ANSES Opinion
Request No 2016-SA-0124

The Director General

Maisons-Alfort, 19 April 2017

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the identification of bisphenol A as a substance of very high concern (SVHC[1]) for its endocrine-disrupting nature

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES's public health mission involves ensuring environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with the necessary information concerning these risks as well as the requisite expertise and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are published on its website.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 19 April 2017 shall prevail.

On 9 May 2016, ANSES received a formal request from the Ministry of the Environment, Energy and the Sea to conduct the following expert appraisal: submission to ECHA[2] of a dossier identifying bisphenol A (BPA) as a "substance of very high concern" (SVHC), in particular due to its endocrine-disrupting (ED) properties for humans, under European Regulation No 1907/2006 (known as the REACh Regulation).

1. BACKGROUND AND PURPOSE OF THE REQUEST

In its Article 57, the REACh Regulation defines the properties that may lead to the identification of a “substance of very high concern" (SVHC).

Identifying BPA as an SVHC has the direct consequence of requiring industry to notify ECHA of its presence in manufactured or imported articles containing more than 0.1% of BPA (as defined in Art. 7 of REACh) and to inform the purchaser of an article of the presence of BPA (as defined in Art. 33). It opens up the possibility of the substance being subject to authorisation (Annex XIV of the Regulation), which may limit the uses of BPA by making them conditional on the granting of a temporary, renewable authorisation. These provisions concern the uses as defined mainly by Article 56 of REACh. In particular, they do not concern the uses of BPA as an intermediate, especially for its polymer uses.

[1] Substance of Very High Concern as defined in Regulation No 1907/2006 (the REACh Regulation)
[2] European Chemicals Agency

To respond to the formal request, the SVHC nature of bisphenol A (BPA, CAS No 80-05-7) was examined in connection with several properties that would make it eligible for identification as an SVHC:

- BPA has a harmonised European classification for its effects on fertility (toxic for reproduction Category 1B). On the basis of these facts, on 8 August 2016 ANSES submitted an SVHC identification dossier according to the criteria of Art. 57(c) of REACh. The identification of BPA as an SVHC for this property was recognised by consensus and BPA was added to the list of SVHC substances (Candidate List[3]) by the ECHA decision of 4 January 2017 (ED/01/2017).
- A substance with endocrine-disrupting (ED) properties is eligible for an SVHC identification according to Article 57(f) of REACh. Identification according to Article 57(f) has specific regulatory consequences compared with identification according to Article 57(c), in particular on the threshold for application of the measures applicable to an SVHC and, where appropriate, on the context of the assessment of future applications for authorisation. It is also the only regulatory means currently enabling a chemical[4] to be identified as an ED. A detailed collection, analysis and synthesis of evidence in connection with these ED properties was therefore carried out in the framework of this formal request.
- Due to previous work conducted by the German authorities on the potential ED effects on species in the environment, it was decided to exclude this subject from ANSES's analysis, which specifically examined the endocrine-disrupting properties of BPA relevant to human health.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

The expert appraisal falls within the sphere of competence of the Expert Committee on "Assessment of the risks related to chemical substances for the implementation of the REACh Regulation" (CES REACh-CLP), which has competence for SVHC dossiers and is the reference CES for this formal request, as well as the Working Group on "Endocrine disruptors" (WG ED), which has competence on this specific subject and was entrusted with the scientific analysis in this formal request. The work was discussed at the WG ED meetings that took place from 11 March 2016 to 13 January 2017, and its methodical and scientific aspects were presented to the CES REACh-CLP at meetings on 29 March, 8 November and 6 December 2016. The report was adopted by the CES REACh-CLP at its meeting on 24 January 2017.

The analysis was conducted within the framework of the procedures and format established by ECHA at European level.

In this analysis, it was considered that a substance can be identified as an ED if it meets the recommendations[5] of the expert advisory group from the European Commission (JRC 2013), in

[3] The candidate list is defined in Article 59(1) of REACh, and the list of substances formally identified as an SVHC and that may therefore be subject to authorisation is in Annex XIV.

[4] Excluding biocidal substances and pesticides.

[5] "*the elements for identification of an endocrine disruptor were demonstration of an adverse effect for which there was convincing evidence of a biologically plausible causal link to an endocrine disrupting mode of action and for which disruption of the endocrine system was not a secondary consequence of other non-endocrine-mediated systemic toxicity.*

connection with the definition of an ED formulated by the WHO/IPCS[6] in 2002. Accordingly, the available information was analysed with regard to the following points:
- Adverse health effects
- Endocrine mode of action
- Plausible link between adverse effects and endocrine mode of action
- Relevance for humans

The analysis of the harmful effects of BPA involving an ED mode of action was conducted based on ANSES's previous assessments on BPA at national (ANSES 2013b) and European level (ANSES, 2013a and 2014). It was supplemented by a review of the relevant data published up to May 2016. For the section of the report concerning identification of the uses of and alternatives to BPA, earlier work (ANSES, 2011, 2012 and 2013a) was supplemented by the consultation of REACh registration dossiers and by hearings or email exchanges with stakeholders, namely the French National Association of Food Industries (ANIA) jointly with the National Association of Manufacturers of Packaging Boxes and Metal Capping (SNFBM) on 1 June 2016, the National Association of the Medical Technology Industry (SNITEM) on 8 June 2016, Plastics Europe in late May-early June 2016, the French Coordinating Committee of Dental Activities (COMIDENT) in June 2016, and Dr Sylvain Caillol, Assistant Director of the Carnot Institute - Chimie Balard in June 2016. It was also supplemented by the comments received during the public consultation that took place for the identification of BPA as an SVHC-57(c).

