 | 0.034 | | | 0.008 | |
| | n/tot | 5/10 | 10/10 | 10/10 | 2/10 | 10/10 | 10/10 | 7/8 | 8/8 | 3/8 | 0/5 | 3/5 | 2/5 |
| High dose 5000 $\mu\text{g}/(\text{kg}\cdot\text{d})$ 20 $\mu\text{mol}/(\text{kg}\cdot\text{d})$ | Mean | 0.106 | 95.873 | 5.277 | 0.058 | 95.698 | 4.555 | 0.259 | 28.041 | 0.502 | 0.236 | 1.624 | 0.056 |
| | S.E.M. | 0.012 | 11.540 | 0.766 | 0.005 | 14.422 | 0.454 | 0.055 | 2.582 | 0.206 | 0.030 | 0.153 | 0.005 |
| | n/tot | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 5/5 | 5/5 |

385

386 Fig. 3 shows the predicted BPA serum concentrations in ewes treated with BPA (0.5 – 50 or
 387 5000 $\mu\text{g}/(\text{kg}\cdot\text{d})$; S.C.) using a bi-compartmental model based on the PK parameters generated
 388 during experiment 1. The values for BPA serum concentrations measured in a representative
 389 ewe from the 5000 $\mu\text{g}/(\text{kg}\cdot\text{d})$ group at GD 62 were superimposed on the prediction curve and
 390 showed a good fitting of the data by our model. BPA serum concentrations were predicted to
 391 fluctuate between 2 – 32 pg/mL (8.8 pmol/L -0.14 nmol/L), between 0.2- 3.2 ng/mL (0.88-14
 392 nmol/L) and between 20 - 320 ng/mL (88-1400 nmol/L) for the BPA doses of 0.5, 50 and 5000
 393 $\mu\text{g}/(\text{kg}\cdot\text{d})$, respectively.



394
 395 **Fig. 3:** predicted serum concentrations of BPA in pregnant ewes treated with repeated
 396 subcutaneous administrations of BPA (0.5 – 50 and 5000 $\mu\text{g}/(\text{kg}\cdot\text{d})$). Concentrations were
 397 predicted using a bi-compartmental model and appropriate pharmacokinetic parameters
 398 previously established in the ewe for a dose of 5 $\text{mg}/(\text{kg}\cdot\text{d})$. Observed concentrations are the
 399 BPA serum concentrations measured in blood samples collected from one representative ewe
 400 treated with BPA (5000 $\mu\text{g}/(\text{kg}\cdot\text{d})$; S.C.) from GD 28 to 132-134. Blood samples were collected
 401 at GD 62 at time 0 - 1.5 – 4 - 8 and 10 h after the S.C. administration.

