

Maisons-Alfort, le 3 avril 2014

The Director General

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

**in response to the consultation of the European Food Safety Authority  
on its draft Opinion regarding the assessment of risks to human health related to dietary  
exposure to Bisphenol A**

### **1. BACKGROUND OF THE REQUEST**

On 9 June 2009, the Agency received a formal request from the Directorate General for Health (DGS) for a health risk assessment (HRA) of exposure to category 3<sup>1</sup> (R3) reprotoxic (according to Directive 67/548/EC) and/or endocrine disrupting (ED) substances found in consumer products marketed in France. This expertise covered the general population, including vulnerable populations and people in the workplace handling so-called 'mass-market' consumer products in the context of their professional activity (excluding production, processing, distribution and disposal).

In this context, in 2013, ANSES published an Opinion on the risks to human health associated with bisphenol A (BPA) taking into account not only exposure related to consumer products but also exposure from other media (drinking water, foodstuffs, domestic dust, air). This Opinion presented the expertise work undertaken by a Working Group on endocrine disruptors and category 3 reprotoxic substances (ED WG) created by ANSES in 2010. The expert appraisal report on the health effects of BPA produced by the ED WG was submitted to several expert groups at ANSES and validated by the Expert Committee on the Assessment of the risks related to chemical substances in February 2013 (ANSES, 2013).

From 25 July to 15 September 2013, EFSA published an interim report on the assessment of BPA exposure on its website for public consultation. All interested stakeholders were invited to submit their written comments before 15 September 2013.

ANSES contributed to this public consultation by analysing the online report and attaching the observations of the French National Agency for Medicines and Health Products Safety (ANSM), more specifically on the theme of cosmetics.

A table summarising the 42 comments made by ANSES and ANSM that were submitted online on the EFSA website can be found in the annexes of this Opinion (Annex 1).

On 17 January 2014, an EFSA draft Opinion on the risks to health related to BPA in foodstuffs was published on the EFSA website for consultation. This draft Opinion drew on an analysis of published data on BPA up to the end of 2013. The experts conclude that BPA does not pose risks to consumers at the current levels of exposure through food and the handling of thermal receipts

<sup>1</sup> Substances classified as category 3 reprotoxic according to Directive 67/548/EEC are now classified as toxic to reproduction, category 2 according to (EC) Regulation no. 1272/2008, known as the CLP (Classification, Labelling, Packaging) Regulation. In this document, substances are classified based on the CLP Regulation.

containing BPA. In this draft Opinion, EFSA proposes a temporary TDI that relies on the results of the study by Tyl *et al.* (2002, 2008).

On 7 February 2014, ANSES issued an internal request to analyse certain points of the EFSA draft Opinion.

## 2. EXPERT APPRAISAL METHOD

Given the limited time-frame to respond to the consultation, the expert appraisal was undertaken by several expert rapporteurs from the ED WG with expertise in toxicology, modelling (*PB-PK* and *BMD* modelling in particular), uncertainty analysis and kinetics, as well as experts specialising in effects on the mammary gland, the central nervous system, the female reproductive system and metabolic diseases. Each expert was mandated to assess a specific part of the EFSA draft Opinion.

The expert appraisal primarily focused on the main differences in the interpretation of data versus the ANSES reports published in 2011 and 2013. It also specifically addressed new aspects of the risk assessment process proposed in the EFSA draft Opinion.

The results of the expert appraisal presented below take into account the experts' comments. They cover specific points of the EFSA draft Opinion that can influence risk assessment results: the choice of publications taken into account, the selection of the critical effect(s), BMD calculation, the estimation of internal exposure in humans and the treatment of uncertainty. Comments specific to certain studies and additional information provided by ANSES have been attached to this Opinion. Quotes from the EFSA draft Opinion appear italicised and in quotation marks.

Given the short time-frame provided for the consultation organised by EFSA and the considerable background work undertaken by this agency, the experts were mobilised in a context of urgency. This Opinion does not intend to present a full expert assessment of the safety of BPA but highlights some major questions and identifies potential improvements to be made following a reading of the EFSA draft Opinion.

## 3. RESULTS OF THE EXPERT APPRAISAL

### 3.1. General comments

#### 3.1.1. Publications taken into account

The analysis of epidemiological studies is not particularly covered in this Opinion given that EFSA and ANSES interpret the results of these studies in a similar manner. The observations in this section only apply to experimental data for which there are differences in interpretation between the two agencies.

ANSES observes that this new health risk assessment for BPA not only takes into account studies on oral exposure but also studies on subcutaneous exposure, which was not the case in previous EFSA reports. Most of the studies undertaken to examine the toxicity of BPA were not carried out according to the OECD guidelines and/or did not adhere to 'Good Laboratory Practice' (GLP). These studies were nonetheless taken into account in the EFSA assessment, even though EFSA gave greater weight to OECD studies undertaken in accordance with GLP (e.g. Tyl, 2002, 2008). Several recent studies published after the ANSES expert appraisal were also included in the

assessment, potentially providing additional information, particularly on certain critical effects such as metabolism for which little information was available until recently. That said, ANSES considers that so far, none of these studies fundamentally call into question the conclusions of its expert appraisal on the nature of the health effects of BPA. Specific comments by type of effect are given in the rest of this Opinion, although the articles not taken into account in the ANSES 2013 report have not been specifically analysed for this call for comments.

ANSES notes that most of the non-OECD/GLP publications assessed in the EFSA draft Opinion have been criticised for various criteria such as the number of animals and control animals, consideration or non-consideration of the 'litter effect', animal housing conditions such as types of cages and diets (e.g. phyto-oestrogen-free or not), BPA exposure conditions including route of exposure, number of doses, blind evaluation, correlation between biochemical effects and anatomical or functional lesions, etc. However, it is unfortunate that these criteria have not been classified. Furthermore, other criteria that are nonetheless essential for the interpretation of results, such as the exposure period, hormonal sensitivity during development and puberty, etc. appear not to have been given the same importance.

### 3.1.2. Weight-of-evidence assessment

The hazard assessment of BPA proposed by ANSES in 2011 relies on a classification of effects as effects that are 'recognised', 'suspected', 'controversial' or 'effects for which no conclusion can be drawn on the basis of the available data' depending on the number and quality of available studies.

The approach used by EFSA is based on the weight of evidence estimated by the experts considering the quality of the data *corpus* by type of effect. However, while this approach has the advantage of systematically analysing lines of evidence in response to a specific issue, it can cause the *corpus* of data and publications to become over-fragmented, ultimately meaning that there is not sufficient perspective to judge a set of arguments that may be part of a *continuum* of similar effects. For example, regarding the effects of BPA on metabolism, subdivisions are made by period of exposure for animal testing (prenatal exposure and exposure in adulthood), and for each exposure period, new subdivisions are made for each study parameter (weight, glucose tolerance, insulin sensitivity). All of these subdivisions lead to the fragmentation of information included in the same scientific article and can cause confusion for the reader. The same is true for other effects such as effects on the mammary gland and brain. Conversely, grouping together several different effects in the final weight-of-evidence analysis can result in a lack of consistency in the data analysis (e.g. for the mammary gland, grouping of morphological changes, cell proliferation and atypical ductal lesions under the same item).

The classification of effects based on plausibility criteria ("*likely*", "*as likely as not*", etc.) is not clearly justified in the draft Opinion, even though the expert assessment is intended to draw conclusions based on the available data. Therefore, it would be desirable, for the transparency of the expert assessment, to further stress these assessment criteria in the final report. For example, no criteria are offered to consider that the available studies for a given line of evidence have low, medium or high reliability. This is even more surprising considering that, for certain lines of evidence, there are studies that only have weaknesses (see Table 29, "*Starting point*", page 421, and *Line 5*, page 423), while for others, there are studies that have both strengths and weaknesses (see *Lines 1 to 4*, pages 421-423). That said, for the vast majority of the lines of evidence, EFSA grants a low level of reliability to the data, whether the lines of evidence are strong or limited. This approach therefore focuses on the limitations of studies in terms of their level of evidence.

The way in which the studies as a whole have been included to address the issue raised and assess reliability so as to conclude as to the likelihood of an effect ("*overall conclusion on likelihood*") is not clearly described (see Table 30, p. 427).

It is stated (page 208) that the assessment of terms for expressing likelihood ("*very likely, likely, etc.*") fully relies on expert judgement. Two issues remain unclear:

- For each line of evidence, a scientific judgement must be made by experts specialising in the issue (ECHA, 2010). However, in the EFSA draft Opinion, this process is not described.
- The method for addressing potentially diverging opinions among the working group's members is not clearly explained. Did all of the group's experts assess these criteria ("*likely*", etc.) in the same way for the same line of evidence? If that was not the case, how were divergences taken into account, or not?

This degree of subjectivity is supported by the abundant use of terms such as "*acceptable*", "*convincing*", "*evidence...too weak*" used without being defined.

### 3.1.3. Non-monotonic relationships

Several experimental studies on BPA exposure have reported non-monotonic dose-response relationships (Jenkins *et al.*, 2011; Jones *et al.*, 2011; Marmugi *et al.*, 2012, etc.). These studies were taken into account in the ANSES expert appraisal and the statistical and biological likelihood of there being non-monotonic relationships was assessed and confirmed in a number of cases. However, no scientific consensus has been achieved as to the quality of the studies or the extent of evidence supporting the assumption of non-monotonic relationships for BPA. Therefore, EFSA has taken into account, with a lower level of evidence, studies that did not show an increasing dose-response relationship.

## 3.2. Hazard characterisation: choice of critical effects

ANSES observes that some of the critical effects deemed "recognised" in its 2013 expert appraisal are considered "as likely as not" or even "unlikely" by EFSA. Specific comments on this type of effect can be found in the sections that follow. The sections of the EFSA draft Opinion dealing with effects not addressed in the ANSES expert appraisal on health risks related to BPA (ANSES, 2013) are not specifically analysed in this Opinion.

### 3.2.1. Effects on the female reproductive system

In the ANSES expert appraisal (ANSES, 2013), the following effects observed in animals with pre- and/or post-natal exposure were considered sufficiently worrying and relevant to be taken into account:

- Increase in the occurrence of ovarian cysts;
- Increase in the frequency of endometrial hyperplasias;
- Disruption of ovarian cycles.

The study ultimately chosen by ANSES for the HRA was the study by Rubin *et al.* (2001) which showed a disruption of the ovarian cycle with lengthening of the oestrous cycle. This study on oral exposure gave a NOAEL of 100 µg/kg bw/day and a LOAEL of 1200 µg/kg/day after treatment from GD6 until weaning in Sprague-Dawley rats.

The divergences in the scope of conclusions between the ANSES report and the EFSA draft Opinion are due to different methodologies. It appears that the classification established by the EFSA working group requires there to be a negative biological modification in conjunction with the effects observed. And yet studies rarely explore in detail gonadotrope activity function in terms of

fertility. It also appears that some divergences in the classification of studies are linked to the way in which the two groups approach methodological biases. Indeed, the EFSA working group considers that not considering properly the litter effect or the statistical analysis is a major methodological limitation that impacts in particular the strength of the study by Rubin *et al.* (2001) chosen by ANSES's experts as a key study for the identification of hazards to the female reproductive system.

The two agencies use different methodological bases to classify effects. The ANSES ED WG established a classification based on a structured decision tree whereas EFSA issues an overall score by system (see page 436 overall conclusion on the effects of BPA on the male and female reproductive system) for exposure to BPA in the development phase while ANSES's assessment is based on an analysis by type of effect (effects on the genital tract and ovaries, effects on the hypothalamic-pituitary-gonadal axis, effects on the onset of puberty, etc.). EFSA mentions that the lack of convergence between studies is a source of too much uncertainty. This assessment may appear justified when considering the system as a whole. However, this uncertainty is significantly reduced if the data in the literature are analysed effect by effect. As for the EFSA analysis of the functional significance of the observed effects, it is undeniable that this type of information may be the cornerstone to hazard assessment. However, rejecting effects because this information is not available can mean disregarding recognised scientific facts where knowledge of functional physiology suggests they may have negative consequences on the effectiveness of this function.

The analysis of the scientific literature from 2011 to 2012 undertaken by the ED WG highlighted an effect on folliculogenesis with developmental exposure. According to the decision tree adopted by the ED WG that was used for the classification of effects, these effects could be considered "recognised". The EFSA experts rightly point out that the functional significance of this type of effect, particularly in terms of fertility impairment, remains to be determined. It still remains true that the mechanisms highlighted in the various studies undertaken in different species are often associated with changes in follicular dynamics and sometimes depletion of follicular reserves. A good-quality publication identified by the ED WG indicates that bisphenol A at low doses (25 ng/kg subcutaneously) with exposure during the development phase (GD8-PND16) could accentuate the decline in ageing-related fertility in CD-1 mice (Cabaton *et al.*, 2011). Although it is impossible, in the current state of knowledge, to establish a direct cause-and-effect relationship, the assumption that such an effect could be related to changes in follicular dynamics underlines the importance of not neglecting the possible impact of BPA on folliculogenesis. Furthermore, it appears that the effects of BPA on ovarian follicles can also appear with exposure in adulthood. For example, the EFSA assessment mentions a good-quality study that shows that subchronic (90 days) oral exposure to low doses (1 and 100 µg/kg bw/day) in young adult female rats (Lee *et al.*, 2013) caused augmentation of follicular atresia and luteal regression while reducing ovarian steroidogenesis and stimulating apoptosis. These ovarian changes were associated with an increase in the synthesis and release of pituitary LH and lengthening of the oestrous phase. According to the rules for the classification of effects adopted by the ED WG, these effects cannot be classified as recognised due to a lack of other converging data on effects on fertility decline and effects on ovarian follicles in adults. However, the string of assumptions and the likelihood of an impact on fertility are sufficiently significant to draw the attention of experts to the effects of BPA on ovarian follicles and their possible consequences in terms of fertility.

### 3.2.2. Effects on the central nervous system

Of all of the observed effects regarding the toxicity of BPA to the central nervous system, the critical effect selected by the ANSES experts involves the impairment of memory and learning, concurrent with a decrease in the expression of various subunits of glutamate NMDA (N-methyl-D-aspartate) receptors, which are particularly involved in synaptic and neuronal plasticity and in memory and learning processes. These effects are also reinforced by the action of BPA in neural systems expressing nitric oxide synthase (NO synthase) with sex- and region-dependent effects in the hypothalamus and limbic system (Martini *et al.*, 2010).



The study by Xu *et al.* (2010a) was chosen by ANSES as the key study. This study was undertaken by oral administration (gavage) in ICR mice (n=10 animals/group) and included four exposure doses in addition to the control group: 0.05; 0.5; 5 and 50 mg/kg bw/day. Ten gestating mice per dose level were exposed from GD7 to PND21. This study did not adhere to the OECD guidelines or GLP. Nonetheless, the study protocol is clearly described and many molecular (NMDA receptors, oestrogen receptor  $\beta$ ) and physiological effects were investigated. The reduced expression of NMDA receptors observed in the hippocampus in this study was reproduced by the same team in Sprague Dawley rats (Xu *et al.*, 2010b), in similar conditions, and by other teams (Tian *et al.*, 2010).

The choice of the Xu *et al.*, 2010a study is supported by studies whose results provide a string of assumptions on the brain damage induced by BPA in relation to cognitive effects. The study by Martini *et al.* (2010) shows changes in the expression of cerebral NO synthase (NOAEL 10  $\mu\text{g}/\text{kg}/\text{day}$ ) in mice exposed orally. The study by Tian *et al.* (2010) highlights changes in the dopaminergic and glutamatergic systems (NMDA) together with cognitive deficits and decreased anxiety in mice exposed orally (LOAEL 100  $\mu\text{g}/\text{kg}/\text{day}$ ). The study by Xu *et al.* (2010b) shows a decrease in the expression of certain glutamate NMDA receptor subunits and oestrogen receptor  $\beta$  (ER  $\beta$ ) in rats exposed orally (LOAEL 50  $\mu\text{g}/\text{kg}/\text{day}$ ). Studies on subcutaneous exposure, such as that by Zhou *et al.* (2011), make a connection between changes in synaptic and neuronal plasticity and behaviour in rats with an LOAEL of 2  $\mu\text{g}/\text{kg}/\text{day}$ .