ANSES analysed the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.
The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

3. ANALYSIS OF THE WG ED AND CONCLUSIONS OF THE CES REACH-CLP

3.1 Analysis by the WG ED of the effects of BPA related to an ED mode of action with a view to an SVHC identification

The present analysis proposes to identify BPA as an SVHC on the basis of its endocrine-disrupting properties relevant to human health. The adverse effects of BPA analysed in detail to support the SVHC identification in connection with the endocrine-disrupting (ED) properties were selected on the basis of the following criteria:

- the adverse effect has been characterised with a sufficient level of evidence in European regulatory discussions, i.e. this effect is acknowledged by either a harmonised classification or by consideration of the effect in the risk assessment process by the Risk Assessment Committee (RAC) in the context of the restriction of BPA (ECHA, 2015);

- there is relevant evidence that these effects are the result of an ED mode of action.

In the event of future applications for authorisation, it is important for the scope of the health effects associated with the endocrine-disrupting action of BPA to be defined adequately and as comprehensively as possible. In addition, a joint analysis of the various adverse effects of BPA

Relevance of the data to humans should be assumed in the absence of appropriate data demonstrating non-relevance."
- Report from the European Commission's Endocrine Disruptors Expert Advisory Group (JRC 2013)

[6] "*An exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*" – Global assessment of the State-of-the-Science of Endocrine Disruptors (WHO/IPCS 2002)

resulting from an ED effect is all the more justified since these effects share certain characteristics that usefully substantiate the likelihood of an overall effect. Therefore, the following effects were selected:

- effect on reproductive function as evidenced more specifically by alteration of estrous cyclicity;
- alterations to mammary gland development leading to an increased susceptibility to carcinogens through morphological modifications;
- alterations to brain tissue development and cognitive functions, shown more specifically in connection with the impairment of learning and memory;
- metabolic alterations, shown more specifically in connection with an impairment of the ability to synthesise insulin and of insulin sensitivity.

- Alteration of estrous cycles

In humans, one epidemiological study analysed the link between BPA and the characteristics of menstrual cycles (Jukic *et al.,* 2015). An association between urinary BPA and shorter luteal phases was observed. However, no definitive conclusion in humans can be drawn on the basis of a single study. In animals, although there were some disparities between the studies, reliable results have repeatedly been reported in several experimental studies in rats and mice showing an adverse effect of BPA on the estrous cycle, including irregular and prolonged cycles, after different periods of exposure:

- after exposure of adult females (Tyl *et al*., 2008; Laws *et al*., 2000; Lee *et al*., 2013),
- after exposure during the developmental phase of the reproductive system, i.e. after *in utero* (Honma *et al*., 2002; Nikaido *et al*., 2004; Wang *et al.,* 2014a), perinatal (Rubin *et al*., 2001; Mendoza *et al*., 2011; Patisaul *et al*., 2014; Delclos *et al*., 2014), postnatal (Nah *et al*., 2011; Adewale *et al*., 2009; Fernandez *et al*., 2009) or prepubertal exposure (Zaid *et al*., 2014)

This effect was recognised by the RAC in its 2014 opinion in support of classification of BPA as toxic for reproduction, Category 1B (Repr 1B) – H360F, and in its 2015 opinion on restriction of BPA (ECHA 2015).

Maintaining proper cyclicity is considered essential to achieve successful ovulation. An alteration of cyclicity may therefore induce a reduction in fertility through disrupted (delayed or absent) ovulation. As summarised by Kortenkamp *et al*., (2012), an association between menstrual cycle characteristics, sub-fecundity and spontaneous abortion has been observed in women of childbearing age. In addition, certain characteristics of the menstrual cycle have been associated with chronic diseases, including breast and ovarian cancer, uterine fibroids, diabetes and cardiovascular disease. Alteration of cyclicity is therefore considered to be a clearly adverse effect.

In relation to alteration of the cycle following adult exposure, a negative effect of BPA on ovarian estrogen production is clearly demonstrated in rodents *in vivo* (Lee *et al*., 2013) and confirmed by similar effects observed on rodent and human ovarian cells *in vitro* (Kwintkiewicz *et al.*, 2010; Mansur *et al.,* 2016). Whereas the effect of BPA on theca-interstitial cells depends on the test model and the protocol, BPA reduces the conversion of androgens into estrogens in granulosa cells, consistently in all the studies identified in the literature.

In both primates and non-primate mammals, follicle selection, growth and maturation, as well as ovulation, oocyte quality and subsequent *corpus luteum* function, all depend on gonadotropin

regulation[7] and intra-ovarian regulation factors, exercised by reciprocal, sequential controls. Furthermore, the ovaries and the hypothalamic-pituitary system are in permanent endocrine dialogue with each other. Consequently, any disruption to the cycle is related to disruption to the endo/para/autocrine activities of the ovaries and/or the hypothalamic-pituitary system. Given the regulatory scheme of the estrous cycle, an alteration to the steroidogenic activity of the preovulatory follicle, as observed after exposure of adult animals to BPA (Lee *et al.,* 2013), can cause disruption to the estrous cycle. These results demonstrate an endocrine mode of action, namely alteration of ovarian steroidogenic activity, which underlies the estrous cycle disruption in adult rodents.