402

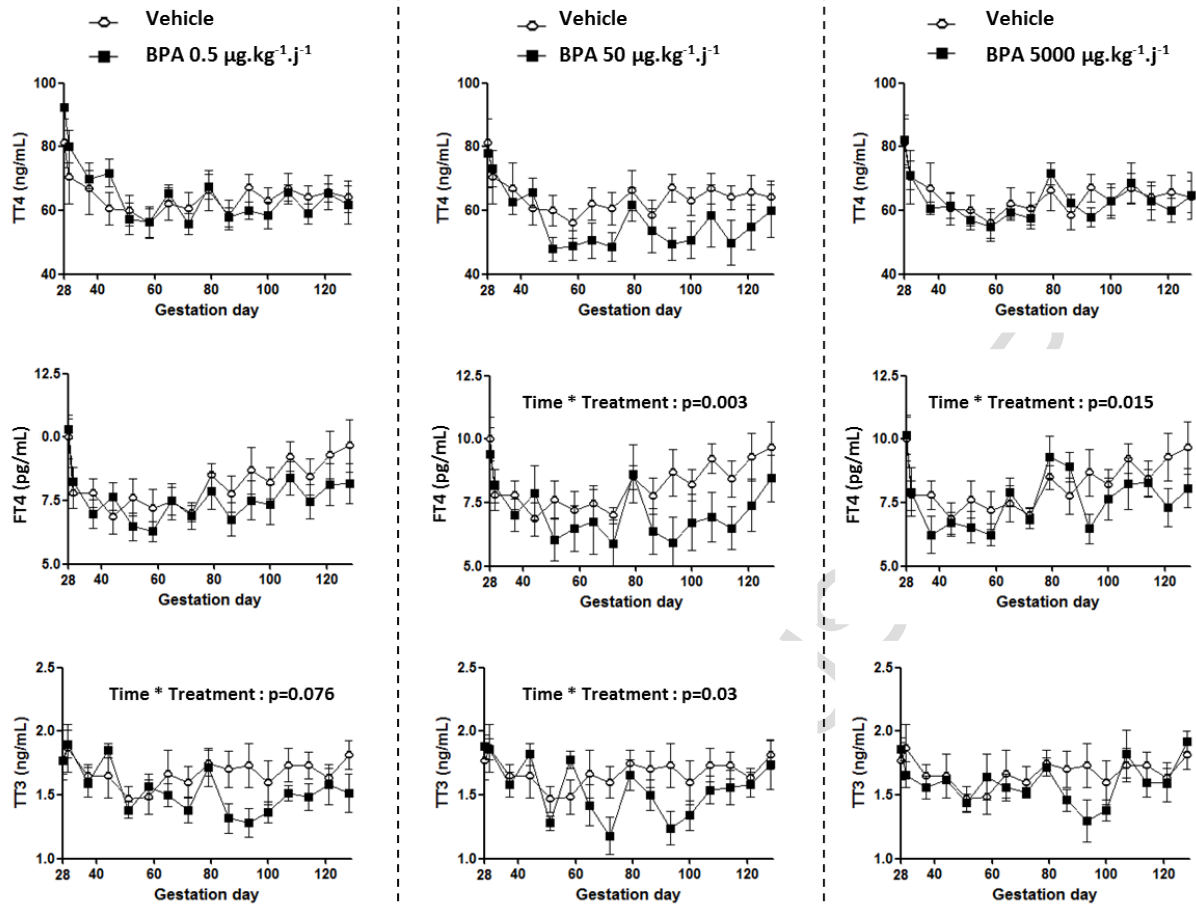
403 3.3. Maternal and fetal thyroid status (Experiment 2)

404 Fig. 4 shows the time course of mean totalT4, free T4and total T3 concentrations measured in
405 the maternal serum samples throughout the treatment period for each BPA group compared
406 individually to the control group. For total T4, no significant interaction between time and
407 treatment could be evidenced ($p=0.37$).

408 The time course of maternal free T4 serum concentrations appeared to be affected by the
409 treatments (interaction time x treatment, $p<0.001$). A significant difference between the control
410 group and the medium ($p=0.003$) and high ($p=0.015$) dose groups was observed which was
411 characterized by a decrease in fT4 concentrations. Visual inspection of the graph indicates that
412 this decrease was initiated around GD 40 (i.e. after about 12 days of treatment) in BPA-treated
413 animals. The time course of the ratio of fT4 to TT4 concentrations in maternal blood was not
414 modified by the treatment (interaction time* treatment, $p=0.7$).

415 Throughout pregnancy, the time course of TT3 concentrations in the mothers was significantly
416 affected by the treatment (interaction time x treatment, $p=0.04$). The profile of the medium dose
417 group differed from that of the control group ($p=0.03$). Overall, TT3 concentrations were lower
418 in BPA-treated animals from GD 60-70 (visual inspection). This interaction was not significant
419 for the high dose group ($p=0.44$).

420 No effect of BPA at any dose on serum TSH concentrations (Fig. 5) could be evidenced in
421 maternal blood samples collected at GD 34, 65,97 and 132 (interaction time * treatment, $p=0.23$
422 and treatment effect $p=0.19$).



423

424 **Fig. 4:** time course of mean (\pm SEM) total T4, free T4 and total T3 serum concentrations in
 425 pregnant ewes treated subcutaneously with vehicle ($n=6$) or BPA ($0.5 \mu\text{g}/(\text{kg.d})$, $n=6$). 50
 426 $\mu\text{g}/(\text{kg.d})$, $n=5$ or $5000 \mu\text{g}/(\text{kg.d})$, $n=5$) from GD 28 to 132-134. Blood samples were collected
 427 on GD 28 – 30 – 37 – 44 – 51 – 58 – 65 – 72 – 79 – 86 – 93 – 100 – 107 – 114 – 121 and 128.
 428 Time * treatment significant interactions ($P<0.05$) were evidenced in several instances
 429 signifying that the time course of the hormones differed between the BPA and vehicle-treated
 430 animals.