In its draft Opinion (see page 303, EFSA identifies several weaknesses in the study by Xu *et al.* (2010a):

- *"However, in the absence of a correlation with a functional adverse effect, the Panel did not consider the available data as convincing evidence of neurobehavioural toxicity of BPA."*

ANSES comments: one of the strengths of the study by Xu *et al.* (2010a) is precisely that it showed a link between changes in synaptic and neuronal plasticity mechanisms in specific cerebral regions (hippocampus) and functional behavioural impairment (spatial learning and conditioning). This is a surprising comment from the EFSA experts, since this study does indeed link various aspects of cerebral function in molecular and behavioural terms.

- *"Study design (no wash-out period between different test procedures)"*

ANSES comments: the wash-out period between different procedures has never been given special attention by EFSA in the studies taken into account in previous reports. It could be considered that a wash-out period would be necessary if successive tests were undertaken with the same study parameter, which is not the case of the key study chosen by ANSES. Indeed, even though the tests carried out by Xu *et al.* (2010a, 2010b) studied the learning and memorisation capacities of animals, two types of memory were successively explored on PND21 and PND56 in the same animals that had been exposed early on to BPA: spatial memory with the Morris water maze and emotional and contextual memory with a conditioning test associating negative reinforcement with the reinforcement context. The Morris water maze, which is above all dependent on the plasticity of the hippocampus, a key region for spatial learning, was used 1<sup>st</sup> while the 2<sup>nd</sup> test examined emotional memory and the activity of the limbic system involving the amygdalae, even though this system interacts with the hippocampus. These considerations suggest that successively undertaking the two tests in the same animals does not bias the results and that the lack of a wash-out period between the two tests is not a study weakness. As a precaution, Xu *et al.* could have alternated the order in which the groups took the two tests so as to offset the effects of potential interactions between them. However, even if different tests investigate the same type of memory, it is not at all mandatory to have a wash-out period between them. Indeed, an experimental protocol can be designed so as to successively carry out various tests using the same parameter to see if different types of events can modify the same parameter (e.g. working memory, anxiety, depression, etc.). Thus, the lack of a wash-out period is not a study weakness.

- “Test performed in one sex only (only male offspring)”

ANSES comments: a study performed in males only is not a weakness but is intended to focus on effects that can be induced in a specific population. Furthermore, the results obtained by Xu *et al.* are reliable enough to be used for the expert appraisal even if they only apply to males.

- “Insufficient study reporting (reproductive outcome not shown, e.g. maternal bw, no pre-weaning body weight data shown)”

ANSES comments: the data on the body weight of pups, produced on PND21, show a significant decrease at the lowest dose of BPA (0.05 mg/kg bw/day) and a significant increase at the highest dose (50 mg/kg bw/day) versus the controls. The same variations were observed on PND56 but the difference at the highest dose was no longer significant. It is indeed unfortunate that data on litter growth in the first three weeks of life were not provided by the authors so as to be able to attribute these variations to BPA exposure only and not other biases, such as differences in litter weight at birth depending on the number of pups and differences in maternal behaviour. Even so, the cerebral and behavioural differences observed in the groups exposed to BPA were such that they could not be attributed to differences in body weight related to a larger litter or under-developed maternal behaviour. Indeed, the brain is an organ whose growth is preserved in the early phase of development in the event of under-nutrition for example.

- “Statistical analysis (litter effect not considered, i.e. no information about one male pup/litter)”

ANSES comments: although they did not adhere to the OECD 426 guideline, the authors included ten gestating female mice per exposure group in the study and used one male per litter to make up the experimental groups whose behaviour was tested. By doing so, Xu *et al.* (2010a) considered the mother as the statistical unit. The inclusion of ten mothers per group, each represented by one pup from each litter, thus eliminated the litter effect, which would not have been the case and would have made testing necessary if all of the pups in each litter had been evaluated for their behaviour. The study's only weakness is the lack of information about the selection of the pup in each litter.

- “Information about type of water bottles is missing”

ANSES comments: in the study by Xu *et al.* (2010a), no information is given regarding the materials used for the water bottles. However, the study was chosen based on the following arguments:

- The study links the effects of BPA on memory to significant changes in NMDA receptor expression in the hippocampus, a cerebral structure involved in memory and learning (40% decrease in the expression of some of this receptor's subunits). A shortage of NMDA receptors induces considerable and sometimes permanent cognitive impairment.
- Although the effects on NMDA receptor expression are the most significant, the effects on memory were chosen by the ED WG as critical effects since it is always difficult to know whether a physiological, cellular or biochemical change can have harmful consequences for an individual.
- These effects are part of a *continuum* of effects, also observed in other studies, on cognitive function and causing histochemical changes in various cerebral structures (Adewale *et al.* 2011; Martini *et al.*, 2010; Bai *et al.*, 2011; Zhou *et al.*, 2011; Rubin *et al.*, 2006).

Lastly, the study was also taken into account by ANSES's experts, despite the poorly controlled BPA environment, considering the following two cases: (i) Environmental BPA induces the same

effects as those described in the study. In this case, the BPA received experimentally aggravates the effects induced by environmental BPA, which leads to differences in effects between the controls and exposed individuals. (ii) Environmental BPA induces effects opposite to those observed. In this case, the BPA received experimentally first cancels out these effects and then induces opposite effects, which also leads to a difference between the controls and treated individuals.

It is surprising that this study was not taken into consideration in the weight-of-evidence analysis, given that other studies with this type of weakness were used in the EFSA draft Opinion (see 11.2 Table 34).

A more recent study by the same team (Xu *et al.*, 2013) was evaluated by the EFSA experts, who mention that one of this study's weaknesses is that the doses were not adjusted to the weight of the animals, whereas the doses do seem to have been adjusted to the weight of the individuals: “*The body weight of each mouse was weighed every week to adjust the drug volume*”. According to ANSES's experts, this was a well-conducted study in which the authors took many precautions to avoid environmental contamination with phyto-oestrogens and BPA. The results are in line with the study by Xu *et al.*, 2010 in mice. Effects on the markers of synaptic function were observed from 0.4 mg/kg/day. Effects on glutamate receptors were observed at 0.4 and 40 mg/kg/day. This study has the advantage of combining cognitive effects with histological changes. ANSES's experts are surprised by this comment regarding the study by Xu *et al.* published in 2013 in *Hormones and Behavior*, as the same statistical procedure was used in other studies published by the same authors and mentioned by EFSA with no such comments. Substantively, Xu *et al.* 2013, like in the key study chosen by ANSES, used Tukey's test to make *a posteriori* comparisons in the various variance analysis models used. Tukey's test is a conservative test that was developed to guarantee the probability of risk  $\alpha$  for all possible comparisons unlike the Newman-Keuls test for example.

Other studies reported effects of BPA on learning and memory after a single exposure (Eilam-Stock, 2012; Inagaki, 2012), which the EFSA experts consider to be a weakness. According to ANSES's experts, this type of exposure is not necessarily a weakness insofar as the aim is to take into account the toxicity induced by acute exposure, which can be quite relevant when considering that single exposure can induce harmful effects that are sometimes irreversible.

Furthermore, the use of positive controls is considered a strength in the studies evaluated by the EFSA expert committee. And yet the inclusion of a positive control in a study implies that the positive control and the substance of interest induce the same type of effect. Thus, the types of effects induced by the substance of interest are prejudged and any deviation from the effects induced by the positive control reduces the level of confidence attributed to the effects induced by the substance.

Thus, the lack of a positive control is considered to be a weakness for a study while its presence is a strength. However, several objections limit the usefulness of a positive control:

- The use of a positive control prejudices the substance's mode of action which is far from being characterised and therefore far from being known.
- For bisphenol A, it is clear that there are effects not related to oestrogenic action.
- In the event that the positive control and substance have the same mode of action, the doses (and therefore the internal concentrations) at which effects are induced may be different depending on the affinity of the positive control or substance for the same targets.
- The same substance can induce different effects at different doses since the biological targets are not the same depending on their affinity for the substance. This is particularly true for hormones and endocrine disruptors. Thus, it is difficult to compare the effect of a substance, which could be the same as that of the positive control at one



dose and different at another dose. This is true for both the substance and the positive control. For example, LHRH agonists first induce an increase in testosterone and then at high doses or with extended exposure almost completely reduce plasma testosterone.

More broadly, ANSES's experts consider it unfortunate that the EFSA expert assessment does not consider the effects of BPA in terms of impaired cerebral development further to pre- or peri-natal exposure to be relevant effects for the risk assessment. Whereas significant consideration is given to studies reporting this type of effect in Section 3.4.2.2 of the EFSA draft Opinion (sub-section "*Effects on brain biochemistry, neurogenesis, neuroanatomy and gene expression*", pages 96-97), these effects are not included in the WoE approach in Section 11 of the same draft Opinion.

Other comments on studies assessing the effects of BPA on cerebral function not considered in the ANSES report published in 2013 can be found in the annexes of this Opinion.

### 3.2.3. Effects on metabolism

As stated above in the general comments, the approach used by EFSA on the weight of evidence for a given effect separates various effects that can be related and be part of a *continuum* that should also be analysed as a whole. For example, a sub-section of the draft Opinion is devoted to the 'weight gain' parameter and the EFSA experts cite various articles reporting or not reporting weight changes. The following point, 'insulin', reports whether changes in insulin secretion and glucose tolerance have been described. The various studies that monitored this parameter are reported. And yet it is obvious that if an animal gains weight after a treatment, this could have repercussions on insulin resistance and glucose tolerance. It is therefore important to analyse all study parameters to have an overall idea of the metabolic impact of BPA. With the subdivision presented in the EFSA draft Opinion, it is difficult for the reader to form an opinion of the effects of BPA on metabolism, even more so given that lipid metabolism is closely related to carbohydrate metabolism.

In addition, the EFSA expert committee draws the following conclusions for each sub-section:

- the lack of a dose-response relationship (see line 4763),
- obtaining contradictory results that are difficult to reconcile (see line 4768, line 4797),
- a non-conclusive statistical analysis (see line 4805),
- a small magnitude of effects (see lines 4817-4818),
- a 'litter' effect not taken into account,
- that it is difficult to understand the underlying mechanisms (see line 4919).

Some other terms used should be clarified, such as: "*not clear cut*" (see line 4847), "*unclear*" (see line 4851) and "*methodological deficiencies*" (see line 4869). In the end, the expert committee indicates "*the assumption of non-monotonicity is not supported by the data*" (see line 4961) and "*the high fat feed intake cannot be considered as a good model for human health assessment*" (see line 4963).

Moreover and regarding *in vitro* studies, EFSA recognises that it is highly likely that nanomolar concentrations of BPA can affect insulin secretion *in vitro* (see line 5008) but that considering the limitations of *in vitro* models, the relevance of results obtained on the impact of BPA on the physiology of pancreatic  $\beta$  cells remains to be specified ("*is currently unclear*", see line 5010).

Regarding non-monotonic relationships, the EFSA expert committee rejects studies for two reasons:

- U-shaped or bell curves cannot be superimposed with the various biological parameters studied. And yet hormonal sensitivity depends on the tissue that is studied and the hormonal context (development, puberty, adulthood) and the use of feedback in tissues.

- effects observed in response to a fatty diet cannot be taken into account. The diets given to rodents are very different even when considered as standard as opposed to fatty diets, particularly due to their level of soya and dietary fibres. This is a significant point since the metabolism of animals closely depends on diet (Zimmermann C *et al.*, 2012). There is therefore no reason to discard fatty diets and only consider standard diets, especially when studying the obesogenic action of BPA. Moreover, a number of metabolic changes are only highlighted in response to a fatty diet, i.e. when an animal is subject to an imbalanced diet to see its ability to adapt to a new nutritional environment.

In the end, the EFSA expert committee concludes that metabolic effects are "*as likely as not*" while ANSES considers that the available experimental data are sufficient to consider that BPA can have effects on metabolism.

The EFSA expert committee concludes that there is "*reasonable evidence*" that BPA has effects on glucose and insulin regulation and/or pancreatic morphology and function, based on the results of short-term studies, while long-term studies do not show any effects (see line 5020). Even so, in the end, the expert committee concludes that the effects of BPA on metabolism are "*as likely as not*". It would be worthwhile to explain why the effects observed with short-term studies are not relevant.

#### 3.2.4. Effects on the mammary gland

In its expert appraisal published in 2011, ANSES considered that the effects of BPA on mammary gland maturation were recognised effects in animals and should be taken into account to assess risks to human health. ANSES observes that in its draft Opinion, EFSA also considers that the effects of BPA on mammary gland development are "*likely*" and that these effects can be transposed to humans. However, ANSES considers that it is important to acknowledge the possibility of increased cancer risk in the descendants of women who have a high level of endogenous oestrogens or xeno-oestrogens during pregnancy and are then exposed to tumour-initiating agents. And yet the EFSA experts only include the analysis of the direct carcinogenic effects of BPA on the development of neoplastic lesions in their criteria. They do not take into consideration enhanced susceptibility after early pre- and/or postnatal exposure to BPA, even at low doses, followed by exposure to a carcinogenic agent (e.g. DMBA or NMU) during puberty. This notion, which was already explained in previous reports (EFSA 2006, 2010), is a point of disagreement with ANSES. Effects on the mammary gland are the most significant effects identified by ANSES to assess the risks of BPA. Situations of at-risk exposure have been identified based on these effects.

Moreover, the arguments set out in the EFSA report according to which the rodent model is not a good model for mammary carcinogenesis because it only develops a limited number of cancer sub-types compared to the thirty or so sub-types of human tumours are not justified (page 139, lines 5822-5824). Firstly, in nature, no studies have estimated the diversity of tumours in rodents exposed to a complex environment. Secondly, no animal models used in specific conditions with little variety can mimic the diversity of mammary cancers in women exposed to a complex environment. Most international experts consider that mammary development and carcinogenesis are similar in rodents and humans (Russo and Russo, 1996; Singh *et al.*, 2000; Rudel *et al.*, 2011). Furthermore, different rodent strains (rats and mice) can have different sensitivity and susceptibility to carcinogenesis, which should be taken into account in the interpretation of experimental studies.

More specifically regarding effects on maturation and architectural modifications in the mammary gland, after foetal or neo-natal exposure to BPA, changes reported in the terminal ducts (TEBs, where carcinogenesis is likely initiated) and mammary branches at puberty are clearly described in the report (pages 139-140). However, other changes in the organisation of the mammary gland, such as changes in the epithelial-stromal organisation or the maturation of adipose tissue, hormonal

changes and metabolic changes that can result in abnormalities in adulthood, are not described in the EFSA report.

ANSES notes that the EFSA report includes the preliminary results of a recent study on chronic carcinogenesis undertaken in 2013 in the USA (US FDA/NCTR, 2013) in Sprague-Dawley rats. Since ANSES's experts have not assessed this study, it is difficult to comment on EFSA's analysis of it but ANSES considers that it should be analysed against other recent publications that appear to show neoplastic lesions (Acevado and Soto, 2013). Furthermore, other publications have not been taken into account, such as the study by Lamartinière *et al.* (2011) which shows an increase in proliferation after exposure during lactation in Wistar rats, while this study does not have the weaknesses noted by EFSA for studies from the same group (Betancourt *et al.*, 2010 and Jenkins *et al.*, 2009).

The spread of data on the mammary gland in the EFSA report is unfortunate as it makes them difficult to interpret and integrate into effects on the mammary gland, an organ that is highly complicated to study and whose particularities should be taken into account. Conversely, the grouping of morphological changes (TEBs, Abs), cellular proliferation (including simple ductal hyperplasia) and atypical ductal lesions in the same line of evidence can interfere with the interpretation of data.

### 3.3. Estimation of exposure

#### 3.3.1. Toxicokinetics and metabolism

An analysis of recent data does not show major differences in interpretation between ANSES and EFSA regarding the absorption, distribution, metabolism and elimination of BPA. However, the following explanations and comments should be made:

- *“Because of the high activity of the conjugation enzymes the percentage of unconjugated BPA in the blood is only a few percent of total BPA (sum of conjugated and unconjugated BPA)”.*

ANSES comments: to comment on the free *versus* total ratio in the blood, it is not enough to describe the activity of conjugation enzymes; it would be better to speak of clearance and write "due to the relatively high BPA clearance compared to the relatively low BPA-glucuronide clearance, the percentage of unconjugated BPA in the blood is only a few percent of total BPA (sum of conjugated and unconjugated BPA)".