Although most of the available evidence comes from rodent studies, there are *in vitro* data that also show the same negative effect of BPA on estrogen production in human follicle cells. In addition, in some women, exposure to BPA was associated with a decreased ability of the follicles to produce estrogens (Mok-Lin *et al.,* 2010; Ehrlich *et al.,* 2012). Lastly, the role of estrogens in regulation of the cycle is similar in rodents and women. Thus, it is likely that BPA may alter the ovarian cycle in women through disruption of the endocrine activity of the ovarian follicle.

The role of endocrine or neuroendocrine mechanisms in the cycle alteration observed following exposure to BPA during development is less clearly demonstrated than after adult exposure. Nevertheless, many studies show that the (neuro)endocrine mechanisms behind the finely-tuned regulation of the gonadotropic function underlying the estrous cycle can be altered in response to BPA exposure, after *in utero* and perinatal exposure. BPA has been shown to affect the hypothalamic expression of kisspeptin, a key neuropeptide in the regulation of the hypothalamic–pituitary–gonad (HPG) axis, and the establishment of hormonal circuits subsequently enabling hormonal regulation of an efficient cycle. In particular, studies by Monje *et al.* (2010) and Fernandez *et al.*, (2009) describe a link between neuroendocrine changes and cycle alteration through concomitant observation of an alteration of HPG-axis hormones and cycle disruption. The affected targets are similar to a large extent to the targets affected by an estrogen agonist or by estrogenic positive controls. Overall, animal and *in vitro* data support the hypothesis of the endocrine system's involvement in disruption of estrus cyclicity after intra-uterine exposure to BPA.

In humans, the role of kisspeptin in neuroendocrine control of the HPG axis has been demonstrated. In addition, the recent study by Kurian *et al.* (2015) on pubertal female rhesus monkeys suggests that persistent exposure to BPA could impair the female reproductive function by directly influencing hypothalamic neuroendocrine function, as shown by an alteration of kisspeptin release and GnRH pulsatility. Therefore, it can be considered that BPA-induced alterations of the hypothalamic kisspeptin/GnRH system are also relevant in women. They may affect estrous cyclicity a long time after intra-uterine or perinatal exposure and are then considered irreversible.

- Alteration of mammary gland development leading to increased susceptibility to carcinogens through morphological modifications

Very few epidemiological studies are available on the risk of breast cancer after exposure to BPA early in life. Based on those studies (mainly case-control or cross-sectional studies), a link between exposure to BPA and the occurrence of breast cancer could not be determined (Aschengrau *et al.*, 1998, Yang *et al.,* 2009, Sprague *et al*., 2013 and Trabert *et al*., 2014). It should be noted that the existing studies evaluate exposure to BPA on the date of the study, whereas the examined effects are long-term effects appearing several decades after exposure. A longitudinal epidemiological study measuring BPA *in utero* and the occurrence of breast cancer cases several years later would be more biologically relevant. The mammary gland develops in three distinctive stages during the

[7] In particular follicle-stimulating hormone (FSH) and luteinising hormone (LH)

life cycle: foetal, peri-pubertal, and pregnancy (Fenton, 2006), with hormonal involvement such as mammary epithelial estradiol signalling and progesterone (PR) and prolactin (PL) receptor involvement, depending on the developmental period. Moreover, the mammary epithelium responds differently to hormonal stimulus depending on its developmental stage.

In terms of hormonal regulation, there seem to be substantial similarities across species. In most mammals, the ovaries first secrete estrogens in response to increased secretion of gonadotropins, and sexual maturity coincides with the establishment of cyclic peaks of ovarian progesterone secretion. Proliferative activity is observed in the mammary epithelium in women (Masters *et al*., 1977; Longacre and Bartow, 1986) concomitantly with the peak of progesterone during the luteal phase. Thus, the mammary epithelia of rodents and humans seem to be similarly regulated, at least with regard to hormonal control of cell proliferation.

Although the anatomy of the human female breast is more complex than the rodent mammary gland, some human breast structures (terminal ductal lobular units or TDLUs) are structurally similar to rodent structures (terminal end buds or TEBs) during the same life stages. These structures are undifferentiated and highly proliferative. As such, they are sensitive to the effects of carcinogens and other chemicals.

Based on an analysis of BPA data, there is evidence from rodent and primate studies that prenatal and postnatal exposure to BPA causes, depending on the period of exposure, modifications in the tissue of the mammary gland, including an increased number of TEBs relative to the ductal area, fewer apoptotic TEB cells, increased lateral branching and ductal hyperplasia, increased cell proliferation and decreased apoptosis in the glandular epithelium, and ductal (and occasionally lobuloalveolar) and intraductal hyperplasia. These changes subsequently increase their susceptibility to chemical carcinogens, as previously reported (ANSES, 2013b and Soto *et al.,* 2013) and increase the number of ductal carcinoma *in situ* (DCIS) in rats treated with BPA (Durando *et al.,* 2007, Jenkins *et al.*, 2009 and 2012, Betancourt *et al*., 2010). Delclos *et al.* (2014) observed ductal hyperplasia accompanied by a very limited number of mammary adenocarcinoma. Overall, based on all the available *in vivo* studies, the structural similarity between humans and rodents, and some common hormonal controls of cell proliferation, suggest that there is substantial evidence from rodent studies that early-life exposure to BPA leads to increased susceptibility to breast cancer in women.