431

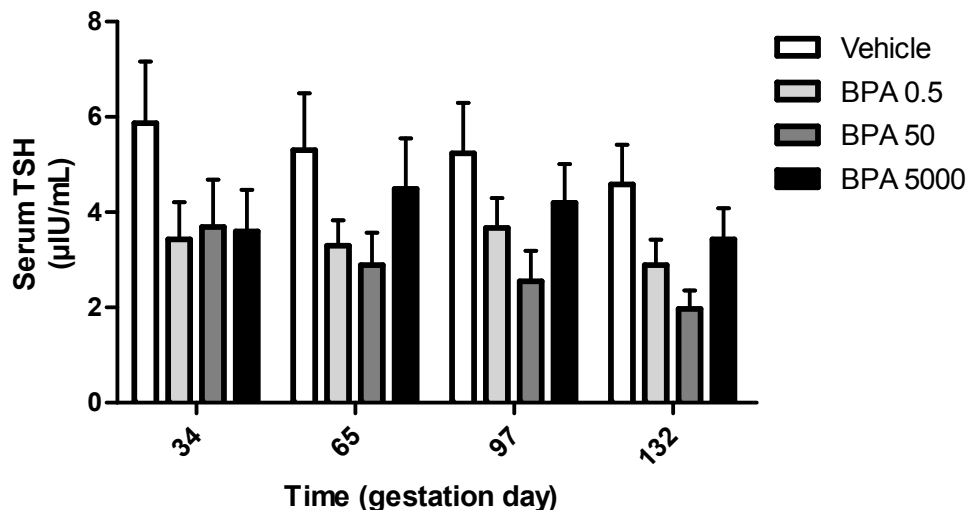
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DOI : 10.1016/i.chemosphere.2017.05.028

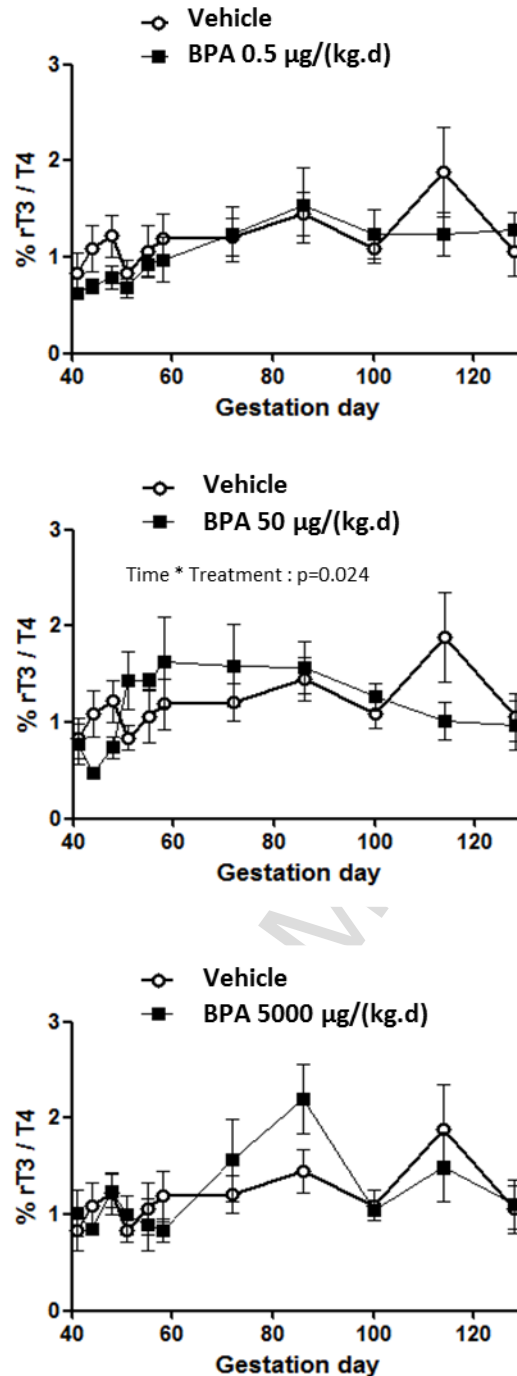


434

435 **Fig. 5:** mean (\pm SEM) serum TSH concentrations in pregnant ewes treated subcutaneously with
 436 vehicle (n=6) or BPA S.C. (0.5 μ g/(kg.d), n=6; 50 μ g/(kg.d), n=5 or 5000 μ g/(kg.d), n=5) from
 437 GD 28 to 132-134. Blood samples were collected on GD 34 – 65 – 97 and 132. No significant
 438 effect of BPA treatments could be evidenced.