- *“Based on the analysis of oral versus intravenous toxicokinetic data, the oral systemic bioavailability of unconjugated BPA in rats is 2.8%, in mice 0.2% and in monkeys 0.9%.”*

ANSES comments: this point also appears questionable and is not supported by the recent study by Gayrard *et al.* (2013). The bioavailability values that appear here are those measured after gavage and not by contamination of food. It would therefore be best to write: “Based on the analysis of oral (gavage) *versus* intravenous toxicokinetic data, the oral systemic bioavailability of unconjugated BPA in rats is 2.8%, in mice 0.2% and in monkeys 0.9%”.

Moreover, an article in press by Vom Saal (2014) in monkeys indicates oral (bolus) bioavailability of 5%.

More specifically regarding the study by Gayrard *et al.* (2013), ANSES's experts are surprised that EFSA rejected the only study that has explored sublingual absorption, on the pretext that this exposure scenario is unlikely with oral treatment. Gavage is not a likely route of exposure either and the significance of this study is precisely that it shows the possibility of a high-peak concentration of

free BPA near the mouth, for example when holding a receipt, plastic pen or polycarbonate spoon in the mouth. In this case, the brain or thyroid can be exposed to high concentrations for a short time and a direct or indirect effect on these organs cannot be excluded.

### 3.3.2. Exposure scenarios

The exposure scenarios taken into account in the ANSES and EFSA expert assessments are different in that EFSA only took into account a 'consumer/general population' scenario while ANSES also assessed risks to people in the workplace who handle thermal receipts as part of their job. ANSES particularly assessed risks related to BPA exposure for women holding cashier positions subject to much higher exposure levels than the general population.

There are differences between the exposure scenarios assessed by ANSES in its expert appraisal and those taken into account by EFSA.

In its expertise work, ANSES calculated exposure for children over the age of three years (3 to 18 years old), adults (men and women combined) and pregnant women. For these three population categories, the exposure sources taken into account when calculating exposure doses were as follows: food, the ingestion of settled dust and the inhalation of air (exterior and interior). For these three exposure media, an aggregated internal exposure dose was calculated. Regarding the handling of thermal receipts, an internal exposure dose was calculated for pregnant women and adults as consumers, excluding situations of exposure in the workplace.

Exposure scenarios corresponding to people in the workplace handling thermal receipts (pregnant women and adults), such as cashiers, were also developed. The internal doses calculated through skin contact with thermal paper were not aggregated with the other exposure doses calculated by ANSES due in particular to a lower level of confidence associated with these results.

All exposure calculations were made applying a probabilistic approach.

In the end, ANSES undertook a risk assessment for pregnant women only, with three exposure scenarios: pregnant women exposed through food, the ingestion of dust and the inhalation of air; pregnant women as consumers exposed dermally by handling thermal paper; and pregnant women in the workplace (cashiers) exposed dermally by handling thermal paper.

In its "*DRAFT scientific opinion on the risks to public health related to the presence of BPA in foodstuffs – Part: exposure assessment*", EFSA calculated the following BPA exposure sources:

Table 1: exposure sources and population sub-groups considered by EFSA for the assessment of BPA exposure

	Infants (0-6 months) Maternal milk			Infants Infant formula	Children	Children	Children	Adolescents	Women	Men	Other adults	Elderly people
	1-5 days	6 days- 3 months	4-6 months	0-6 months	(6-12 months)	(1-3 years)	(3-10 years)	(10-18 years)	18-45 years	18-45 years	45-65 years	+ 65 years
<b>Ingestion</b>												
dust		x	x	x	x	x	x	x	x	x	x	x
toys		x	x	x	x	x						
food	x	x	x	x	x	x	x	x	x	x	x	x
<b>Inhalation</b>												
air	x	x	x	x	x	x	x	x	x	x	x	x
<b>Skin contact</b>												
thermal paper							x	x	x	x	x	x
cosmetics		x	x	x	x	x	x	x	x	x	x	x
Total exposure		x	x	x	x	x	x	x	x	x	x	x

All exposure calculations were made using external doses and a deterministic approach. For each exposure estimate, a mean level and high level were calculated.

**ANSES comments:**

As stated in Appendix VI of the EFSA report, even though all of the comments received on its "Exposure assessment" report had been examined, EFSA was not able to revise this specific part on exposure assessment so it could be included in its risk assessment report covered in this Opinion. This amendment work is currently underway at EFSA.

Therefore, it is not possible to evaluate whether the comments submitted to EFSA by ANSES in September 2013 regarding requests for clarifications, justifications, reformulations, details and additional references to be inserted in the text have been taken into consideration. However, Appendix VI of the document states that the EFSA experts considered that some of the comments received were relevant, and could lead to a change in the calculations. This Appendix presents the changes taken into account that resulted in new exposure figures. It also presents the EFSA experts' rationale for not taking into account certain comments such as those indicating that the assessment should not include some instances of occupational exposure, exposure from medical devices and exposure from dental sealants.

The comments made by ANSES can be found below, although it is not possible to assess whether or not they have been taken into account. Comments on dermal exposure are not addressed here, since this item is covered in a separate part of this Opinion.

Regarding the overall approach to the estimation of exposure, ANSES recommended implementing a probabilistic approach to calculate exposure rather than the deterministic approach used by EFSA. The risk assessment undertaken by EFSA is based on a deterministic approach to calculate exposure, with a mean level and a high level.

EFSA does not take into account any scenarios in the workplace (cashiers handling thermal receipts), considering that this is not part of its scope of expertise.

Regarding BPA exposure through cosmetic products, given that BPA may be found in containers, ANSES's comments generally insisted on uncertainties regarding the presence of BPA in cosmetic products, such that it did not seem possible to calculate a reliable and representative level of exposure to BPA through the use of these products (only six products, choice of body lotions as a benchmark for exposure, etc.). EFSA considers that the assumptions used are the most reliable that can be made based on the current data. An assessment of exposure through the use of cosmetic products is maintained.

Regarding the respiratory volume used in EFSA's calculations, taken from the publication by Trudel *et al*, 2008 and considered to be over-estimated and not representative of a daily respiratory volume as required in the calculation, ANSES recommended referring to the *Exposure Factor Handbook – 2011 edition*. This comment was taken into consideration and the calculations for respiratory exposure were amended by EFSA (see Tables 23A and 23B in Appendix VI).

Regarding the level of ingestion of settled dust used in EFSA's calculations, taken from the publication by Trudel *et al*, 2008, as for respiratory volume, ANSES considered that the values used were unsuitable and taken from an inappropriate publication. This comment was taken into consideration. The calculations for the ingestion of settled dust were amended by EFSA in its report (see Tables 23A and 23B of Appendix VI).



### 3.3.3. Biosurveillance data

Although exposure is generally determined by assaying BPA in urine, where it is mainly found in conjugated form, a number of studies also report blood concentrations of BPA in adults and in the umbilical cord blood of newborns. In its expert appraisal report on BPA (ANSES, 2013), ANSES thus devoted a paragraph to blood assays and particularly the share of the various forms of BPA (conjugated and unconjugated) in this matrix. Since the toxicity of BPA has been attributed to its unconjugated form, the share of this form in the blood, related among other things to the individual's metabolising capacity, is an essential parameter to be taken into account when assessing the potential effects of exposure.

In its expert appraisal report, ANSES presented mean values of blood concentrations of unconjugated BPA reported by various studies undertaken between 2002 and 2012 in Asia, Europe and the USA ranging from 0.32 to 2.5 ng/mL in adults. A study carried out in Taiwan in a sample of 97 pregnant women (Chou *et al.*, 2011) reported a maximum value of 29.4 ng/mL.

In umbilical cord blood, the study by Fénichel *et al.* (2012) cited in the ANSES report (ANSES, 2013) presented, for a population of 152 newborns, blood concentrations of unconjugated BPA ranging from 0.14 to 4.76 ng/mL, with a mean greater than 1.1 ng/mL.

In its report (Section 3.1.2.4, pages 42 to 44), EFSA concludes that the data published since 2010 confirm the fact that, after oral exposure to BPA, the unconjugated form of BPA in the plasma is so low that it cannot be detected/quantified with analytical methods having a limit of detection below 0.3 ng/mL. These conclusions, at odds with the ANSES report (ANSES, 2013), are based on a single study (Teegarden *et al.*, 2011) undertaken in the USA in 20 subjects in whom successive blood assays over a 24-hr. period had shown concentrations of unconjugated BPA below the 0.3 ng/mL limit of detection for all of the 320 serum samples analysed.

The study by Teegarden *et al.* (2011), also taken into account in ANSES's expert appraisal, was the only one of the studies that reported such low values. The other studies cited in the ANSES report are not taken into account in the EFSA report.

In the paragraph devoted to BPA in the blood of pregnant women and umbilical cord blood, the EFSA report cites the study by Kosarac *et al.* (2012), reporting serum concentrations of total BPA in 12 pregnant women ranging from <0.026 ng/mL to 10.4 ng/mL (median = 0.548 ng/mL, detection frequency: 67%) at mid-pregnancy and from <0.026 ng/mL to 3.05 ng/mL (median = 1.46 ng/mL, detection frequency: 58%) at delivery. Umbilical cord blood concentrations ranged from <0.026 ng/mL to 2.57 ng/mL (median = 1.82 ng/mL, detection frequency: 42%). Most of the detected total BPA was considered unconjugated BPA since conjugated BPA was only detected in two out of 12 serum samples at concentrations of 0.12 ng/mL and 0.22 ng/mL respectively (this last point is not specified in the EFSA report).

However, the EFSA experts consider that, despite the good quality of the analytical methodology, the data in the study by Kosarac *et al.* have low credibility due to a lack of information with respect to sample collection and handling, and discrepancies with the study by Teegarden *et al.* (2011), in which free BPA was never detected and total BPA was only detected in six out of 20 subjects who had peak concentrations of 0.6 to 1.3 ng/mL. In Appendix II of the EFSA report, the low number of subjects in the Kosarac study is also considered a weakness.

In general, the conclusions of the EFSA report on blood concentrations of total BPA and free BPA and the ratio of these two forms are based only on the results of the study by Teegarden *et al.* (2011). The few studies cited in the report that present high concentrations of unconjugated BPA in biological fluids are all considered as having many methodological shortcomings. This position is particularly questionable insofar as the study by Teegarden *et al.* ultimately appears to be an exception in the literature compared to the vast majority of other studies, most of which are not covered in the EFSA report.

### 3.3.4. Skin penetration of BPA

In its report, EFSA considers that the diet (oral route) is the main source of exposure in the general population, while dermal exposure from thermal paper is considered the second source of exposure in the population above three years of age (see line 373). Of the five *in vitro* publications on the percutaneous penetration of BPA, EFSA relied on the article by Demierre *et al.* (2012) to estimate the contribution of the dermal route to total daily exposure. For EFSA, the total absorbed quantity over a 24-hr. period is 10% of the dose applied on the skin based on the 8.6% absorbed within 24 hrs. (quantity in the receptor fluid) and the 0.6% in the skin (excluding the *stratum corneum*). According to EFSA, the quantity of BPA in the *stratum corneum* (39.4% of the applied dose) should not be taken into account for systemic absorption (see line 2370).

The study by Demierre *et al.* (2012) is considered the key study for EFSA for whom it is a good-quality publication. Likewise, the use by Demierre *et al.* (2012) of water as a vehicle of BPA is more comparable to a scenario of consumer exposure to thermal paper than acetone (Marquet *et al.*, 2011) or diluted hydro-ethanol solutions (Mork *et al.*, 2010, Zalko *et al.*, 2011), and the applied surface density of 1.83  $\mu\text{g}/\text{cm}^2$  is comparable to exposure estimates as derived for thermal paper (1.37-5.5  $\mu\text{g}/\text{cm}^2$  finger tip).

For ANSES, the choice of the study by Demierre *et al.* (2012) as the key study and the rejection of the study by Zalko *et al.* (2011) (see line 18936) are questionable. First of all, the study by Demierre *et al.* (2012), which was supposedly undertaken in accordance with the OECD TG 428 guideline, has several weaknesses (see Annex 5). Secondly, a comparison of the results obtained by Mork *et al.* (2010), Zalko *et al.* (2011) and Demierre *et al.* (2012) does not favour a study undertaken with a diluted aqueous solution of BPA (Demierre *et al.*, 2012) over studies undertaken with varying concentrations of hydro-ethanol solutions of BPA (Mork *et al.*, 2010, Zalko *et al.*, 2011). Indeed, the permeability coefficient of BPA is independent of the type of vehicle used (aqueous or hydro-alcohol) or the concentration of BPA in the applied BPA solution. Thus, the  $K_p$  calculated from the experimental data reported by Zalko *et al.* (2011) is  $0.9 \cdot 10^{-4} \text{ cm/h}$ . This  $K_p$  value is the same as the value obtained with Demierre *et al.* (2012) ( $k_p=1.1 \cdot 10^{-4} \text{ cm/h}$ ) who used a 194  $\mu\text{g}/\text{mL}$  aqueous solution of BPA, and Mork *et al.* (2010) ( $k_p=1.75 \cdot 10^{-4} \text{ cm/h}$ ) who used a 3995  $\mu\text{g}/\text{mL}$  hydro-ethanol solution. Likewise, the fraction of BPA absorbed within 24 hrs. is comparable for Mork *et al.* (2010) (approximately 6.5%= 13 X 24 h/48 h), Demierre *et al.* (2012) (8.6%) and Zalko *et al.* (2011) (15.2%= 45.6% X 24 h/72 h).

EFSA's affirmation that the use of water as a vehicle for BPA is more comparable to a scenario of exposure to thermal paper than acetone needs to be justified. Marquet *et al.* (2011) applied BPA as a solution in acetone. The acetone immediately evaporated. In these conditions, BPA in solid form was directly put into contact with the *stratum corneum*, as in the case of BPA transferred from thermal paper to the *stratum corneum* of the finger. The absorption flux of BPA ( $0.12 \mu\text{g}/\text{cm}^2/\text{h}$ ) applied at a rate of  $200 \mu\text{g}/\text{cm}^2$  of skin (after evaporation of acetone) was approximately 6-7 times smaller than the BPA flux of  $0.70 \mu\text{g}/\text{cm}^2/\text{h}$  (13%/48h X  $259 \mu\text{g}/\text{cm}^2$ ) obtained after applying BPA in a hydro-alcoholic solution at a rate of  $259 \mu\text{g BPA}/\text{cm}^2$ . This difference in flux can be attributed to the need to first dissolve solid BPA before it penetrates the skin.

EFSA estimates that only 10% of the BPA dose applied on the skin is bioavailable within 24 hrs. This value is based on the quantity found in the receptor fluid (8.6% of the dose) and the skin (0.6% of the dose) reported by Demierre *et al.* (2012). This quantity in the skin is small compared to the values reported by Kaddar *et al.* (2008) and Mork *et al.* (2010) which are, excluding the *stratum corneum* and epidermis, 8.8% after 10 hrs. of exposure and 17.2% after 48 hrs. of exposure respectively. A significant reservoir effect was also reported *in vivo* in rats in which over 80% of the

quantity of BPA in the skin after 8 hrs. of exposure was absorbed within 68 hrs. (Marquet *et al.*, 2011). In light of the data in the literature, failure to take into account a skin reservoir effect could cause the daily dose of absorbed BPA to be under-estimated.

In its 2013 expert appraisal report, ANSES used a triangular distribution for skin penetration rates with 27% as the most likely value and 10% and 60% as the lower and upper limits, weighted by the daily duration of skin penetration. ANSES's experts considered 27% to be the most likely value as it was taken from a study in volunteers handling receipts in exposure conditions similar to those of real life (Biedermann *et al.*, 2010). The study by Demierre *et al.* used by EFSA was undertaken using human skin explants on which BPA was applied in the form of an aqueous solution. This formulation was different from that of receipts, and therefore the choice of this study for this assessment did not adhere to the guidelines (OECD 428, EHC235), which underline the need for studies to reflect real-life exposure conditions in terms of doses, durations and formulations.