In vitro experiments conducted on normal-like (MCF-10F or MCF-12A) breast cells show increased cell proliferation due to exposure to BPA and the mediation of ERα and its co-activator (Sengupta *et al*., 2013). In breast cancer cells and cancer-associated fibroblasts that lack the classical ER, it was shown that the G protein-coupled estrogen receptor (GPER) is required for growth effects and migration (Pupo *et al*., 2012). *In vitro* 3D models for breast glandular structure development, using non-transformed breast epithelial MCF-10F and MCF-12A cells, showed that BPA may alter the ductular and alveolar patterns in the collagen matrix, with a similar pattern to E2 (Fernandez and Russo, 2010). When BPA or 17β-estradiol treatment was combined with ER or GPER inhibitors (ICI 182 780 and G15, respectively), the effects (deformed acini) were reversed, suggesting that ER and GPER play a role in the disruption induced by BPA. As GPER is required for the growth and migration of cancer cells (SKBR3) and cancer-associated fibroblasts (CAFs), and since proliferative effects induced by BPA were cancelled out when GPER expression was silenced by a molecular biology technique, it can be concluded that BPA induces stimulatory effects as a GPER agonist in these breast cancer cells and CAFs. The deformation of the acini and the proliferation of cancer cells induced by BPA therefore involve hormone receptors in the *in vitro* models used.

Some studies using transcriptional analyses on stromal and epithelial compartments isolated from the foetal mammary gland (mouse) have demonstrated that exposure to BPA in dams modifies the expression of genes involved in apoptosis (increased expression of the anti-apoptotic gene, Birc2, Abl1), myoepithelial differentiation, the composition of the extracellular matrix (ECM) and adipogenesis of mesenchymal and epithelial cells (Wadia *et al*., 2013).

A series of studies conducted in the same scientific group (Mandal's team) described how BPA can alter the epigenetic programming of the promoter of HOTAIR and EZH2, genes whose expression depends on ethinyl estradiol (EE2). In addition, these genes have been described as being involved when cell proliferation increases, during the increased invasiveness seen in some breast tumours, and contributing to breast cancer progression.

Furthermore, exposure to BPA during foetal life provokes increased expression of both RankL (a critical connection between progesterone and epithelial cell proliferation) and Wnt4 (involved in progesterone-induced side branching in early adult life) at the adult stage (see respectively Jenkins *et al*., 2009 and Ayyanan *et al.,* 2011).

In conclusion, the available data support the plausibility that BPA, through interaction with the nuclear ERs or GPER and indirectly with PR, modulates estrogen and progestin agonist activities. Emerging epigenetic studies have reported that BPA-induced changes to estrogen-dependent genes (such as EZH2 and HOTAIR), as well as HOX genes (involved in embryogenesis and postnatal development) can be associated with abnormal development and increased cancer susceptibility of the mammary gland.

- Alteration of learning and memory performance

Epidemiological evidence about a link between exposure to BPA, particularly in early life, and performance related to learning and memory, are insufficient to draw any conclusions. However, a substantial majority of rodent studies (a total of 26/35, i.e. 74%) reported impaired spatial and non-spatial memory following exposure to BPA, regardless of the period of exposure. The number of studies supporting identification of this effect have risen substantially in recent years and overall they tend to conclude that BPA impairs memory in rodents. In addition, as the studies reporting impaired cognitive behaviour following BPA exposure were performed under various experimental conditions (various doses, routes and periods of exposure tested), this supports the plausibility of this effect. It is interesting to note that, depending on the studies, this effect may only appear in males/females or may differ according to the sex. Furthermore, although not systematically assessed in all the studies, effects on the neuronal structures were observed in association with the behavioural alterations. They consist in a reduction in the level of expression of NMDAR subunits, kinases, enzymes involved in neurotransmitter regulation and synaptic proteins, as well as decreased spine density or neurogenesis. Such molecular, cellular and structural changes are fully relevant and could underlie the reduced learning and memory performance observed in the same animals.

Finally, although there are a limited number of studies conducted in non-human primates, these have shown that BPA has detrimental effects on the midbrain dopaminergic system and on spine synapses in the hippocampus, following prenatal exposure, while it has no effect when applied at a juvenile stage (Elsworth *et al.,* 2013). In addition, adult BPA-exposed monkeys displayed significant cognitive impairment (Elsworth *et al*., 2015). It is also interesting to note that another study by the same laboratory reported that BPA cancelled out the effect of estradiol-induced stimulation of the spine synapses in the hippocampus and prefrontal cortex in adult ovariectomised monkeys (Leranth *et al.,* 2008).