439

440 Disruption of the maternal thyroid status was further characterized by measuring rT3 jointly to
 441 TT4 using HPLC/MS in samples collected from the 41st day of gestation, i.e. after FT4
 442 concentrations have started to decrease in the medium dose group. A treatment effect could be
 443 evidenced on TT4 concentrations with no interaction with the time. On rT3 profiles, a trend
 444 ($p=0.09$) toward an interaction between time and treatment was evidenced suggesting that
 445 modifications of the rT3 patterns might occur later than for T4. The time course of the rT3/TT4
 446 ratio (Fig. 6) was significantly affected by BPA exposure (interaction time x treatment $p=0.04$)
 447 over the period considered. These modifications were due to differences between the control
 448 and medium dose groups with a time-dependent effect on the rT3 to TT4 ratio ($p=0.024$)
 449 characterized by a higher rT3 to TT4 ratio in the BPA group from GD50 to 90, approximately.



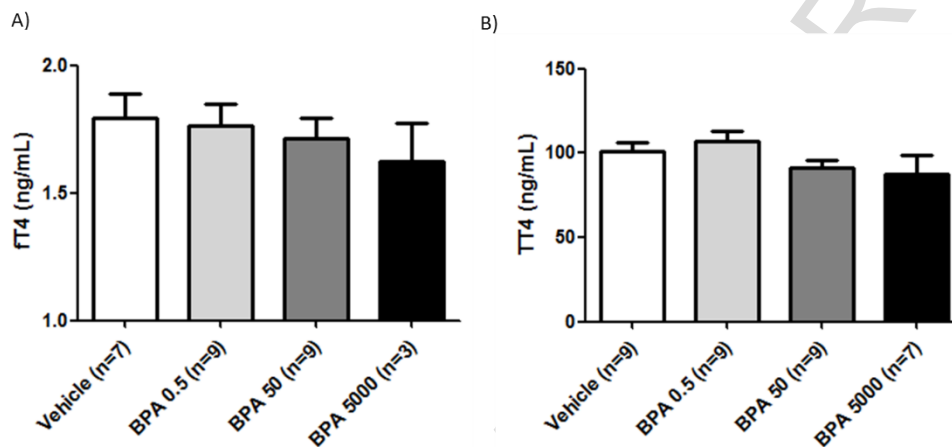
450

451 **Fig. 6:** time course of mean (\pm S.E.M.) ratio (%) of rT3 to total T4 serum concentrations
 452 measured by HPLC/MS in pregnant ewes treated subcutaneously with control or BPA 0.5 – 50
 453 and 5000 $\mu\text{g}/(\text{kg}\cdot\text{d})$ from GD 40 to 132-134. Blood samples were collected on GD 41 – 44 –
 454 48 – 51 – 55 – 58 – 72 – 86 – 100 – 114 and 128. A time * treatment significant interaction was
 455 evidenced only in the groupe treated with the middle dose ($p=0.024$) signifying that the time
 456 course of the rT3/T4 ratio in this group differed from vehicle animals.

457

458 Neither TT4 nor fT4 in fetal jugular blood (Fig. 7) were affected by BPA treatments at any of
 459 the doses tested (individual comparison with the control group, $p > 0.05$). TT3 was never
 460 detectable in fetal jugular blood.

461



462

463 **Fig. 7:** mean (\pm S.E.M.) free (A) and total (B) T4 serum concentrations measured by a RIA
 464 method in fetal jugular blood collected at GD132-134. Fetuses came from mothers treated with
 465 vehicle or BPA 0.5 – 50 and 5000 $\mu\text{g}/(\text{kg}\cdot\text{d})$ from GD 28 to 132-134 S.C. Data were analyzed
 466 using a one factor ANOVA. No significant effect of the treatments could be evidenced ($p > 0.05$).

467

468 The thyroid hormone concentrations in cord blood were measured by HPLC/MS. TT3 in the
 469 fetuses was almost always below our assay LOD. No effect of the treatment on rT3, TT4
 470 concentrations and rT3/TT4 ratio could be evidenced in cord blood.