The choice of the study by Demierre *et al.* as the key study and the estimate of 10% as a conservative value are defended but remain questionable considering the methods and results reported in the BPA skin absorption studies (see Annex 5).

High uncertainty remains as to the fate of BPA after skin penetration and the degree of metabolism by the skin. Few studies have properly investigated the metabolism of BPA and ANSES approves EFSA's recommendations as to the need to further explore this issue (see line 6876). No toxicokinetic studies have measured the dermal bioavailability of BPA. Therefore, the evidence once again seems limited to affirm, as stated in the EFSA report, that the value of 10% skin penetration is conservative (see line 6489 lines 6860-6862).

For information, ANSES's approach resulted in a percutaneous absorption rate of 0.02% to 27% (probabilistic approach) over a 24-hr. period, which can be compared to EFSA's rate of 10% (deterministic approach)<sup>2</sup>. In the end, this difference between the EFSA and ANSES approaches hardly influences the difference in results between the respective risk assessments, which is mainly related to the choice of toxicological benchmark dose. Furthermore, ANSES observes that in the recent SCENIHR Opinion on the safety of bisphenol A in medical devices, the experts chose a skin penetration value of 25-30% taken from the study by Demierre *et al.* based on the same *corpus* of data.

### 3.4. Risk assessment

#### 3.4.1. Use of a BMD

To model the dose-response relationship from the study by Tyl *et al.* (2008), EFSA chose to use RIVM's PROAST software ([www.proast.nl](http://www.proast.nl)) in which the choice of response level (or BMR) is defined as a percent change in the response compared to the response observed in the controls. The idea is to choose a value above which the observed response is considered abnormal. This choice of BMR must be clearly explained.

To calculate the BMD (and BMDL) based on the study by Tyl *et al.* (2008), EFSA chose a BMR of 10% related to an increase in absolute kidney weight. EFSA defends this choice of 10% (page 67), considering that below 10%, the effects observed are not harmful to health ("*less than 10% should not be regarded as adverse*") which may indeed be justified given the lack of histopathologically visible kidney lesions.

<sup>2</sup> In the ANSES exposure model, taking into account the penetration period used and the absorption rate of 27%, the 24-hr. absorption rate is approximately 0.02% to 27%, which is a range of equally probable outcomes (this is an estimate and the model would need to be run again with triangular distribution for an exact result (mode: 27%, min 10% and max 60%)).

However, according to the EFSA recommendations<sup>3</sup>, a default 5% BMR is recommended for continuous data (see Section 5.2 "For continuous data the BMR could be defined in various ways. The way recommended here is to define it as a percent change in the average magnitude of the response variable as compared to the predicted background response. The recommended default value is a BMR of 5%"). Depending on the choice made in terms of BMR (5% or 10%), the BMD and BMDL values differ greatly. An alternative choice of BMR could have been made based on the upper limit of (95% or 99%) confidence intervals around mean values for increases in kidney weight in male and female control animals (F0 and F1). BMR calculations for these various choices are given in the annexes (see Annex 6).

EFSA chose to calculate BMD and BMDL values using sex and generation (F0 and F1) as covariates. This makes it possible to see whether either the two sexes or two generations is more sensitive to BPA. Table 54 of the EFSA report shows that generation F0 males are most sensitive to BPA, with a BMDL rounded to 4 mg/kg bw/day.

For clarity purposes, it would have been helpful to present the same calculations in the appendices, modelling F0 males (the most sensitive) and then F0 males with generation F1 males as the covariate and lastly F0 males with F0 females as the covariate. This approach would make it possible to compare the various BMD and BMDL pairs and choose the most reliable one.

Regarding the choice of data on the critical effect (absolute weights *versus* relative weights), the study by Tyl *et al.* (2008) provides figures on the relative weights of each organ (Tables 19-20).

It would have been beneficial to perform the same calculations comparing absolute weights and relative weights.

In conclusion, the data modelling on kidney weight of Tyl *et al.* (2008) was performed with the software PROAST, distinguishing between four subgroups (F0 and F1 males, F0 and F1 females). The appeal of this approach (taking into account covariates) is that it measures the influence of sex (male or female) and generation (F0 or F1) on equation parameters (exponential and Hill). The EFSA analysis shows that generation F0 males are more sensitive to BPA than F0 females and F1 males.

The table below shows BMD and BMDL values recalculated by ANSES based on the use of covariates, a BMR of 10% or 5% and the effect (absolute weight and relative weight).

The values vary by several orders of magnitude depending on the choices made. It should be noted that the BMD/BMDL ratios are all less than ten when relative weight is considered as the critical effect (and so these values have a lower level of uncertainty than if absolute weight were the critical effect).

It can be noted that a 5% BMR (as recommended by EFSA in its methodological guide<sup>4</sup>) with an increase in relative weight as the critical effect results in a BMD<sub>5%</sub>L<sub>90%</sub> of 286 µg/kg/day, i.e. a value that is one tenth of that used by EFSA.

<sup>3</sup> Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. The EFSA Journal (2009) 1150, 1-72

<sup>4</sup> Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. The EFSA Journal (2009) 1150, 1-72

Table 2: Summary of BMD and BMDL values based on the use of covariates (F1 and sex), the effect (absolute versus relative weight) and the response level (BMR).

Effect	Covariate	BMR (CES)	BMD (µg/kg.bw/day)	BMDL (µg/kg.bw/day)	BMD/BMDL ratio
Increase in absolute (left) kidney weight in F0 males	Females, and F0/F1	10%	23600	3633	6.5
		5%	1040	43	24
	F1 males	10%	19000	2732	6.9
		5%	1050	33	31
	F0 females	10%	48900	9272	5.2
		5%	4520	262	17
	none	10%	48400	9694	5
		5%	5740	348	16
Increase in relative (% of total weight) (left) kidney weight in F0 males*	Females, and F0/F1	10%	35500	10000	3.5
		5%	2170	286	7.6
	F1 males	10%	36400	10520	3.4
		5%	2300	260	8.8
	F0 females	10%	54900	14250	3.8
		5%	5370	539	9.9
	none	10%	51900	16720	3
		5%	10600	1316	8

\*Note: for the calculation of BMD values with relative weight, the F1 generation (males) is the most sensitive, but the values described in the table are those for F0 (for comparison purposes). Only values taken from the exponential equation are shown in this table. Irrespective of the model used (exponential or Hill), the results have the same order of magnitude.

The report does not consider effects on the mammary gland (*mammary gland ductal proliferation*) for the risk assessment on the grounds that the BMDL<sub>10</sub> obtained with the various models varies significantly (more than ten orders of magnitude) (see p. 161, p. 515). The fact that the choice of models has an impact on BMD results is known (Foronda *et al.*, 2007, Sand *et al.*, 2008). This is not reason enough to not use this critical effect for the risk assessment. To address the impact of the model on BMD values, a sensitivity analysis could have been undertaken and a range of values could have been included in the risk assessment for this critical effect.

#### 3.4.2. Animal - human extrapolation: PBPK modelling

The approach used by EFSA consists in calculating a human equivalent dose from the critical dose (BMDL) established in mice according to the study by Tyl *et al.* (2008). To do so, an equivalence factor was calculated from area-under-the-curve (AUC) ratios for free BPA in serum for the same single dose of 100 µg/kg body weight/day.

Like EFSA, ANSES recommends using allometric adjustment by default based on the ratio of body weights between mice and humans to the ¼ power. However, if one or more PBPK (physiologically-based pharmacokinetic modelling) models are available, they are preferably used to establish the human equivalent dose. EFSA therefore used the PBPK models of Yang *et al.* (2013) and Fisher *et al.* (2011) (same team) to calculate a human equivalent dose factor (HEDF) (ratio of animal AUCs/human AUCs) from a single dose of 100 µg/kg bw/day for the two species, which assumes a linear toxicokinetic dose response which is far from being certain, particularly due to the possible



saturation of metabolism. A table listing uncertainties and their potential impact on HEDF determination is presented in the report (see Table 50, page 499).

From ANSES's perspective, the approach would have involved converting the external exposure dose in mice (the BMDL already established) (Tyl *et al.* 2008) into an internal dose using the mouse PBPK model (Yang *et al.*, 2013). This internal dose corresponds to an AUC. In humans, it can be expected that this same AUC would have similar effects (or no effects), provided that a 2.5 uncertainty factor is applied for the toxicodynamic component. A human PBPK model (Yang *et al.*, 2013) could then be used to establish the corresponding exposure dose for BPA.

In general, the requirements for using a PBPK model can be summarised through these 'guidelines' taken from the WHO document<sup>5</sup> (see Figure 1).

The level of confidence associated with a model relies on an analysis of the model's overall structure, a simulation and validation, and lastly an evaluation of reliability including a sensitivity and uncertainty analysis (see Figure 1).

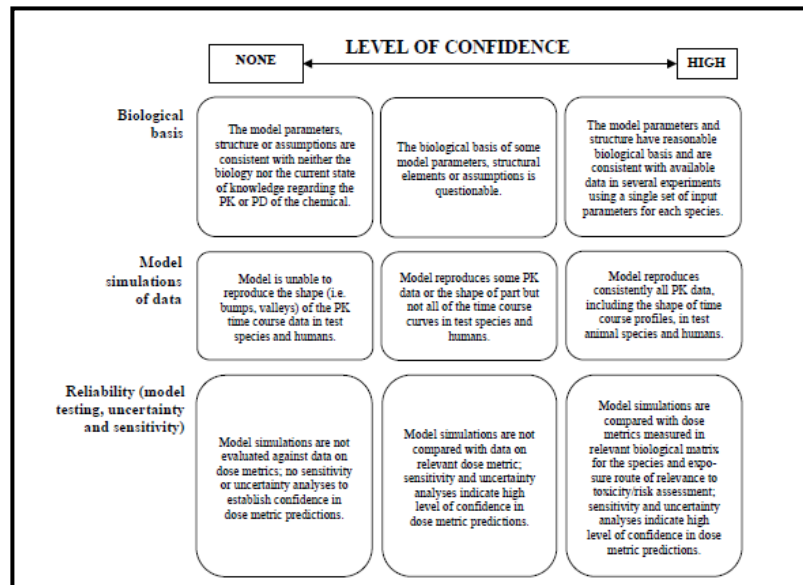


Figure 1: level of confidence in a PBPK model – source WHO<sup>5</sup>, 2013

### Description of the PBPK models used

The models (rats and humans) used in the EFSA report are those described in the articles of Fisher *et al.* (2011) for humans and Yang *et al.* (2013) for rats, in addition to one human model from Mielke *et al.* (2011).

<sup>5</sup> Characterization and application of physiologically based pharmacokinetic models in risk assessment, WHO 2010.

## Fisher group models

- **Description of the Fisher team's PBPK models**

The first two PBPK models used were intended to identify the starting dose resulting from the work of the Fisher group. This group first produced a PBPK model for monkeys and extrapolated it to humans, and then for rats exposed to BPA.

The model developed for monkeys and humans has a structure with seven compartments: the blood compartment (serum), reproductive tract (gonad), brain, fatty tissues, richly perfused tissues, slowly perfused tissues and liver. This model also has three pseudo-compartments: the small intestine, stomach and a compartment that the authors call volume of distribution (Vd). This last pseudo-compartment represents the metabolised fraction of BPA as BPA-c (Fisher *et al.*, 2011). However, it does not take into account the enterohepatic cycle (Fisher *et al.* 2011).

The rodent PBPK model, published by Yang *et al.* (2013), is the same as that of Fisher *et al.* (2011). For the metabolite (BPA-c), the authors described three compartments: the plasma, 'body' and liver, and a pseudo-compartment called the digestive tract. Note that the plasma compartment and liver are the same compartments as those given for the parent product (free BPA) but that the 'body' compartment is an agglomeration of the other compartments. In this version, the model contains a description of an enterohepatic cycle (Yang *et al.*, 2013). Moreover, each of the compartments is described as having limited perfusion ("*well-stirred model*"), meaning that the quantity of BPA distributed in the tissues is related to the perfusion capacity of the organ, which implies and assumes that the BPA that enters the compartments is evenly and instantly distributed.

The physiological parameters are those traditionally found in the literature. The metabolic parameters used for rodents (Vmax and Km) have been taken from a review of the literature or optimised from the published kinetic data. The physicochemical parameters (partition coefficient) used for the two models have been taken from two prior publications by these same authors (Doerge *et al.* 2011; Fisher *et al.* 2011). The equations are described in an attachment (for Yang *et al.* 2013) and do not appear to include syntactic errors: they are basic equations for PBPK models.

In conclusion, the physiological basis for the two models appears acceptable. However, we did not analyse or audit the equations and parameters of the said models. Based on Figure 1 in reference to the WHO document, the level of confidence for the physiological basis of the models would be 'medium' (WHO-IPCS, 2010).

- **Calibration, evaluation and predictability of the models**

The PBPK model for rodents and humans is used to estimate the internal plasma concentration (Cb) of BPA and its area under the curve (AUC) according to various exposure scenarios. The models were calibrated from a single dose of 100 µg/kg bw/day in rats and monkeys (Doerge *et al.*, 2010a; Doerge *et al.*, 2010b). However, a calibration has no predictive value for the model and it is necessary to compare the measured data with those calculated by the model. Fisher's model was calibrated by visual inspection for several parameters and therefore its calibration remains questionable. It could have been optimised with the software used (ACSLX), which would have increased the confidence level.

Visual examination of Figures 7, 8 and 9 in the model by Fisher *et al.* (2011) is satisfactory<sup>6</sup> for exposure to varying concentrations of 10 mg/kg bw/day, 400 mg/kg bw/day or a total of 5 mg. There is good fit between what is measured and what is modelled. Visual examination of Figures 7, 8 and

<sup>6</sup> It is recommended that the WHO-IPCS ratio between predicted value and measured value be less than two. ANSES does not have access to gross data to establish this ratio.

10 in Yang's model is satisfactory<sup>4</sup>: for exposure to varying concentrations (1 mg/kg bw/day, 10 mg/kg bw/day), there is good fit between what is measured and what has been calculated.

In conclusion, based on Figure 1 (WHO-IPCS document, 2010), the level of confidence for the 'Simulation and validation' of the models would be 'medium-low'.

- **Reliability analysis including a sensitivity and uncertainty study**

The following uncertainty factors are discussed in the EFSA report:

- *Uncertainty as to the measurement of concentrations in animals.*

Analytical accuracy is 20% for the method used for all the studies. Moreover, the method used protects from risks of exterior contamination of samples.

- *Uncertainty as to the calculation of AUCs*

This uncertainty stems from the variability between animals and the calculation method that introduces uncertainties, particularly for the calculation to infinity. The authors consider that taking into account the standard deviation covers these two aspects, which is acceptable. Another source of uncertainty relates to the handling of missing values (below the limit of detection), underestimating the value of the AUC.

The oral absorption procedure appears consistent between the experimental studies in animals and the human PBPK model, which does not generate additional uncertainty. For the human model, only the impact of inter-individual variability is evaluated. Therefore, several evaluations of uncertainty are missing, particularly regarding the PBPK model in humans.

To first legitimise the calculation of the equivalence factor at the concentration of 100 µg/kg/day, this assumes a linear toxicokinetic dose response which is far from being certain, particularly due to the possible saturation of metabolism. The starting concentration for the hazard characterisation is greater than 3500 µg/kg/day (Tyl *et al.*, 2008). The use of PBPK models for each species (mice and humans), valid over a range including this starting concentration for the extrapolation, would have eliminated this uncertainty factor which is ignored here.

- *Uncertainty as to PBPK modelling*

Monte Carlo analysis is the most commonly used probabilistic approach with PBPK models since it incorporates variability into these models. The aim of this Monte Carlo analysis is to qualitatively and quantitatively characterise variability and uncertainty in estimations. It is possible to measure uncertainty, by changing a physiological parameter, a (physicochemical) partition coefficient or a biochemical parameter with realistic values. It is then possible to theoretically consider how these changes influence the outputs. In this case, the result is not a single concentration but rather a distribution of probability, with a median and 95<sup>th</sup> percentile.

According to a WHO report on PBPK modelling, the ratio of the 95<sup>th</sup> percentile and the median easily provides a measure of this uncertainty, which is high, medium or low<sup>7</sup> (WHO/IPCS 2010). However, this ratio does not appear in the EFSA report.