Overall, it is considered that BPA has been demonstrated to impair memory and learning after exposure at the perinatal or pubertal stages, or in adulthood, based on multiple converging experimental studies. These also report molecular and cellular changes in the brain in line with the functional changes observed, pointing to a coherent adverse effect.

The possibility that BPA alters the cellular and molecular mechanisms involved in learning and memory processes through disruption of estrogen-dependent pathways was first suggested in the

study by Xu *et al.* (2010a and 2010b). They report decreased expression of ERβ. In the study by Xu *et al.* (2014b), the link between BPA-induced effects on learning and memory processes and estrogenic pathway disruption was clearly established: use of the ER antagonist (ICI 182,780) reversed BPA-induced effects on both ERα modulation and memory. Three other studies performed in adult animals showed that BPA can also counter estradiol-induced effects on behaviour and spine density in rodents (Xu *et al*., 2015b; Inagaki *et al*., 2012) and on synaptogenesis in non-human primates (Leranth *et al*., 2008). Additional evidence was provided by *in vitro* studies showing that BPA-induced effects on NMDAR signalling and synaptic proteins were reversed by the ER antagonist. In one *in vitro* study, BPA-induced disruption also concerned other non-classical estrogen receptors (ERRγ).

The modulatory effects of estrogens on cognitive processes and behaviour in adults are now well established. Although they have been studied more extensively in female animals (Galea *et al*., 2013; Pawluski *et al*., 2009), the importance of the estrogenic pathway in the regulation of cognitive behaviour and synaptic plasticity has also been reported in male rodents (Picot *et al*., 2016). Detailed effects of adult estrogens on learning and memory and the mechanisms underlying these effects in both males and females have been described in recent reviews (Frick *et al*., 2015; Hamson *et al*., 2016). Sex differences in hormonal impregnation during the critical periods of development and their influence on cognitive performance have been described by Roof and Havens (1992). This may explain the sex differences observed in the expression of cognitive behaviours and their alteration by BPA.

Altogether, these data strongly support the plausibility that alteration of learning and memory by BPA is mediated by disruption of the estrogenic pathways. Further information presented in the dossier also show that other steroid pathways might be involved.

Cognitive function in humans involves signalling pathways, which seem similar to those described in rodents. The involvement of the NMDAR signalling pathway in memory processes in healthy and diseased brain has been widely reviewed (e.g. in Gilmour *et al.,* 2012; Campos *et al.,* 2016; Arnsten *et al.,* 2016). Estrogens were shown to modulate hippocampus-dependent learning in women and non-human primates (Hampson, 1990; Lacreuse, 2006 and review by Hamson *et al*., 2016). Testosterone also modulates cognitive functions in men. Given these similarities in the modulatory effects of sex steroids on cognitive functions between rodents and humans, it is likely that the endocrine disruption observed in rodents occurs in humans and affects the cognitive processes in humans in a similar way as in rodents. In support of this hypothesis, the *in vivo* study conducted on adult female non-human primates showed that subcutaneous exposure to BPA (50 µg/kg/d) counteracts the synaptogenic effect of estradiol in the hippocampus and prefrontal cortex (Leranth *et al*., 2008).

Based on a) the significant amount of *in vivo* and *in vitro* animal data showing on the one hand impairment of learning and memory following exposure to BPA, and on the other hand the potential alteration of the cellular and molecular mechanisms underlying these processes through disruption of the estrogenic pathway, b) similarities in the signalling pathways underlying human cognitive function and c) the numerous data showing sex steroid regulation of these behaviours, it can be considered that exposure to BPA may alter human cognitive abilities through disruption of estrogenic pathways.

- Effects on metabolism

Based on the results of studies conducted on animals exposed during the prenatal and/or perinatal period or in adulthood, there is now evidence that BPA may increase the incidence of type-2 diabetes *via* an ED effect. In particular, BPA has been shown to alter insulin synthesis and/or release by β-pancreatic cells, or insulin signalling mechanisms within insulin-sensitive organs (i.e. liver, muscle, adipose tissues). These changes resulted in variations in the expression of hepatic or

adipose tissue markers, which are indicative of a state of insulin resistance. These effects are considered by the experts to be hallmarks of hormonal adverse effects, especially if there is a combination of effects, each leading to insulin resistance of the different insulin-sensitive tissues. In addition, while most studies were performed on males, a few studies have also examined the impact of BPA on both sexes, or specifically on female animals. However, more studies should be undertaken before concluding as to any sex-specificity of the metabolic impact of BPA on insulin synthesis/release and signalling.

Recent experimental *in vivo* and *in vitro* studies indicate that these effects may involve ERα, ERβ or GPR30 pathways. Other hormones such as leptin or adiponectin, involved in resistance to insulin and lipogenesis, are also modified following BPA exposure. This shows that BPA could disrupt the balance between insulin secretion and insulin action controlling glycaemia.

Overall, the data suggest that the endocrine functions of the pancreas are a target for BPA and that the mechanisms could differ depending on whether exposure occurs during foetal life or in adulthood. Foetal differentiation of the pancreas appears highly sensitive to exposure to BPA based on the outcomes analysed, e.g. β-cell proliferation and apoptosis. There are limited data available on the impact of BPA on α-cells and glucagon secretion. In addition, BPA can elicit histopathological modifications during foetal life with consequences on insulin synthesis and/or release.