471

472

4. Discussion

473 Our study is one of the rare studies to directly assess the equivalence between a route of
474 exposure commonly used in experimental studies of BPA toxicity and the dietary route
475 acknowledged as the main route of exposure for human populations. Our findings suggest that
476 the bioavailability of BPA might be 30 fold lower when administered by dietary route than by
477 subcutaneous route. Our current results indicate that the difference between the two routes of
478 exposure is mainly related to the overall BPA exposure and much less to the peak serum
479 concentrations. Our results also show that BPA-GLUC exposure, unlike that of BPA, is very
480 similar for both routes of administration. BPA-GLUC is usually assumed to be devoid of
481 biological activity. However, in some sensitive tissues, the balance between BPA detoxification
482 by conjugation into BPA-GLUC and BPA-GLUC reactivation by deconjugation into BPA is in
483 favor of deconjugation (Corbel et al., 2014). Although the mechanism enabling BPA-GLUC (a
484 hydrophilic compound) to reach the intracellular space remains unknown, these observations
485 indicate that internal exposure to BPA metabolite might also be of biological significance and
486 should be more thoroughly considered in toxicological studies. Furthermore, it is noteworthy
487 that BPA-GLUC blood profiles differ markedly after gavage administration as compared to the
488 dietary one (Guignard et al., 2016). Thus when considering potential effects of BPA-GLUC,
489 gavage might not be better than the subcutaneous route for evaluating BPA oral toxicity.

490 Our TK model enabled us to estimate the internal exposure of ewes treated by chronic exposure
491 via the subcutaneous route. Interestingly, both the medium and low doses selected were
492 predicted to reproduce BPA serum concentrations in ewes within the range of concentrations
493 commonly reported in the most recent studies in pregnant women i.e., from 100 pg/mL
494 (Teeguarden et al., 2016) to 4 ng/mL (Shekhar et al., 2016). However, in both those studies,
495 free BPA would account for about 60-80% of total BPA while in our study the ratio of BPA-
496 GLUC to BPA was markedly in favor of BPA-GLUC in both the mother and the fetus at
497 delivery, i.e. at least 24 h after the last BPA administration.

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498 Concerning fetal exposure, our exposure schemes made it possible to encompass the range of
499 aglycone BPA concentrations reported in fetal compartments in humans, whether in amniotic
500 fluid or cord blood. Indeed, for BPA, the geometric mean of about 4.8 ng/mL in the amniotic
501 fluid of full-term human newborns (Shekhar et al., 2016) is similar to the concentrations
502 measured in the amniotic fluid of lambs from the medium dose group in our study. Similarly,
503 cord blood concentrations in human newborns (about 1.2 ng/mL; Chevalier et al., 2015) are
504 very close to the few measurable values obtained in the cord blood of lambs from our medium
505 dose group. Our study evidenced a high accumulation rate of BPA-GLUC in the fetal
506 compartment in agreement with other study with short term exposure. Indeed, in our current
507 study, as in the short term fetal exposure study performed at GD120 (Corbel et al., 2013),
508 amniotic fluid concentrations were about 4 fold lower than concentration in fetal blood (jugular
509 or cord blood). However, in a first study with chronic gestational exposure (Viguié et al., 2013),
510 BPA-GLUC concentrations in full term lambs were 2 fold higher in amniotic fluid than in fetal
511 blood. The most likely explanation for such fetal age –dependent differences in fetal exposure
512 to BPA-GLUC could rely on the ontogeny of UGT activity in the fetal sheep with a late rise
513 toward the end of pregnancy (Pretheeban et al., 2011) similarly to human (Divakaran et al.,
514 2014). The biological significance of the very high fetal exposure to BPA-GLUC produced in
515 our sheep model and its relevance to humans remains to be elucidated. From this standpoint, it
516 is noteworthy that some data in monkeys suggest that, similarly to what is observed in the ovine
517 fetus, BPA-GLUC accumulates in the fetal compartment (Patterson et al., 2013).

518 BPA-SULF appeared to accumulate as well in the fetal compartment. However, it is noteworthy
519 that it does not follow the same pattern in the fetal compartment than BPA-GLUC. BPA-SULF
520 concentrations in the amniotic fluid are indeed about 10 fold lower than in the fetal
521 compartment. This suggests that BPA-SULF might not be eliminated from the fetal blood

522 through the kidney and thus the amniotic fluid or that BPA-SULF is very rapidly and efficiently
523 cleared from the amniotic fluid.