Sensitivity analysis makes it possible to determine the parameters that most influence the measured indicator (e.g. C<sub>b</sub>, AUC). The approach consists in changing one parameter at a time (perhaps a physiological, physicochemical or biochemical parameter) and seeing how this change influences the measured indicator. The closer the value is to 1 in absolute value, the more the parameter influences the measured indicator. According to the WHO criteria, this sensitivity can be

<sup>7</sup> Uncertainty analysis results are summarised as high uncertainty (value could be a factor of 2 or higher), medium uncertainty (value could be a factor between 0.3 and 2) or low uncertainty (value could be a factor of 0.3 or lower)

classified as high, medium or low<sup>8</sup> (WHO-IPCS 2010). The authors of the original articles carried out a sensitivity analysis for each of the rat (Yang *et al.* 2013) and human (Fisher *et al.* 2011) models.

For the Fisher group's rat model and human model

CRITERIA	CONFIDENCE LEVEL
PHYSIOLOGICAL BASE	Medium to high
SIMULATION AND VALIDATION	Medium to low
RELIABILITY (UNCERTAINTY AND SENSITIVITY ANALYSIS)	Medium

#### Model of Mielke *et al.* 2011

- **Description of the PBPK model of Mielke *et al.* 2011**

The human model (which was used for dermal exposure (Mielke *et al.* 2011)) has eight compartments: muscle, skin, adipose tissue, skeleton, brain, kidneys, liver and an 'other organs' compartment. Two routes of exposure are described including oral and dermal exposure. All of the compartments are perfusion-limited. Metabolism occurs only in the liver.

- **Reliability analysis including a sensitivity and uncertainty study**

The authors of the 2011 publication indicate that a sensitivity analysis was performed in the 2009 publication (Mielke and Gundert-Remy, 2009). However, a review of the article does not show any sensitivity analysis. This is a limitation for using this model in a risk assessment and does not reflect a standardised WHO strategy. The model of Mielke *et al.* (2011) is worthwhile to generate assumptions but is a significant source of uncertainty that EFSA does not explain.

All things considered, the level of confidence that can be associated with a model is a combination of sensitivity and uncertainty analyses on a scale from low to high according to the criteria set by WHO. In conclusion, based on the WHO recommendations, the following confidence levels can be assigned:

For Mielke's model

CRITERIA	CONFIDENCE LEVEL
PHYSIOLOGICAL BASE	Medium
SIMULATION AND VALIDATION	Low
RELIABILITY (UNCERTAINTY AND SENSITIVITY ANALYSIS)	Very low, no evidence that this was performed

#### Conclusion

The two models of the team of Fisher *et al.* (2011 and 2013) give a good physiological description and have predictability for blood only. This model is not predictive for the other compartments. This poses a problem of confidence in the model. This limitation is partly due to the lack of data in the literature and possibly a methodological limitation. The use of pseudo-compartments also reduces confidence in the model. However, the authors nonetheless have good predictability for the blood compartment (serum or plasma).

<sup>8</sup> High (absolute value greater than or equal to 0.5), medium (absolute value greater than or equal to 0.2 but less than 0.5) or low (absolute value greater than or equal to 0.1 but less than 0.2)

Regarding the model of Mielke (2010), i.e. the PBPK model in humans that establishes overall exposure (oral and dermal), it would have been simpler to use the same model (with the same physiological basis) to determine this aggregate exposure, by including, for example, dermal exposure from Fisher's model for which a predictability assessment and sensitivity study were undertaken.

Mielke's model appears less reliable than Fisher's model for the following reasons:

- Fisher's model was compared to experimental data, which means the model can be tested
- The predictability of Mielke's model is demonstrated by comparing a measured point taken from the findings of Volkel. Furthermore, no sensitivity studies appear to have been performed with Mielke's model, which does not increase the level of confidence in the model.

#### 3.4.3. Application of an additional uncertainty factor

ANSES, in its expert appraisal report published in March 2013, chose to apply an additional uncertainty factor of 3 to take into account all the uncertainties in connection with the effects of BPA observed at lower doses than those selected for the HRA and the existence of non-monotonic dose-response relationships, the existence of *in vitro* and *ex vivo* data in favour of a much greater sensitivity (beyond a factor of 10 already considered in the inter-species variability factor) of tissues of human origin with respect to BPA, compared to animal tissues. In the end, an overall uncertainty factor of 300 was applied in ANSES's expert appraisal.

In the EFSA report, uncertainties as to effects are described in several places in narrative mode. This is the case for effects on reproduction and development (p. 5), neurotoxic effects (p. 6), effects on immunity (p. 6), cardiovascular effects (p. 6), effects on metabolism (p. 7) and carcinogenic effects (p. 7). One might have expected for these uncertainties to be taken into consideration in the risk assessment, for example with a specific uncertainty factor to take into account the state of knowledge. Such is not the case, based on the argument that the calculation of the human equivalent dose covers this due to its conservative nature. The report specifies that the HEDF of 0.03 that is used is conservative. And yet this argument is questionable; just because the HEDF developed for one effect (increase in kidney weight) is conservative, does not mean that it is conservative for all other effects.

#### 3.4.4. Overall consideration of uncertainty

Despite what is said (see p. 9), uncertainty is only partially evaluated in the EFSA report. It would have been helpful to define the term 'uncertainty' and better describe the method used to choose uncertainties. The reasons why some uncertainties are described and others are not are not clear upon reading the report.

The aim of any risk assessment is to draw conclusions when 'perfect' and therefore 'certain' information is not available. In other words, a risk assessment is intended to produce a conclusion in a situation of uncertainty. It is therefore questionable to refuse to consider available knowledge on the pretext that it is uncertain. And yet, in the EFSA report, uncertainty is often used as an argument to consider that an effect is not likely (effects on reproduction and development, p. 5) or even exclude an effect that is considered likely from the risk assessment (effect on mammary hyperplasia, p. 8). In addition, when the uncertainty as to the effect is high (see p. 5), what arguments did the experts use to consider effects unlikely?



#### 4. CONCLUSIONS OF THE EXPERT APPRAISAL

ANSES agrees with the observations made by the rapporteurs of the Working Group on endocrine disruptors and category 3 reprotoxic substances further to the analysis, on a complex topic in a short time-frame, of the draft opinion on the health risks related to BPA submitted to public consultation by EFSA on 17 January 2014.

This analysis dealt with the assessment approach developed by EFSA, hazard and exposure characterisation, biokinetic data and risk assessment. It covered specific points of the EFSA report that can influence the results of the risk assessment: the choice of publications taken into account, the selection of the critical effect(s), BMD calculation, the estimation of internal exposure in humans and the treatment of uncertainty. Comments specific to certain studies and additional information provided by ANSES have been attached to this Opinion.

Regarding the characterisation of effects, ANSES acknowledges the systematic nature of the approach used by EFSA to characterise, study by study, lines of evidence associated with the effects of BPA. Nonetheless, the approach implemented has a number of limitations, such as the sometimes over-fragmentation of the data analysis, making it difficult to characterise effects by organ or system (reproductive system, mammary gland, etc.) in a consistent manner. Furthermore, biochemical and/or histological signs that can lead to biological changes preceding effects harmful to health are not considered by EFSA as significant enough to be taken into account for the risk assessment. ANSES considers that some of these effects (e.g. effects on the central nervous system, effects on the mammary gland) should be taken into consideration for the assessment of risks related to BPA. Effects on the mammary gland are the most significant effects identified by ANSES to assess the risks of BPA. Situations of at-risk exposure have been identified based on these effects. Likewise, uncertainties as to the effects of BPA related to the quality of the studies analysed are mentioned several times in the EFSA report. In this context of uncertainty, it would be helpful if the choices made by the EFSA experts throughout the expert assessment process were better described, documented and justified. In the EFSA report, uncertainty is often used as an argument to consider that an effect is not likely or even exclude an effect that is considered likely from the risk assessment.

ANSES observes that this new health risk assessment for BPA not only takes into account studies on oral exposure but also studies on subcutaneous exposure, which was not the case in previous EFSA opinions. Most of the studies undertaken to examine the toxicity of BPA were not conducted in accordance with the OECD guidelines and did not systematically adhere to 'Good Laboratory Practice' (GLP); these studies were nonetheless taken into account in the EFSA expert assessment, even though EFSA gave greater weight to studies following the OECD recommendations and/or carried out according to GLP (e.g. Tyl, 2002, 2008). Many studies have been published since June 2012, the deadline for publications taken into account by ANSES in its expert appraisal report on the assessment of health risks related to BPA published in March 2013. These recent studies included in the EFSA expert assessment provide additional information, particularly on certain critical effects such as metabolism for which fairly little information was available until recently.

Subject to an assessment of these new publications, which have not been analysed in this Opinion by the Working Group's experts, ANSES considers that the conclusions of its assessment published in March 2013 remain valid. ANSES nonetheless takes note of the number of publications since its report on the health effects of BPA (ANSES, 2011), which is justification for maintaining an active watch to update the data on this substance.

Lastly, ANSES considers it is necessary to define objective criteria to qualify studies investigating the effects of potential endocrine disrupting substances, given the differences in interpretation noted by the experts particularly with regard to the methodological limitations of BPA toxicity studies, the number of necessary doses and animals, the lack of positive controls and the lack of increasing dose-response relationships. These criteria should be standardised between EFSA and national health and safety agencies.

**The Director General**

**Marc MORTUREUX**



**KEY-WORDS****Bisphenol A, Risk assessment, EFSA Opinion****REFERENCES**

Acevedo N, Davis B, Schaeberle C M, Sonnenschein C, and Soto AM (2013) Perinatally Administered Bisphenol A as a Potential Mammary Gland Carcinogen in Rats *Environ Health Perspect.* 121(9): 1040–1046.

ANSES (2011) Effets sanitaires du bisphénol A – rapport d’expertise collective

ANSES (2013) Evaluation des risques du bisphénol A (BPA) pour la santé humaine – Tome 1 rapport d’expertise collective

Bai Y, Chang F, Zhou R, Jin PP, Matsumoto H, Sokabe M, Chen L (2011) Increase of anteroventral periventricular kisspeptin neurons and generation of E2-induced LH-surge system in male rats exposed perinatally to environmental dose of bisphenol-A. *Endocrinology* 152(4), 1562-1571.

Benoit B, Plaisancié P, Awada M, Géoën A, Estienne M, Capel F, Malpuech-Brugère C, Debard C, Pesenti S, Morio B, Vidal H, Rieusset J, Michalski MC. (2013) High-fat diet action on adiposity, inflammation, and insulin sensitivity depends on the control low-fat diet. *Nutr Res.* 33(11):952-60.

Betancourt AM, Eltoum IA, Desmond RA, Russo J, Lamartiniere CA (2010) *In utero* exposure to bisphenol A shifts the window of susceptibility for mammary carcinogenesis in the rat. *Environmental Health Perspectives* 118(11), 1614-1619.

Biedermann S, Tschudin P, Grob K (2010) Transfer of bisphenol A from thermal printer paper to the skin. *Analytical and Bioanalytical Chemistry* 398(1), 571-576.

Cabaton NJ, Wadia PR, Rubin BS, Zalko D, Schaeberle CM, Askenase MH, Gadbois JL, Tharp AP, Whitt GS, Sonnenschein C, Soto AM (2011) Perinatal exposure to environmentally relevant levels of bisphenol A decreases fertility and fecundity in CD-1 mice. *Environ Health Perspect.* 119(4): 547-52.

Chou WC1, Chen JL, Lin CF, Chen YC, Shih FC, Chuang CY (2011) Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan. *Environ Health.*;10:94.

Demierre A-L, Peter R, Oberli A, Bourqui-Pittet M (2012) Dermal penetration of bisphenol A in human skin contributes marginally to total exposure. *Toxicology Letters* 213(3), 305-308.

Doerge, DR., Twaddle, NC, Vanlandingham, M, Brown, RP, and Fisher, JW (2011). Distribution of bisphenol A into tissues of adult, neonatal, and fetal Sprague-Dawley rats. *Toxicol Appl. Pharmacol* 255(3), 261-270.

Doerge, DR, Twaddle, NC, Vanlandingham, M, and Fisher, JW (2010a) Pharmacokinetics of bisphenol A in neonatal and adult Sprague-Dawley rats. *Toxicol Appl. Pharmacol* 247(2), 158-165.

Doerge, DR, Twaddle, NC, Woodling, KA, and Fisher, JW (2010b) Pharmacokinetics of bisphenol A in neonatal and adult rhesus monkeys. *Toxicology and Applied Pharmacology* 248(1), 1-11.

ECHA (2010). Practical guide 2: How to report weight of evidence. URL : [https://echa.europa.eu/documents/10162/13655/pg\\_report\\_weight\\_of\\_evidence\\_en.pdf](https://echa.europa.eu/documents/10162/13655/pg_report_weight_of_evidence_en.pdf)

Eilam-Stock T, Serrano P, Frankfurt M, Luine V (2012) Bisphenol-A impairs memory and reduces dendritic spine density in adult male rats. *Behav Neurosci.* 126(1):175-85.

Fenichel P, Dechaux H, Harthe C, Gal J, Ferrari P, Pacini P, Wagner-Mahler K, Pugeat M, Brucker-Davis F (2012) Unconjugated bisphenol A cord blood levels in boys with descended or undescended testes. *Hum Reprod* 27(4), 983-90

Fisher, JW, Twaddle, NC, Vanlandingham, M, and Doerge, DR. (2011). Pharmacokinetic modeling: Prediction and evaluation of route dependent dosimetry of bisphenol A in monkeys with extrapolation to humans. *Toxicology and Applied Pharmacology*(0).

Foronda NM, Fowles J, Smith N, Taylor M, Temple W (2007). A benchmark dose analysis for sodium monofluoroacetate (1080) using dichotomous toxicity data. *Regul Toxicol Pharmacol.* 7(1):84-9.

Gayrard V, Lacroix MZ, Collet SH, Viguié C, Bousquet-Melou A, Toutain PL, Picard-Hagen N (2013) High bioavailability of bisphenol A from sublingual exposure. *Environ Health Perspect.* 121(8):951-6.

Heather B. Adewale, Karina L. Todd, Jillian A. Mickens, Heather B. Patisaul HB. (2011) The impact of neonatal bisphenol-A exposure on sexually dimorphic hypothalamic nuclei in the female rat *NeuroToxicology* 32, 38-49

Inagaki T, Frankfurt M, Luine V. (2012) Estrogen-induced memory enhancements are blocked by acute bisphenol A in adult female rats: role of dendritic spines. *Endocrinology.* 153(7):3357-67.

Jenkins S, Raghuraman N, Eltoum I, Carpenter M, Russo J, Lamartiniere CA (2009) Oral Exposure to Bisphenol A Increases Dimethylbenzanthracene-Induced Mammary Cancer in Rats. *Environmental Health Perspectives* 117, 910-915.

Jenkins S, Wang J, Eltoum I, Desmond R, Lamartiniere CA (2011) Chronic oral exposure to Bisphenol a results in a nonmonotonic dose response in mammary carcinogenesis and metastasis in mmtv-erbB2 mice. *Environmental Health Perspectives* 119(11), 1604-1609.

Jones BA, Shimell JJ, Watson NV (2011) Pre- and postnatal bisphenol A treatment results in persistent deficits in the sexual behavior of male rats, but not female rats, in adulthood. *Hormones and Behavior* 59(2), 246-251.

Kosarac I, Kubwabo C, Lalonde K, Foster W (2012) A novel method for the quantitative determination of free and conjugated bisphenol A in human maternal and umbilical cord blood serum using a two-step solid phase extraction and gas chromatography/tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 898:90-4.

Lamartiniere CA, Jenkins S, Betancourt AM, Wang J, Russo J (2011) Exposure to the Endocrine Disruptor Bisphenol A Alters Susceptibility for Mammary Cancer. *Horm Mol Biol Clin Investig* 1;5(2):45-52.

Lee SG, Kim JY, Chung JY, Kim YJ, Park JE, Oh S, Yoon YD, Yoo KS, Yoo YH, Kim JM (2013). Bisphenol A exposure during adulthood causes augmentation of follicular atresia

and luteal regression by decreasing  $17\beta$ -estradiol synthesis via downregulation of aromatase in rat ovary. *Environ Health Perspect.* 121(6):663-9.