Moreover, most of the *in vitro* studies showing adverse effects of BPA on adipocyte differentiation and function reveal an alteration of endocrine mechanisms (e.g., adiponectin release, insulin signalling-cascade effectors). However, uncertainties remain as to whether BPA activates PPARγ and/or other nuclear receptors (possible cross-talks between nuclear receptors).

The available epidemiological studies are unable to reach any definitive conclusions. The identified effects are considered relevant for humans because similarities exist in homeostatic regulation of insulin production and sensitivity between rodents (mostly investigated in the experimental studies) and humans, and because of *in vitro* experimental data using human cells or tissues.

- General conclusion concerning ED-mediated effects of BPA

Extensive evidence shows that BPA can affect several physiological functions and systems in mammals through estrogenic pathways. As explained above, BPA alters the reproductive function, mammary gland development, cognitive functions and metabolism through an ED mode of action.

Most importantly, although the steps of the respective mechanisms of action are specific to each effect, the disruption of the estrogenic pathways is consistently involved in each of the four effects. The primary target of BPA is however still not known with certainty. BPA binds to estrogen receptors (ER) but with weak affinity. In addition, BPA also binds to other types of ER such as GPER or ERR-γ with a higher affinity. These receptors may then also be involved in BPA-mediated effects, particularly at low doses. The complexity of the toxic response to BPA also suggests multiple modes of action that may interact. However, the evidence detailed for each effect specifically makes it possible to establish that disruption of estrogenic regulation is central and common. Other mechanistic evidence has also been identified relating to BPA-mediated effects, e.g. epigenetic modifications. It is not known whether they may be estrogen-dependent but the existence of additional mechanistic pathways does not contradict the importance of the estrogenic pathways in the BPA-mediated effects.

Estrogens are known to be central to the regulation of the reproduction function and interact with many other physiological functions, in particular the nervous system and metabolism. The pattern of effects observed with BPA is therefore consistent with an ED mode of action through estrogenic pathways.

In addition, there is "emerging" evidence that BPA may have immunotoxic effects (Ménard *et al*., 2014a and 2014b). The variability of the effects observed makes interpretation and transposition of these effects to humans uncertain. It should however be noted that the role of estrogens has often been reported in immunocompetence and in the development of the innate and adaptive immune response (Fish *et al*., 2008). The possibility cannot be excluded, therefore, that the range of effects related to the ED-properties of BPA may be broader than that described in this dossier.

Effects on the environment are not addressed in this report. However, experimental data identify estrogen-related modes of action involved in the reproductive disruption observed in several taxonomic groups. In particular, some data point toward an estrogen-agonist effect of BPA in fish (severe effect on reproduction affecting the population, in Yokota *et al*., 2000 and vitellogenin induction, in Kashiwada *et al*., 2002), in amphibians (skewed sex ratio towards females, in Levy *et al*., 2004 and vitellogenin induction, in Oehlmann *et al*., 2009), in molluscs (stimulatory effect of BPA on egg production antagonized by anti-estrogen, in Oehlmann *et al*., 2006) and possibly an estrogen-like action in echinoderms (abnormal larvae development, in Roepke *et al*., 2005).

Altogether, there is consistent evidence showing that BPA affects the reproductive function, mammary gland development, cognitive functions and metabolism. These alterations are mediated through disruption of estrogens and estrogenic pathways. These effects are considered serious health effects. Although they may be exerted through direct exposure (alteration of estrous cycles and of memory/learning performance), they are also observed for the four effects, after intra-uterine exposure, with consequences later in the life of offspring.

- Demonstration of a level of concern equivalent to that for CMR (carcinogenic, mutagenic, reprotoxic) properties

To fulfil the conditions for identification of an SVHC according to Article 57(f), it is necessary to demonstrate that the adverse effects mediated by the ED properties of BPA give rise to a level of concern equivalent to that for CMR properties. For this purpose, the following parameters are discussed:

- Type of effect: in relation to the ED mode of action, BPA exerts a range of effects considered serious. BPA can affect regulation of the reproductive function and can be involved in alteration of fertility, as reflected by its harmonised classification as a Category 1B reprotoxic substance (Repr 1B). On this basis, it unequivocally fulfils the criteria of severity, and therefore of equivalent level of concern (ELoC) compared to CMR substances. BPA alters the development and structure of the mammary gland and is considered to present a substantial risk factor by increasing the susceptibility to induction of breast cancer tumours. BPA is also implicated in alteration of learning, memory and brain neuronal structure. All these changes are recognised as adverse neurotoxic effects by the experts within the WHO Environmental Health Criteria Programme and EFSA (JRC, 2015). The effects of BPA on metabolism after exposure in animals (rodents and non-rodents) during the prenatal and/or perinatal period or in adulthood are associated with serious chronic diseases such as type-2 diabetes. Diabetes is accompanied by major co-morbidities and reduced life span. Altogether, the ED mode of action of BPA is considered to be responsible for multiple serious health effects.
- Irreversibility and delay: some of the ED-related effects of BPA have been identified after direct exposure. However, they are also all characteristically observed after exposure to BPA during developmental phases, with consequences that are observed later in life (estrous cycle, mammary gland, neurotoxicity, insulin synthesis and resistance), without direct exposure. As the effects appear a long time after exposure, they are indeed considered permanent and irreversible.