524 Our results concerning the thyroid status of the mother reveal a certain degree of thyroid
525 disruption oriented toward a decrease in concentrations of the circulating hormones, fT4 and
526 TT3. The medium dose (50 $\mu\text{g}/(\text{kg}\cdot\text{d})$) seemed to be pivotal as this level of BPA treatment
527 significantly affected the time courses of both hormones. Interestingly, the time course of TT3,
528 the biologically active hormone, tended to be significantly modified by our lowest dose (0.5
529 $\mu\text{g}/(\text{kg}\cdot\text{d})$). This suggests that pregnant females might be sensitive to BPA-induced thyroid
530 disruption even at environmentally-relevant exposure. Our study did not provide an assessment
531 of the minimal active BPA dose. It is noteworthy however that the effect observed at our lowest
532 dose are limited to a trend in TT3 decrease while both fT4 and TT3 were modified with the
533 middle dose. Although our highest dose (5000 $\mu\text{g}/(\text{kg}\cdot\text{d})$) enabled us to reproduce the
534 hypothyroxinemia described in a previous study, surprisingly, modifications of the time course
535 were only observed for fT4 and not for TT4. The fact that BPA induced a disrupting effect on
536 some thyroid parameters at the medium and/or low doses but not at the high dose suggests a
537 non-monotonic effect of BPA on thyroid function in pregnant animals. Nevertheless, this
538 interpretation needs to be treated with caution and more doses and a larger number of animals
539 would be required to evidence and characterize such a complicated dose-response relationship.

540 No effect of BPA treatments on fetal thyroid status could be evidenced in cord and jugular
541 blood samples. This contrasts with our previous results in sheep (Viguié et al., 2013). One major
542 difference between this study and the previous study in sheep is related to the gestational stage
543 and fetal age and/or the different fetal exposure to BPA/BPA-GLUC. In our earlier experiment,
544 the lambs were extracted at term, whereas in the current experiment they were extracted 10 to
545 14 days before term. Thus, the final stage of thyroid maturation might not yet have occurred in
546 these animals. This explanation is fully consistent with the fact that TT3 could not be detected

547 in the fetal blood samples. Indeed, TT3 concentration in the blood of sheep fetuses, as well as
548 human fetuses (Fisher, 2002), is only detectable in the later stages of pregnancy (the last week
549 for sheep; (Fisher et al., 1994). It is noteworthy that, in rodents, BPA-induced thyroid disruption
550 due to maternal exposure was mainly evidenced during the postnatal period, which corresponds
551 in this species to the final stage of maturation of thyroid function (Xu et al., 2007; Zoeller et
552 al., 2005), i.e., equivalent to the two missing weeks required, in the current experiment, for the
553 ewes to attain full term pregnancy.

554 Another explanation for such a discrepancy might arise from the difference in mineral supply
555 provided to the animal. Indeed, in the current experiment, ewes received a pellet ration
556 containing a mineral supply adapted to pregnancy requirements and thus enriched in mineral
557 iodine (calcium iodate about 1.5 mg/day) and selenium (sodium selenite about 0.62 mg/day).
558 Iodine supply might have somehow compensated at least in part the BPA-induced thyroid
559 disruptive process or made the fetal thyroid function less sensitive to thyroid disruption. This
560 kind of relationship between sensitivity to thyroid disruption and iodine status is supported by
561 epidemiological observations in humans. The inverse maternal urinary BPA /newborn girl TSH
562 relationship was shown to depend, at least in part, on the maternal iodine status in the human
563 biomonitoring "HOME" survey (Romano et al., 2015), suggesting that the maternal iodine
564 supply might somehow affect the sensitivity of the developing fetus to chemical thyroid
565 disruption. Other examples of interaction between the iodine status and diverse thyroid stressors
566 have been found in humans such as mercury in pregnant women (Llop et al., 2015) or
567 perfluoroalkyl acids in anti-TPO antibody positive humans (Webster et al., 2016).