Marmugi A, Ducheix S, Lasserre F, Polizzi A, Paris A, Priymenko N, Bertrand-Michel J, Pineau T, Guillou H, Martin PG, Mselli-Lakhal L (2012) Low doses of bisphenol A induce gene expression related to lipid synthesis and trigger triglyceride accumulation in adult mouse liver. *Hepatology.* 55(2), 395-407.

Marquet F, Payan JP, Beydon D, Wathier L, Grandclaude MC, Ferrari E (2011) In vivo and ex vivo percutaneous absorption of [ $^{14}C$ ]-bisphenol A in rats: A possible extrapolation to human absorption? *Archives of Toxicology* 85(9), 1035-1043.

Martini M, Miceli D, Gotti S, Viglietti-Panzica C, Fissore E, Palanza P, Panzica G (2010) Effects of perinatal administration of Bisphenol A on the neuronal nitric oxide synthase expressing system in the hypothalamus and limbic system of CD1 mice. *Journal of Neuroendocrinology* 22(9), 1004-1012.

Mielke, H., and Gundert-Remy, U. (2009) Bisphenol A levels in blood depend on age and exposure. *Toxicol. Lett.* 190(1), 32-40.

Mielke, H., Partosch, F., and Gundert-Remy, U. (2011). The contribution of dermal exposure to the internal exposure of bisphenol A in man. *Toxicology letters* 204(2-3), 190-198.

Mok-Lin E, Ehrlich S, Williams PL, Petrozza J, Wright DL, Calafat AM, Ye X, Hauser R (2010) Urinary bisphenol A concentrations and ovarian response among women undergoing IVF. *International Journal of Andrology* 33(2), 385-393.

Rubin BS, Murray MK, Damassa DA, King JC, Soto AM (2001) Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. *Environmental Health Perspectives* 109, 675-680.

Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, Soto AM (2006) Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. *Endocrinology* 147(8), 3681-3691.

Rudel RA, Fenton SE, Ackerman JM, Euling SY, Makris SL (2011) Environmental exposures and mammary gland development: State of the science, public health implications, and research recommendations. *Environmental Health Perspectives* 119(8), 1053-1061.

Russo IH, Russo J (1996) Mammary gland neoplasia in long-term rodent studies. *Environmental Health Perspectives* 104(9), 938-967.

Sand S, Victorin K, Filipsson AF (2008) The current state of knowledge on the use of the benchmark dose concept in risk assessment. *J Appl Toxicol.* 28(4):405-21. Review.

Singh M, McGinley JN, Thompson HJ (2000) A comparison of the histopathology of premalignant and malignant mammary gland lesions induced in sexually immature rats with those occurring in the human. *Laboratory Investigation* 80(2), 221-231.

Teeguarden JG, Calafat AM, Ye X, Doerge D, Churchwell MI, Gunawan R, Graham MK (2011) Twenty-four hour human urine and serum profiles of bisphenol A during high-dietary exposure. *Toxicological Sciences* 123, 48-57.

Tian YH, Baek JH, Lee SY, Jang CG (2010) Prenatal and postnatal exposure to bisphenol A induces anxiolytic behaviors and cognitive deficits in mice. *Synapse* 64(6), 432-439.

Tyl RW, Myers CB, et al. (2008) Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (swiss) mice. *Toxicological Sciences* 104(2), 362-384.

Tyl RW, Myers CB, et al. (2002) Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. *Toxicological Sciences* 68(1), 121-146.

U.S.FDA/NCTR (National center for Toxicological Research –national Toxicology Program), 2013. Evaluation of the toxicity of bisphenol A (BPA) in male and female Sprague-Dawley rats exposed orally from gestation day 6 trough postnatal day 90.

Vom Saal FS, VandeVoort CA, Taylor JA, Welshons WV, Toutain PL, f, Hunt PA (2014). Bisphenol A (BPA) pharmacokinetics with daily oral bolus or continuous exposure *via* silastic capsules in pregnant rhesus monkeys: Relevance for human exposures. *Reprod Toxicol* , in press

WHO/IPCS. Characterization and Application of Physiologically Based Pharmacokinetic Models in Risk Assessment. WHO. No. 9, 1-97. 2010. IPCS harmonization project document.

Ref Type: Report

Xu XH, Zhang J, Wang YM, Ye YP, Luo QQ (2010a) Perinatal exposure to bisphenol-A impairs learning-memory by concomitant down-regulation of N-methyl-D-aspartate receptors of hippocampus in male offspring mice. *Hormones and Behavior* 58(2), 326-333.

Xu XH, Wang YM, Zhang J, Luo QQ, Ye YP, Ruan Q (2010b) Perinatal exposure to bisphenol-A changes N-methyl-D-aspartate receptor expression in the hippocampus of male rat offspring. *Environmental Toxicology and Chemistry* 29(1), 176-181.

Xu X, Liu X, Zhang Q, Zhang G, Lu Y, Ruan Q, Dong F and Yang Y. (2013a) Sex-specific effects of bisphenol-A on memory and synaptic structural modification in hippocampus of adult mice. *Hormones and Behavior*, 63, 766-775

Yang, X., Doerge, D. R., and Fisher, J. W. (2013) Prediction and evaluation of route dependent dosimetry of BPA in rats at different life stages using a physiologically based pharmacokinetic model. *Toxicol Appl. Pharmacol.*

Zalko D, Jacques C, Duplan H, Bruel S, Perdu E (2011) Viable skin efficiently absorbs and metabolizes bisphenol A. *Chemosphere* 82(3), 424-430.

Zhou R, Bai Y, Yang R, Zhu Y, Chi X, Li L, Chen L, Sokabe M (2011) Abnormal synaptic plasticity in basolateral amygdala may account for hyperactivity and attention-deficit in male rat exposed perinatally to low-dose bisphenol-A. *Neuropharmacology* 60(5), 789-798.

Zimmermann C, Cederroth CR, Bourgoin L, Foti M, Nef S (2012) Prevention of diabetes in db/db mice by dietary soy is independent of isoflavone levels. *Endocrinology*. 153(11):5200-11.



**ANNEX 1 – COMMENTS MADE TO EFSA IN SEPTEMBER 2013 VIA THE INTERNET WEBSITE**

Tableau des commentaires de l'Anses transmis à l'Efsa sur le rapport « DRAFT Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs – Part : Exposure Assessment »

CHAPTER OR CONCERNED PARAGRAPH (n° and title)	Line numbers of the text on which the comment is	ANSES COMMENTS
SUMMARY - General approach taken for the assessment	66	<p>The approaches used are different between the two institutes. EFSA used a deterministic approach while Anses used a probabilistic approach.</p> <p>The average exposure assessment conducted by EFSA is classic, combining the average values of each exposure parameters and calculating the total exposure adding the average exposure of each sources investigated. However, the high exposure assessment is less conventional. Some parameters are taken at a high percentile and others at their average. Moreover, the total exposure is calculated adding high levels of some exposures and average levels of the others. This method doesn't permit to take into account some possible correlations between parameters. Further, a parameter involved in the calculation of the exposure of each source, as the body weight, is not taken at the same values according the level of the exposure.</p> <p>The aggregation of the exposure via the media and some products (toys, thermal papers) can generate some difficulties about the risk management. Moreover, the total exposure thus calculated is subject to more uncertainties.</p>
SUMMARY - Dietary exposure	157	Could you precise the term "PC filters" : jug water filters, filters in drinking water plants or filters withing buildings

ASSESSMENT		
2 Physical and chemical characterisation	566 to 570	Could you add that in common chlorinated drinking water, the half-life of BPA would be less than 3 h. Some studies show that the chlorinated BPA are not detected in the drinking water networks (Dupuis, 2012). A. Dupuis, V. Migeot, A. Cariot, M. Albouy-Llaty, B. Legube, S. Rabouan (2012). Quantification of bisphenol A, 353-nonylphenol and their chlorinated derivatives in drinking water treatment plants. <i>Environmental Science and Pollution Research</i> , 19(9), pp. 4193-4205.
3 Potential sources of exposure - 3.1 Polycarbonate plastics	595 & 596	The use of pipes in PC plastic in public water distribution networks doesn't exist in France. Could you specify the countries where this practice exists.
3 Potential sources of exposure - 3.1 Epoxy resins	631 & 632	Could you specify the frequency and conditions of use of epoxy resins in drinking water pipes and tanks and mention the differences between countries.
3 Potential sources of exposure - 3.1 Polysulfone resins	706	Could you specify if pumps, valves and pipes mentioned are for drinking water. In France, PSU is used in the fittings or in the membranes but not in the pipes for public water distribution networks.
3.1. Materials and uses	729	In Cacho study (2013), <u>thirty</u> cosmetic products have been collected from " <u>local</u> supermarket". We question whether such data could be considered as a realistic sample for a European safety assessment.  <i>Cacho, J.I., Campillo, N., Viñas, P., and Hernández-Córdoba, M. (2013). Stir bar sorptive extraction with EG-Silicone coating for bisphenols determination in personal care products by GC-MS. Journal of Pharmaceutical and Biomedical Analysis 78-79, 255-260.</i>
3 Potential sources of exposure - 3.1 Other uses	737	Could you specify if tanks and piping mentioned are for drinking water.
4.3.4. Data on occurrence in and migration from food contact materials into food simulants	874	It is specified that "Consumers tend to be loyal to the type of water they consume", is there any reference about it?

Table 3	1145	Typing error for legumes, nuts & oil seeds: Mean is 121 whereas max is 103.
4 Exposure assessment -4.3 Occurrence data - 4.3.5 Occurrence data in food Table 3: Summary of average BPA concentrations (g/kg) from the literature and EFSA's call for data	1145 - 1146	As mentioned in appendix III, lines 5162 to 5165, 10 results for samples of canned drinking water come from "Efsa's call for data".
4 Exposure assessment -4.3 Occurrence data - 4.3.5 Occurrence data in food Table 3: Summary of average BPA concentrations (g/kg) from the literature and EFSA's call for data	1145 - 1146	Taking into account the differences between BPA concentrations in drinking water from distribution networks, PET bottles or PC coolers, it would be interesting to consider them separately.
4.3.6. Occurrence, migration and transfer data from non-dietary sources Indoor air sub-chapter	1233-1234	The choice of the French data is based on the argument of the only study available in Europe but not based on quality criteria as mentioned in the first paragraph. Only the average level is taken into account without explanation for the exposure calculation.

<p>4.3.6. Occurrence, migration and transfer data from non-dietary sources Dust sub-chapter</p>	<p>1235-1257</p>	<p>Some references are lacking in the description of available data in the scientific literature (cf Rudel et al. 2003, Wilson et al. 2007 referenced in the Anses report), Efsa quotes the result from Völkel et al. (2008) although the author indicates in his publication that they had problems of samples contamination. Otherwise, this reference provides no information on the measurement method, The choice of Geens et al. (2009a) data is based on the comparison with the other average median concentrations but not based on quality criteria as mentioned in the first paragraph. Indeed, the argument mentioned to support this choice is that the author reported the average median concentrations among the recent dust studies available for Europe. However, as described in lines 1253-1254, the French Agency ANSES recently reported average and median concentrations of 5,8 and 4,7 mg/kg respectively (ANSES, 2013). The final choice of the data used does not appear to be based on an analysis of interest criteria such as the description of the methods of sampling and measurement, Such descriptions and criteria are essential but are not always presented in the publications, Only the median level is taken into account without explanation for the exposure calculation. For indoor air, the average level was retained while median level is available from the French study. This point should be explained.</p>
<p>4.3.6. Occurrence, migration and transfer data from non-dietary sources Children's toys and articles intended to be mouthed sub-chapter</p>	<p>1328-1332</p>	<p>The choice of KEMI (2012) data for rattle and Lassen et al. (2011) for pacifiers is not explained. Only the average middle bound level is taken into account without explanation for the exposure calculation.</p>

<p>4.3.6. Occurrence, migration and transfer data from non-dietary sources</p> <p>“The concentration of 31 µg/kg found in facial lotion by Cacho et al. (2013) was chosen for exposure calculation from e.g. the use of body lotion.”</p>	<p>1351-1353</p>	<p>We wonder about the selection of the body lotion (as a reference for the exposure) because there is no BPA concentrations data for body products in this publication (Cacho et al., 2013). Moreover, SCCS's notes of guidance for the testing of cosmetic substances and their safety evaluation, 8th revision (2012) proposed to calculate a global daily exposure value for all cosmetic products (in the specific case of preservatives) that one person may daily apply on the skin [SCCNFP/0321/00]. Taking into account the latest exposure values and considering the worst-case scenario in which the consumer would use a set of cosmetic products containing the same preservative, an aggregate value of 17.4 g/day or 269 mg/kg bw/day will have to be used in the calculation of the MoS.</p>
<p>4.3.6 Occurrence, migration and transfer data from non-dietary sources</p>	<p>1396</p>	<p>The origin of the 22 volunteers in the Kang et al(2011) study is not mentioned. This information could be useful to assess the reliability of the study to the European market.</p>
<p>4.3.6. Occurrence, migration and transfer data from non-dietary sources</p> <p>Table 4: Overview of BPA concentrations and sources considered for the present exposure assessment</p>	<p>1407</p>	<p>We wonder about the selected BPA concentration for cosmetic products which is 31µg/kg. Which criteria has been chosen to select the lowest value as an exposure value? Indeed, Cacho's publication concluded that “The analyzed personal care samples contained BPA at concentration levels ranging from 30.9 to 88.3 ng g<sup>-1</sup>”.</p>
<p>4.5.2. Dermal absorption</p>	<p>1438-1439</p>	<p>The study of Demierre (2012) has been conducted over 8h and 24 h. So "8h" should be added in the brackets line 1439.</p>

<p>4.5.2. Dermal absorption</p>	<p>1439-1441 ; 1466-1468</p>	<p>In the study of Zalko et al (2011), only &lt;3% of the applied dose remain on the skin surface after a 72h incubation period (which is over the 24h recommended to preserve the integrity of skin explants according to the OECD guideline 428). In the study of Marquet et al (2011), the dose applied on skin can be considered as a saturating concentration (200 µg.cm-2) and the study provides maximal absorption flows (µg.cm-2.h-1). So, arguing that in vitro studies fail to provide a reliable upper boundary for dermal absorption (dermal exposure assessment) because no study was conducted over a large enough time span to reach the maximum absorption, seems poorly supported. This comment is not contrary to the use of the 30% absorption fraction (Biedermann et al, 2010) for dermal exposure from thermal paper specifically (line 1468).</p>
<p>4.5.2 Dermal Absorption</p>	<p>1466-1470</p>	<p>With regard to the risk assessment on the <b>general population</b> relating to cutaneous contact with thermic papers containing BPA, the estimate of the percutaneous absorption flow (expressed in % absorbed by the dose transferred onto the skin, and not in the quantity absorbed by surface unit of skin and time) corresponds to values of the least probable rate of a minimum of 10 % and a maximum of 60 %, encompassing a most probable value of 27 %. The rate of 27 % was used in an experimental study (Biedermann et al., 2010). The data from this study cannot be considered as representative on a population scale. However, the experimental protocol is considered to be similar to the conditions of exposure for a person handling cashier's tickets on an occasional basis during the day, different to cashiers. This rate was estimated from the quantity of BPA transferred to the skin of the finger after a single contact of 5 seconds with a ticket, and the quantity of BPA which was no longer removable from the skin by soap and water 2 hours after this contact. The maximum rate of 60 % corresponds to the rate estimated by Biedermann et al. (2010) 2 hours after immersion of the finger in a solution of BPA in acetone; while the minimum level of 10 % corresponds to a (default) recommended value by the European Commission when a substance has a molecular weight over to 500 g.mol-1 and an octanol-water distribution coefficient lower than -1 or higher than 4 (EC, 2004). Therefore, with the absorption rates being estimated by Biedermann et al. (2010) for a period of exposure to the skin to BPA of 2 hours, they must be weighted by an adapted</p>



		period of exposure for the general population in the handling of BPA-based thermic papers.
4.6.1. General assumptions for calculation	1476	It could be precised that a deterministic calculation is done for each scenario that explained the choice of one value of each parameter which are taken into account in the calculation.
4.6. Exposure assessment	1471	As a general comment, EFSA used the deterministic approach to calculate exposures. This approach has the advantage to determine the main contributors to the exposure and to identify specify consumption habits. However, EFSA did not take advantage of this aspect. A probabilistic approach would have been preferable with this respect as discussed in further comments.
4.6.1. General assumptions for calculation	1476	To a certain extent, two scenarios were developed for average exposure and for high exposure, to account for the variability and/or uncertainty of exposure. Considering the available data, a probabilistic approach seems possible, especially for dietary exposure, and more relevant, why this approach has not been implemented?
4.6.2. Exposure estimation from dietary sources Dietary exposure from water coolers with PC reservoirs, PC water filters and old waterpipes repaired with epoxy resins	1575 to 1581	As concentrations of BPA in water from PC coolers are included in general concentration of BPA in drinking water, is not it redundant to consider a specific scenario for PC coolers users ?