- Quality of life affected: disruption of ovarian cycles may result in various outcomes being triggered in women, with impacts of varying severity on everyday life, such as abnormal bleeding, disruption of fertility (e.g. due to fewer ovarian cycles and thus a lower probability of becoming pregnant in the case of elongation of cycles), disruption of sexuality, discomfort and inconvenience, and generally a lower quality of life. The increased vulnerability of the mammary gland due to developmental and/or peri-postnatal exposure increases the risk of developing breast cancer at a later stage of life. Besides the intensive potential treatments, breast cancer may also have many other adverse consequences on people's lives, such as absenteeism, social isolation, psychological depression, anxiety, and more generally a lower quality of life. A decrease in cognitive functions can have an impact on daily life by impairing the ability of an individual to effectively and autonomously cope with daily tasks and difficulties. It may occur through many different forms, such as temporal and spatial disorientation, poor memory, disrupted learning capacities, etc. New evidence is available on anxiety, which can also be associated with a possible impact on the well-being and social abilities of these individuals. Exposure to BPA may enhance diabetes risk due to an increase in insulin resistance. Diabetes may require daily glycaemic control as well as careful adherence to specific dietary habits that may also affect social life. The quality of life may also be impaired by symptoms of low or very high blood glucose and fears about potential or real complications. Altogether, the adverse effects of BPA as demonstrated in the report may lead to a reduced quality of life.
- Societal concern: the adverse effects of BPA as demonstrated in the report all raise an indisputable societal concern. In particular, they are associated with conditions (in particular breast cancer, neurobehavioural disorders, diabetes) that have been observed with increasing trends during the last few decades and at high prevalence. The incidence of breast cancer is 90 per 100,000 in European women and it is the cancer type causing the most deaths in women (17.2%). Learning difficulties potentially affect up to 10% of US school children. In 2014, 8.5% of adults in the EU were diabetic. There is however no estimate of the share attributed to BPA in these diseases. In addition, treatment of these adverse effects has been shown to be very costly for society as a whole. Although the part that can be attributed to BPA has not specifically been assessed, the Health and Environment Alliance (HEAL) study evaluated the cost associated with pathologies related to ED chemicals in the EU28. The study concluded that the direct cost of ED-related male and female infertility associated with assisted reproductive technologies ranges between 48 and 155 million euros per year, the direct (healthcare) cost of ED-related female breast cancers ranges between 128 and 320 million euros per year, and the direct and indirect cost of diabetes due to EDs is estimated at between 6 and 15 billion euros per year. In another analysis, Rijk *et al*. (2016) stated that behavioural disorders comprise the largest contributors to the total estimated ED-associated socio-economic cost. In particular, the contribution of IQ loss dominates this cost, accounting for between 32 and 184 billion euros per year for the EU28 (indirect cost).
- Is it possible to derive a safe concentration value for exposed individuals? With regard to alteration of reproductive function, the uncertainty in the dose response was acknowledged by the RAC in its BPA restriction opinion (ECHA, 2015), which concluded that “since effects on the reproductive system have been observed at and below the range where kidney effects occur, RAC considers it prudent to take them into account in hazard and risk assessment and in health impact assessment. RAC however acknowledges that the available information does not allow a quantification of the dose-response relationship.” A similar conclusion (studies were not sufficiently convincing for quantifying the dose-response relationship, but it is prudent to take the effect into account when setting assessment factors) was reached regarding the proliferative

effect on mammary tissue, effects on behaviour and metabolic effects. In addition, several studies report effects at doses below the starting point used for deriving the DNEL[8] – BMDL[9]10 for kidney effects in mice). The shape of the dose-response relationship and the parameters impacting the dose-response (period of exposure and concomitant presence of estrogen, in particular) are still under discussion. Altogether, the database shows considerable uncertainties in establishing a quantitative dose-response relationship, as well as safe levels, with some studies identifying effects at doses below the point of departure used for deriving the DNEL.

Overall, the effects of BPA induced by the ED mode of action it causes are therefore considered to meet the criteria for an equivalent level of concern to CMR properties. Therefore, it is justified to identify BPA as an SVHC according to Article 57(f) of REACh for effects on human health exerted through an ED mode of action.

3.2 Conclusion of the CES REACh-CLP

On the basis of the analysis presented above, the CES confirms that the available data are sufficient for BPA to be identified as an SVHC according to Article 57(f) of REACh due to its ED properties for human health.

In addition, the CES notes:

- the exemplary and innovative nature of this dossier, bringing together health effects in humans with an ED mode of action for each adverse effect selected, in phase with the current European definition. This dossier illustrates the amount of data and the aggregation effort needed to substantiate an ED identification dossier;
- that this dossier usefully relies on research studies, not necessarily applying the standardised protocols preferred by the REACh Regulation. Many of them concern experiments conducted in animals;
- that as it stands, the regulatory impact of this SVHC identification measure, if it were accepted at European level, may be very limited. Indeed, authorisation is the most binding measure that can be associated with the SVHC status and it does not apply to monomers and intermediates. A significant amount (estimated at 90%) of BPA is placed on the market as a monomer and intermediate. In spite of clear and well-documented exposure of consumers and the environment to an endocrine-disrupting substance, the derogation granted to monomers in the REACh Regulation severely restricts the management options by ruling out authorisation as well as restriction;
- the very high quality and completeness of the SVHC identification dossier for BPA according to Article 57(f). The work to draft this dossier, mobilising a large number of human resources in a short timeframe, was possible because of the abundance of data on this substance;
- that the toxicological profiles of the chemicals that are candidates for substituting BPA are insufficiently documented.