568 The mechanisms by which BPA exposure induces maternal thyroid disruption remain unclear.
569 From our results, we could not get any evidence that either TSH secretion or TH binding to
570 carrier serum proteins might be substantially altered in BPA-treated ewes. The absence of
571 significant modifications of TSH in our BPA-treated pregnant ewes should not be considered

572 as an ultimate proof that the thyroid axis alteration were of no potential functional
573 consequences. Disruption of thyroid homeostasis indeed occurs in the absence of modification
574 of blood TSH in so called states of hypothyroxinemia (decreased free-T4 concentration with or
575 without modifications of TSH). There are some experimental evidences that such maternal
576 hypothyroxinemia might be of detrimental consequences on fetal neural development and as so
577 should not be regarded as trivial (Morreale de Escobar et al., 2000; Lavado-Autric et al., 2003;
578 Gilbert et al., 2012). Accordingly, some epidemiological surveys highlight a link between
579 maternal hypothyroxinemia during pregnancy and increased risk of autism impaired
580 psychomotor development, Attention Deficit Hyperactivity Disorder, language delay, lower
581 mental scores (Zoeller et al., 2002; Henrichs et al., 2013; Román et al., 2013).

582 The balance between deiodinase activities converting T4 into either the bioactive T3 or
583 inactive rT3 is a key factor in the maintenance of fetal thyroid homeostasis. This is why we
584 measured rT3 and T4 concentrations in cord blood and in some bi-weekly maternal samples,
585 using MS detection, in an attempt to gain new insights into the balance between T4 and rT3 and
586 the potential mechanisms of BPA action. The results obtained with MS in maternal samples,
587 collected from GD 41 to 128, suggest that a modification of the deiodination balance,
588 characterized by a time-dependent modulation of the rT3/TT4 ratio, occurred in mothers
589 receiving the medium BPA dose compared to those in the vehicle group. The placenta is a key
590 actor in fetal thyroid balance. It not only expresses deiodinases, in particular type 3, to produce
591 a balance between the active and inactive thyroid hormone supply to the fetus, it also
592 synthesizes transthyretin which regulates the transplacental transfer of maternal thyroxine to
593 the fetal compartments (Landers et al., 2009) and regulates iodide exchanges through NIS (Na/I
594 symporter) expression. *In vitro* data suggest that the placenta is a sensitive target tissue for BPA,
595 including at environmentally relevant doses (Mannelli et al., 2015; Sieppi et al., 2016; Tait et
596 al., 2015). However, no evaluation of the impact of BPA on thyroid-relevant placental functions

597 are currently available and there is no evidence that BPA can alter deiodinase expression and/or
598 activity in any tissues. In the light of our current results indicating that in pregnant ewes BPA
599 exposure modifies the balance between T4 and rT3 at a late stage of pregnancy, it could be very
600 interesting to assess the effect of BPA on placental deiodinase activities.

601

602 **5. Conclusion**

603 Our study carried out in an animal model relevant to humans in terms of thyroid and gestation
604 physiologies shows that BPA can affect maternal thyroid function and equilibrium during
605 pregnancy. This effect was expressed at levels of internal exposure (as assessed by serum
606 aglycone BPA concentrations) similar to those described in humans i.e. for BPA blood
607 concentrations below the ng/ml range. Although within the framework of this study, these
608 maternal modifications could not be tied with alterations in some patterns of fetal TH serum
609 concentration, the possibility remains that TH action and concentrations in target tissues,
610 particularly in the brain, might have been affected. Interestingly, our data suggest that BPA
611 exposure might somehow modify the deiodination balance, thus pinpointing a possible effect
612 of BPA on the thyroid-related endocrine function of the placenta.

613

614

615 **Acknowledgements**

616 We thank the INRA experimental unit of Domaine de Lafage for providing the animals and the
617 staff from the ENVT sheep experimental unit for their involvement in animal care.

618 This research did not receive any specific grant from funding agencies in the public,
619 commercial, or not-for-profit sectors. D Guignard benefited from PhD Grant of the doctoral
620 School SEVAB from the Institut National Polytechnique of Toulouse, France.

621

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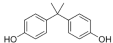
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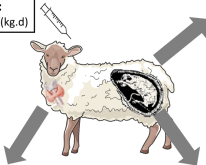
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Bisphenol A:

0.5 – 50 – 5000 $\mu\text{g}/(\text{kg}\cdot\text{d})$



Toxicokinetic analysis

S.C.
route

BPA

Dietary
route

>

BPA-G

=

Effects on thyroid function
at low doses

Maternal thyroid status:

↓ T4

↓ T3