4.6.2 Exposure estimation from dietary sources	1670-1671	Dietary exposure in toddlers was used to estimate dietary exposure in infants aged 6 to 12 months. However the consumption of these 2 populations are quite different and the type of food consumed also. There is a part of the population who consumed only infant meals. Why no calculation has been made for 6-12 months children ? Is there any data on contamination of infant foods except for infant formulae ? Anyway, this point should appear in the list of sources of uncertainty.
4.6.2 Exposure estimation from dietary sources	1687	The objective of this work is to perform risk assessment at the European level. In the present work, the national median of the average exposures were used to determine these exposures. The risk is assessed using mean contamination in combination with high consumptions. This implies that food, environmental (air quality, drinking water etc.) are homogeneous over Europe which is clearly not the reality (considering local food production for instance).
4.6.3. Exposure from non-dietary sources	1761	In the Table 15, ingestion exposure to dust for newborns should be mentioned with exclusion of infants (cf. line 1782-1783)
	1764-1773	Other possible pathways exposures to BPA from non-food sources should be mentioned in this paragraf as well those mentioned and not used then. The following exposure pathways are lacking: dermal exposure to dust; dermal exposure to air (recent publication has been dealing with this pathways (Weschler and Nazaroff, 2012).

	1779-1792	<p>Data for dust ingestion rate are derived from Trudel et al 2008. This publication does not appear to be an adapted reference for parameter such as dust ingestion rate. For example, dust ingestion rates for infants and toddlers (9,0 and 106 mg/d respectively for average and high scenarios) are taken from Calabrese et al 1989, But these values correspond to soil ingestion rates (Yttrium tracer element used) and not dust ingestion rates. For teenagers and adults, there seems to be errors in the publication of Trudel because the values reported as weighted dust ingestion rates are actually ingestion rate of soil directly from a publication of Davis et al, 2006 (see original publication). In any event, it would be fair and appropriate to rely on the Exposure Factors handbook- 2011 edition of US-EPA which provides a detailed analysis of a range of publications including the source publications Calabrese and Davis cited by Trudel. EPA recommends the following means dust ingestion rate: 60 mg/day for individuals 1-21 years old and 30 mg/day for adults, and a general population upper percentile of 100 mg/day.</p>
	1868-1880	<p>As for dust ingestion rate, values for air intakes are taken from Trudel et al. 2008. This publication does not appear to be an adapted reference for parameter such as air intakes. Daily respiratory volumes used by EFSA are quite unusual and surprising, and seem to be very elevated for a whole day. We also do not get to find them since the cited source (Trudel et al, 2008). As for dust rate ingestion, the Exposure Factors Handbook - 2011 edition of US- EPA provides a detailed analysis of a range of publications concerning air intakes. This parameter should be considered with caution. A daily inhalation rate has to be a long-term inhalation rate. The inhalation rates used by Efsa seem to correspond more to a characterisation of a short-term exposure to a median to high level of activity. Moreover, this parameter is correlated with body weight and this must be taken into account in the calculation as much as possible.</p>
4.6.3. Exposure from non-dietary sources - <i>Thermal paper: transfer to food</i>	1847-1867	<p>The exposure estimate is based on numerous assumptions without supporting data, so that the development of this scenario is questionable. It seems useful to further investigate the various modes of BPA food contamination, including food contamination from thermal papers.</p>

4.6.3. Exposure from non-dietary sources	1751	Brand loyalty was considered for some food items (infant formulae for instance). However, one could also consider “brand loyalty” in the context of cosmetic uses, or environmental conditions (The air quality in one city is probably very constant). Here again, a probabilistic approach would have been of great use.
4.6.3. Exposure from non-dietary sources - <i>Dermal - Thermal paper</i>	1882-1904	No exposure scenario were developped for cashiers, a population expected to be more exposed than the general population. The exposure estimate for children is based on assumptions without supporting data for the parameter "number of handling events", so that the development of this scenario is questionable. The dermal absorption fraction of 30% is supported by the Biedermann experiment resulting in a 27% after a 2h absorption duration. So, the exposure equation (line 1898) could overestimate exposure because of the combination of the 30% dermal absorption fraction with the number of handling events.
4.6.3. Exposure from non-dietary sources	1909	For adults, exposure data usually used for safety evaluation of cosmetic are given by the SCCS's notes of guidance for the testing of cosmetic substances and their safety evaluation, 8th revision (2012).
4.7 Total exposure	1953-1954	Why total high exposure was calculated by adding up high levels of exposure from the two highest sources and not from all sources? Is there any reason?
4.7 Total exposure	1946	To calculate the highest exposures, the only 2 main contributors were considered, and the mean exposure for all other routes. This appears surprising since it implies that one can not be highly exposed to more than 2 routes. A probabilistic approach would have solved this issue.
4 Exposure assessment - 4.7. Total exposure	1973 to 1976	Could you specify if your have any information on the frequency of such cases

4.8.1 General introduction	1984	Efsa's document did not take into account the higher bioavailability that could occur via the sublingual route compared to the pure oral route. This was shown in a recent publication « High bioavailability of bisphenol A from sublingual exposure. V Gayrard et al Environ Health Perspect 2013; 121 :951-956 ».
4.8.1 General introduction "In addition, it has been shown that generally less than 1% of total serum BPA in in the unconjugated form after oral administration	2007-2008	Anses has considered for the risk assessment of BPA that 3% of BPA would be available as a free form after oral exposure. On the basis of the available data, only the studies by Doerge <i>et al.</i> (2010) carried out on the Sprague Dawley rat and the Rhesus monkey for an administered dose of BPA of 100 µg/kg and Farbos (2012) carried out on several species (ewe, pig, dog, Wistar rat, CD1 mouse) for an administered dose of BPA of 100 mg/kg enable the determination of an absolute bioavailability of unconjugated BPA for the oral route. These two studies report an absolute bioavailability in unconjugated BPA in rats of the same size, specifically: 2.8% ± 3.1% (Doerge <i>et al.</i> (2010)) and 3.03% (Farbos, 2012) . It should be noted that a study carried out recently on gestating rhesus monkeys (Patterson <i>et al.</i> , 2012) reported an absolute oral bioavailability in unconjugated BPA of 0.48 % (on average), and could therefore support the arguments in favour of a lower bioavailability factor than that used. Furthermore, other authors (Mielke and Gundert-Remy, 2012, Gundert-Remy and Mielke, 2013) note a value of 10% of oral bioavailability of free BPA for humans, based on a PB-PK model. In anticipation of being able to use the PB-PK model currently being developed at the request of Anses, the working group considered that the value of 3% was, in view of current knowledge, the most robust one.
APPENDIX III: FOOD CATEGORIES	5483 to 5489	Pool all the results and use the values of the PC reservoirs for drinking water contamination is not representative of the population exposition because only some people consume DW from PC reservoirs.

<p>APPENDIX III: FOOD CATEGORIES</p>	<p>5566 to 5580</p>	<p>It would be interesting to comment the difference of results between France and Sweden : public network or network inside building (surface-to-volume ratio and residence time of the water differents), chlorinated or not chlorinated drinking water, existence or not of an hygienic authorization of materials and products in contact with drinking water (PDWs),</p>
<p>APPENDIX VIII: EVALUATION OF UNCERTAINTIES IN THE EXPOSURE ASSESSMENT THROUGH 5770 EXPERT JUDGEMENT</p>	<p>5770-5820</p>	<p>In general, the results of the uncertainty analysis are fairly well detailed. In contrast, the method used is too briefly described, which could affect the correct interpretation of results. In particular, it seems necessary to specify how the principles suggested by EFSA 3163 (EFSA, 2006b) were adapted to the needs of the present assessment. It is also necessary to explain how the explanation of experts was conducted. Finally, the method of evaluation of the combined impact of all the uncertainties is described nowhere in the report.</p>
<p>APPENDIX VIII: 4. Uncertainties in the assessment of (average and high) non-dietary exposure Table 58</p>	<p>5879</p>	<p>There are too many uncertainties affecting the assessment, so that it seems not possible to calculate a reliable exposure to BPA in cosmetic products (6 products, choice of body lotion as a reference for exposure, ...)</p>



## ANNEX 2 EFFECTS ON CENTRAL NERVOUS SYSTEM

## STUDIES EXAMINING EFFECTS OF BPA ON ANXIETY-LIKE BEHAVIOUR

**Diaz Weinstein S, Villafane JJ, Juliano N and Bowman RE, 2013. Adolescent exposure to Bisphenol-A increases anxiety and sucrose preference but impairs spatial memory in rats independent of sex. Brain Research, 1529, 56-65.**

Weaknesses of the study :

*“Information about sexual maturation is lacking”*

**Commentaire** : Les rats exposés journalièrement à 40 µg/kg BPA sont âgés de 7 semaines. L'exposition est répétée durant 12 jours. L'âge des rats étant connu, la maturité sexuelle l'est donc aussi.

**Fujimoto T, Kubo K, Nishikawa Y, Aou S, 2013. Postnatal exposure to low-dose bisphenol A influences various emotional conditions. The Journal of Toxicological Sciences, 38(45), 539-546.**

Weaknesses

*“Drinking water consumption (containing BPA) not measured”*

**Commentaire** : Au début de la section Results, il est indiqué : *“In this study, mother rats were administered BPA (0.1 ppm in drinking water) during 7 days soon after the delivery. The average intake of BPA for mother rats was 23.8 ± 0.86 µg/kg/day (mean ± S.E.M.) which was calculated from mother's weight and drinking water volume”*.

**Gioiosa L, Parmigiani S, Vom Saal FS, Palanza P, 2013. The effects of bisphenol A on emotional behavior depend upon the timing of exposure, age and gender in mice. Hormones and Behavior, 63, 598-605.**

*The Panel also noted that these findings are not consistent with those of Ferguson et al. (2012; who used twice the dose (25 µg/kg bw per day) during GD6-21 without finding any effects of BPA).*

**Commentaire** : Une étude peut ne pas avoir mis en évidence un effet qui pourrait être observé dans une autre étude dans d'autre condition. De plus, des effets observés à une dose n'impliquent pas forcément qu'ils soient aussi observés à dose plus forte. Cela est particulièrement vrai quand les relations dose-effet ne sont pas monotones. Enfin, les études de Gioiosa 2013 et Ferguson 2012 diffèrent par les protocoles qui pourraient expliquer, entre autre, les différences de résultats :

- Ferguson 2012 : Rat Sprague-Dawley, doses de 2.5 et 25 µg/kg/j, exposition des F1 via les mères par gavage avec le BPA solubilisé dans une solution de carboxyméthylcellulose à 0.3% (p/v) de GD6-21, et ensuite exposition orale des F1 de PND1-21 à la même dose que les mères.
- Gioiosa 2013 : Souris CD-1, dose de 10 µg/kg/j, exposition orale sans contrainte des mères par le BPA dans de l'huile de maïs de GD11 à PND8.

**Jasarevic E, Williams SA, Vanda GM, Ellersieck MR, Liao C, Kannan K, Roberts RM, Geary DC, Rosenfeld CS, 2013. Sex and dose-dependent effects of developmental exposure to bisphenol A on anxiety and spatial learning in deer mice (peromyscus maniculatus bairdii) offspring. Hormones and Behavior, 63, 180-189.**

*“It is also noted that the authors reported that free BPA in serum at the highest dose was similar to that found in pregnant women, without taking into account that serum BPA is not the optimal biomarker of exposure due to BPA toxicokinetics”*.

**Commentaire** : Il est vrai que, compte tenu de son métabolisme rapide, le BPA sérique ne constitue pas le meilleur marqueur d'exposition au BPA, surtout lorsque la concentration en BPA <

LOD. Cependant, compte tenu aussi du métabolisme rapide, une forte teneur en BPA sérique signe une forte exposition. Aussi, même si dans l'absolu les concentrations en BPA observées dans la souris dans cette étude ne peuvent pas être directement comparées à celle observées chez les femmes enceintes, elles apportent des renseignements importants sur les expositions susceptibles d'induire des concentrations sériques de cet ordre.

**Jones BA and Watson NV, 2012. Perinatal BPA exposure demasculinizes males in measures of affect but has no effect on water maze learning in adulthood. Hormones and Behavior, 61, 605-610**

Strengths

*“Use of non-PC cages and of BPA-free water sacks”*

**Commentaire** : Le polysulfone résulte de la condensation du sel di-sodique de bisphénol A et du bis(4chlorophényl)sulfone (ou bis(4fluorophényl)sulfone). Chacun de des deux composés a des actions similaires à celles du bisphénol A et peuvent se retrouver sous forme de monomères libres dans les plastiques. Ainsi, ce type de matériaux ne garantit pas un meilleur contrôle des sources de composés à action estrogénique.

**Patisaul HB, Sullivan AW, Radford ME, Walker DM, Adewale HB, Winnik B, Coughlin JL, Buckley B and Gore AC, 2012. Anxiogenic effects of developmental bisphenol A exposure are associated with gene expression changes in the juvenile rat amygdala and mitigated by soy. PLoS One, 7, e438**

Weaknesses

*Lack of constant levels of exposure in time (lactational exposure is much lower than the gestational or juvenile exposure).*

*An important limitation was the lack of constant levels of exposure in time (lactational exposure is much lower than the gestational or juvenile exposure). The exposure to BPA was estimated based on water intake and not normalized to body weight for calculation of internal exposure*

**Commentaire** : L'exposition non constante n'est pas un problème car elle a été mesurée. De plus, l'exposition durant la lactation n'est pas inférieure mais supérieure à celle durant la gestation ou chez les petits. La différence d'exposition journalière n'est pas considérable car pour le BPA, par exemple, elle est de 71.8 µg durant la lactation, 35.2 durant la gestation et 22.4 µg chez les petits.

**Viberg H, Fredriksson A, Buratovic S and Eriksson P, 2011. Dose-dependent behavioral disturbances after a single neonatal bisphenol A dose. Toxicology, 290, 187-194.**

*The selection of behavioural tests is appropriate, however given the peculiar profile of BPA exposed mice at either 2 and 5 months is somewhat surprising that no motor activity impairments are present in either the Elevated plus maze and the Morris water maze test (testing performed at different ages). The deficit shown by BPA-treated male mice is specific to the exploration of a novel environment, but the authors did not provide any mechanistic explanation.*

**Commentaire** : Il n'est pas toujours possible de donner une explication plausible à tous les effets spécifiques observés car l'état actuel des connaissances ne permet pas toujours de la faire. Aussi, une absence d'explication ne signifie en rien que l'effet n'existe pas. Les mécanismes d'action ne sont pas connus pour une grande majorité des médicaments sans que ne soit remise en cause leur action thérapeutique. Cependant, les différents tests utilisés dans cette étude n'explorent pas les mêmes fonctions cognitives et les mêmes structures du cerveau, et il n'est pas anormal que le BPA n'induisse pas des effets avec tous les tests. L'exploration d'un nouvel environnement met en jeu des mécanismes de mémoire, d'apprentissage et d'habituation. Ce test implique des structures cérébrales comme l'hippocampe, le bulbe olfactif ou le cortex périrhinal. Il permet d'explorer la capacité d'intégration d'informations sensorielles nouvelles. La piscine de Morris est configurée pour explorer principalement les processus de mémoire et d'apprentissage liés à la mémoire spatiale et met en jeu, sans être exhaustif, des structures cérébrales comme l'hippocampe, le striatum, le cervelet, le cerveau antérieur et le cortex cérébrale et cérébelleux. Enfin, la labyrinthe

en croix surélevé (Elevated Plus Maze) explore les mécanismes d'anxiété et met en jeu des structures cérébrales comme l'amygdale ou le cortex préfrontal droit. Ainsi, selon le contexte, les impacts sur l'activité motrice n'ont pas les mêmes causes, n'impliquent pas les mêmes mécanismes cognitifs et les mêmes structures cérébrales.