Consequently, the CES recommends:

[8] Derived no effect level

[9] Benchmark dose level

- creating a hazard category to identify endocrine-disrupting substances within the regulations. Just as with the other hazard classes (for example CMR), the ED property should be integrated in the regulation with classification criteria and systematically assessed in the same way as the other hazard classes, without necessarily requiring the quantity of data available for BPA;
- undertaking research to document the toxicity/safety of substitutes for BPA;
- for this purpose pursuing work on *in vitro* models with complex cellular and *in silico* models, and promoting their integration in the regulations,
- holding further discussions on the derogation systematically granted to isolated monomers and intermediates in the REACh regulation;
- taking into account in the regulations and in health risk assessments studies that have not been standardised according to a guideline and that result from research work, insofar as they provide informative elements that are not always analysed in the "guideline" studies. Non-standardised studies should be taken into account for their specific scientific quality. The Klimisch rating system is not suited to assessing their reliability, and alternative rating methods should be promoted:
- that registration dossiers comply with the regulatory requirement to list all the existing data, including from the scientific literature.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety concludes, in agreement with the analysis presented in Section 3, that BPA can alter reproductive function, mammary gland development, cognitive functions and metabolism through a mechanism of action involving endocrine disruption, in particular the disruption of estrogens and estrogenic pathways.

Consequently, the French Agency for Food, Environmental and Occupational Health & Safety recommends identifying bisphenol A as an SVHC substance according to Article 57(f) of REACh due to its ED properties for health.

Moreover, in connection with the CES's conclusions, the Agency recommends:

- creating a hazard category for identifying endocrine-disrupting substances within the regulations. As with the other hazard classes (for example CMR), the ED property should be integrated in the regulation with the classification criteria, and systematically assessed in the same way as the other hazard classes, as advocated in ANSES's opinion on the definition of scientific criteria for defining endocrine disruptors of 19 July 2016. In this opinion, ANSES recommends distinguishing EDs into three categories: “known" EDs, "presumed" EDs and "suspected" EDs, relying on the WHO/IPCS definition of an ED while reflecting the level of uncertainties. Such a scheme would enable a harmonised classification to be applied, and management to be tailored to the different regulatory contexts according to the uses and populations;
- undertaking research to document the toxicity/safety of substitutes for BPA;
- for this purpose pursuing work on *in vitro* models with complex cellular models making it possible to investigate the mechanisms of actions attached to an ED;

- holding further discussions on the derogation granted to isolated monomers and intermediates in the REACh regulation in order to enable the risks associated with polymers to be managed;
- taking into account in the regulations and in health risk assessments studies that have not been standardised according to a guideline. Research studies can provide useful data on parameters that are not always analysed in studies conducted according to the guidelines in force, as mentioned in ANSES's opinion of 25 March 2013 on the assessment of the risks associated with bisphenol A for human health. These studies should be taken into account for their specific scientific quality. To allow their reliability to be assessed, alternative rating tools to the Klimisch rating system should be promoted;
- that registration dossiers comply with the regulatory requirement to list all the existing data, including those from the scientific literature.

In addition, the Agency recommends as a supplementary measure that:

- in the framework of the assessment of possible applications for authorisation of uses of BPA under REACh, the relevance of selecting an approach with or without a threshold be discussed. Uncertainties related to characterisation of the dose-response and those related to the scope of potential effects of BPA due to its ED effects should be taken into account in this discussion;
- the endocrine-disrupting properties of BPA on species in the environment should also be presented at European level with a view to a possible supplementary SVHC identification.

Dr Roger GENET

KEYWORDS

Bisphenol A / BPA / CAS 80-05-7
REACh
Substance extrêmement préoccupante / Substance of Very High Concern / SVHC
Perturbateur endocrinien / Endocrine disruptor

REFERENCES

The full bibliography is contained in the report identifying BPA as an SVHC for these ED properties for health.

ANNEX 1

Presentation of participants

PREAMBLE: The expert members of the Expert Committees and Working Groups or designated rapporteurs are all appointed in a personal capacity, *intuitu personae*, and do not represent their parent organisation.

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EXPERT COMMITTEE ON "ASSESSMENT OF THE RISKS RELATED TO CHEMICAL SUBSTANCES FOR THE IMPLEMENTATION OF THE REACH REGULATION" (2013-2017)

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CONTRIBUTIONS FROM OUTSIDE THE GROUP(S)

Purpose of the contribution: "Data on polycarbonate"; Mr Michel LOUBRY- Director West Region – Plastics Europe

Purpose of the contribution: "Use of BPA in the dental sector"; Ms Céline WURTZ – General Delegate – French Coordinating Committee of Dental Activities (COMIDENT)

Purpose of the contribution: "Substitution of BPA"; Dr Sylvain CAILLOL – Deputy Director – Carnot Institute - Chimie Balard