**Xu X, Hong X, Xie L, Li T, Yang Y, Zhang Q, Zhang G, Liu X, 2012. Gestational and Lactational Exposure to Bisphenol-A Affects Anxiety- and Depression-like Behaviors in Mice, Hormones and Behavior, 62, 480-490.**

**Commentaire** : Accord avec les commentaires de l'EFSA. Les résultats sont en accord avec ceux d'histochimie montrant des modifications de l'expression des récepteurs glutamates AMPA et NMDA dans l'hippocampe et l'amygdale.

En ce qui concerne la disparité des résultats avec les différents tests, voir les remarques ci-dessus, même si la plupart des tests sont configurés pour investiguer principalement l'anxiété et la dépression.

D'une manière générale, l'étude est assez complètes mais souffre des faiblesses rapportées par l'EFSA et l'environnement pro-générateur de BPA n'est pas considéré, comme dans l'étude de Xu de 2010.

## STUDIES EXAMINING THE EFFECTS OF BPA ON SOCIAL BEHAVIOUR

**Wolstenholme JT, Edwards M, Shetty SR, Gatewood JD, Taylor JA, Rissman EF, Connelly JJ, 2012. Gestational exposure to bisphenol A produces transgenerational changes in behaviors and gene expression. Endocrinology, 153, 3828-3838.**

Weaknesses

*Animal age and body weight not given*

For each test, and for each generation (F1-F4), the age the animals is indicated: PND20 for juvenile social interactions, PND22 for elevated plus maze, PND24 for the social preference test, GD18.5 for microarray analysis and quantitative real-time PCR.

*The results on social behaviour throughout the generations were inconsistent (social interaction decreased in the F0 generation but increased in the F2 and F4 generations), whereas the effects on gene expression from F1 to F4 appeared per.*

Dans ce commentaire, il est postulé que les effets doivent être identiques ou du même ordre entre les générations. Cependant, (i) les effets peuvent s'atténuer ou s'amplifier entre les générations et (ii) la génération F1 provient de mère exposées et a donc ainsi été exposée directement. La génération F2 n'a pas été exposée mais proviennent d'animaux exposés alors que les F3 et F4 n'ont pas été exposées et ne proviennent pas d'animaux exposés. Ainsi, selon la nature de la perturbation physiologique, il est tout à fait concevable que de certains effets perdurent de générations en générations alors que d'autres disparaissent ou, éventuellement apparaissent. Dans ce cas, les effets induits chez les individus d'une génération peuvent avoir des impacts différents chez les individus de la génération suivante.

### **Annex 3 Effects on female reproductive system**

Tableau comparatif point à point de l'analyse des études publiées après 2010

Reference	ANSES	EFSA	*Species * Route and dose *Period	Observed Significant effect	ANSES opinion	EFSA opinion
Ferguson et	No	Yes		Lower preweaning		

<i>al.</i> 2011				bodyweight		
S FDA/NTCR subchronic toxicity study 2013	No	Yes	SD rats	None for doses lower than equivalent current NOAEL		
Xiao <i>et al.</i> 2011	Yes	Yes	C57Bl6 mice sc 0.025-0.5-10 40-100 mg/kg HD0.5 to GD3.5	None for doses lower than equivalent current NOAEL	Effect were observed on pre implantary embryony loss but only for very HIGH doses	
Kobayashi <i>et al.</i> 2012	Yes	Yes	Sprague Dawley rats 0.02-0.17-1.65 mg/kg/d in diet GD6 to PND21	Decreased Anogenital distance and ovary weight at 5 weeks at 0.17 and 1.65 mg/kg/d reversed at 3 months		No clear biological significance
Veiga-Lopez <i>et al.</i> 2013	No	Yes	Sufolk ewes Sc 0.5 mg/kg/d	Alteration of enzymes expression (CYP19A1 and SRD5A1) and miRNA patterns in fetal ovaries at GD65 but not GD90		No clear biological significance
Nah <i>et al.</i> 2012	yes	Yes	ICR mice Sc 0.1-1-10-100 mg/kg/d a single injection at PND8	Decreased ovary weight and age at puberty for all doses Earlier vaginal opening No other significant effect for doses lower than current NOAEL	Consistent with an effect of postnatal exposure to BPA on puberty processes	
Christiansen <i>et al.</i> 2013	No	Yes	Wistar rats Oral gavage 0.025- 0.25 5-50 mg/kg/d	9% Decrease of anogenital distance in female pups		Indicative of an effect of BPA on the development of female reproductive system BUT No clear biological significance
Cao <i>et al.</i> 2012	Yes	No	Long Evans rats SC 50 µg-50 mg/kg/d PND0 to PND2	Decreased expression of Er $\alpha$ and ER $\beta$ at PND10 in the preoptic area	Indicative of a possible effect of BPA on the gonatropic hypothalamo-pituitary axis	
Losa-Ward <i>et al.</i> 2012	Yes	No	Rat SC 50 µg-50 mg/kg/d PND0 to PND3	Reduced age at vaginal opening decreased density of RFRP3 neurons within the hypothalamus	Indicative of a possible effect of BPA on the development of the gonatropic hypothalamo-pituitary axis	
Aldad <i>et al.</i> 2011	Yes	No	Green Monkeys ovariectomized	BPA antagonizes the effect of E2 on the expression of the PR (progesterone		

			SC infusion 50µg/kg/d	receptor) in the uterus		
Mendoza-Rodriguez et al; 2011	Yes	No	Wistar rats Oral drinking water (10 mg/L ~1.2 mg/kg/d) GD6-PND21	Decreased frequency of oestrus cycle decreased of apoptotic processes in the uterin wall during oestrus and decreased expression of Er $\alpha$	Effects consistent with endometrial hyperplasia and disruption of oestrus cyclicity .	
Varayoud et al. 2011	Yes	No	Wistar rats SC 50 µg and 20 mg/kg/d PND1,3,5 and 7	Decreased expression of Er $\alpha$ and PR in the uterus during the preimplantatory period in adults	No clear biological significance	
Chao et al. 2011	Yes	No	CD-1 mice SC 20 -40 µg/kg/d Daily PND7- PND14 or evry 5 days PND5- PND20	Modification of methylation of imprinted genes in oocytes, increased expression of Er $\alpha$ , alteration of prophase 1 of meiosis resulting in increased transition from primordial to primary follicles	Effect on follicular dynamic in juvenile animals possibly resulting in depletion of follicular stocks	
Rivera et al. 2011	Yes	No	Ovine SC 50µg/kg/d PND1-PND14	Increased transition from primordial to primary follicles Increased frequency of multiocytes follicles	Relevance of the animal model Effect leading possibly to a precocious depletion of follicular stocks	
Zhang et al; 2012 References as 2011 in the ANSES report	Yes	Yes	CD1 mice Oral 20-40-80 µg/kg	Dose dependent increase in the number of retention of oocytes in nests and reduced number of primodial follicle in female offsprings Alteration of the meiotic process	Indicative of an effect of BPA on meiosis	
Hunt et al. 2012	No	Yes	Rhesus macaque Oral diet: 400µg/kg/d Sc implants to provide plasma concentration ~2 -3 ng/ml	Oral BPA: increased multi-oocyte follicles  SC BPA : alterations of meiotic processes		No clear biological relevance in terms of fertility
Signorile et al., 2012	Yes	Yes	Balb-C mices sc 100 and 1000 µg/kg/d GD1 to PND10	Decreased number of primordial follicle and increased number of atretic follicle	Confirmation of the effect of BPA on ovarian development possibly linked to endometriosis phenotypes	

Le tableau ci-dessus regroupant les avis émis par les experts des deux agences sur les études récentes (janvier 2011 à juin 2012 pour l'ANSES et janvier 2011 à Décembre 2013 pour l'EFSA) concernant les effets du BPA sur l'appareil reproducteur femelle souligne que seules 4 études ont été revues par les deux groupes d'experts. Ceci s'explique en partie par le fait que les périodes couvertes pour le recensement de la littérature ne sont pas exactement les mêmes.

Si on suit la démarche validée par le groupe d'expert de l'ANSES concernant la classification des effets, il apparaît que les effets sur la méiose et sur le développement de l'axe hypothalamo-phypophysaire gonadotrope peuvent être considérés comme « avérés » chez l'animal sur la base de plusieurs études de bonne qualité (sans limite méthodologique majeure) donnant à des résultats convergants.



**ANNEX 4** EXPOSURE SCENARIO

En complément des commentaires précédemment formulés et relatifs aux calculs d'exposition ayant in fine servi à l'évaluation de risque conduite par l'Efsa, les 2 tableaux suivants présente les niveaux d'exposition externe calculés par l'Efsa et met en regard les niveaux d'exposition externe calculés par l'Anses dans son évaluation de risque publiée en avril 2013, elle basée sur le calcul de niveaux d'exposition interne.

L'exercice de mise en regard est réalisé pour les sous-groupes de population considérés par chacun des 2 organismes et pouvant être « rapprochés ». Par ailleurs, les doses externes d'exposition présentées relatives aux travaux de l'Anses correspondent à des valeurs recalculées à partir des moyennes et 95ème percentiles des distributions de doses internes d'exposition calculées par l'Anses qui a travaillé selon une approche probabiliste.

Tableau des estimations moyennes des expositions externes (ng / kg PC / j)

	Nourrissons nourris au lait maternel			Nourrisson nourri au lait maternisé	Infants	Jeunes enfants	Autres enfants	Adolescents	Women	Men	Other adults	Elderly people	
	1-5 days	6 days-3 months	4-6 months	0-6 months	6-12 months	1-3 years	3-10 years	10-18 years	18-45 years	18-45 years	45-65 years	+ 65 years	
EFSA (2014)	<b>Ingestion :</b>												
	dust		8.8	8.8	8.8	8.8	7.3	2.9	2.0	0.6	0.6	0.6	0.6
	toys		0.3	0.3	0.3	0.3	0.02						
	food	225	180	158	30	375	375	290	159	132	126	126	116
	$\Sigma$ de toutes les sources par ingestion	225	189	168	39	384	382	293	161	132	127	127	117
	<b>Inhalation :</b>												
	air	0.7	0.7	0.7	0.7	0.7	0.7	0.4	0.4	0.2	0.2	0.2	0.2
	<b>Contact cutané :</b>												
	thermal paper							69	94	59	59	59	59
	cosmetics		4.8	4.8	4.8	4.8	2.8	2.2	2.5	2.0	2.0	2.0	2.0
$\Sigma$ de toutes les sources par contact cutané		4.8	4.8	4.8	4.8	2.8	71	96	61	61	61	61	
ANSES (2013)									Enfants (3-17 ans)	Femmes enceintes	Adultes		
	<b>Ingestion :</b>												
	dust							11.6	2.7	2.7			
	food							55	56	39			
	<b>Inhalation :</b>												
	air							0.4	0.2	0.2			
	<b>Contact cutané :</b>												
Papiers thermiques (scénario consommateurs)									133*	200*			

\*calculs de doses externes cutanées (DE) à partir des doses internes (DI) calculées pour un scénario « consommateur » corrigées d'un taux d'absorption moyen de 30% (Tabs) pondéré par une durée d'absorption moyenne de 1h (Dabs), selon l'équation (modèle b, page 190 du rapport Anses, 2013) :  $DE tickets = \frac{DI tickets}{Tabs * \frac{Dabs}{2}}$   $DE tickets = \frac{DI tickets}{Tabs * \frac{Dabs}{2}}$

Ex : application aux femmes enceintes : DE = 20/(0,3\*1/2)= 133 ng/kg PC/j

Tableau des estimations « hautes » des expositions externes (ng / kg PC / j)

	Nourrissons nourris au lait maternel			Nourrisson nourri au lait maternisé	Infants	Jeunes enfants	Autres enfants	Adolescents	Women	Men	Other adults	Elderly people	
	1-5 days	6 days-3 months	4-6 months	0-6 months	6-12 months	1-3 years	3-10 years	10-18 years	18-45 years	18-45 years	45-65 years	+ 65 years	
EFSA (2014)	<b>Ingestion :</b>												
	dust		14.6	14.6	14.6	14.6	12.2	4.9	3.3	1.0	1.0	1.0	1.0
	toys		1.2	1.2	1.2	1.2	0.5						
	food	435	345	304	80	857	857	813	381	388	335	341	375
	<b>Σ de toutes les sources par ingestion</b>	<b>435</b>	<b>361</b>	<b>319</b>	<b>96</b>	<b>873</b>	<b>870</b>	<b>818</b>	<b>384</b>	<b>389</b>	<b>336</b>	<b>342</b>	<b>376</b>
	<b>Inhalation :</b>												
	air	1.4	1.4	1.4	1.4	1.4	1.1	0.6	0.6	0.3	0.3	0.3	0.3
	<b>Contact cutané :</b>												
	thermal paper							550	863	542	542	542	542
	cosmetics							4.2	4.8	4.0	4.0	4.0	4.0
<b>Σ de toutes les sources par contact cutané</b>							<b>554</b>	<b>868</b>	<b>546</b>	<b>546</b>	<b>546</b>	<b>546</b>	
ANSES (2013)								<b>Enfants (3-17 ans)</b>	<b>Femmes enceintes</b>	<b>Adultes</b>			
	<b>Ingestion :</b>												
	dust							32	7	7.3			
	food							130	123	82			
	<b>Inhalation :</b>												
	air							1.2	0.7	0.6			
	<b>Contact cutané :</b>												
Papiers thermiques (scénario consommateur)									533*	600*			

\*calculs de doses externes cutanées (DE) à partir des doses internes (DI) calculées pour un scénario « consommateur » corrigées d'un taux d'absorption moyen de 30% (T<sub>abs</sub>) pondéré par une durée d'absorption moyenne de 1h (D<sub>abs</sub>), selon l'équation (modèle b, page 190 du rapport Anses, 2013) :  $DE\ tickets = \frac{DI\ tickets}{T_{abs} * \frac{D_{abs}}{2}}$   $DE\ tickets = \frac{DI\ tickets}{T_{abs} * \frac{D_{abs}}{2}}$

Ex : application aux femmes enceintes : DE = 80/(0,3\*1/2)= 533 ng/kg PC/j

## ANNEX 5 DERMAL PENETRATION

Pour conduire l'évaluation des risques liés à la manipulation de tickets thermiques contenant du BPA, l'EFSA a retenu une valeur de 10% d'absorption percutanée sur 24h basée sur l'étude de Demierre *et al.* (2012). Les autres études *in vitro* montrent des taux également proches ou inférieurs à 10% dans le compartiment receveur des cellules de Frantz, alors que la fraction dans les couches de la peau se situe entre 12 % et 35 %. Pourtant cette publication présente plusieurs points faibles qui sont:

- 1- le nombre restreint de donneurs (n=2)
- 2- l'absence d'information sur les donneurs (âge, sexe, race).
- 3- l'utilisation de peau congelée alors qu'un métabolisme cutané a été rapportée par Zalko *et al.* 2011 et Marquet *et al.* 2011.
- 4- la vérification de l'épaisseur des peaux dermatomées n'est pas indiquée ; le réglage du dermatome ne donne qu'une valeur indicatrice de l'épaisseur finale de l'échantillon de peau
- 5- l'intégrité des échantillons de peau et l'absorption du BPA ont été déterminées en mesurant la radioactivité de l'eau tritiée et du [<sup>14</sup>C] du BPA ; aucune précision n'est fournie pour expliquer les mesures prises pour réduire la radio-contamination croisée.
- 6- les deux échantillons de peau donnent des résultats très comparables, ce qui ne traduit pas la forte variation inter-donneurs observée par Marquet *et al.* avec le BPA ou rapportée *ex vivo* avec de la peau humaine avec d'autres molécules (Van de Sandt *et al.*, 2004)
- 7- l'utilisation d'une solution aqueuse de BPA ne correspond pas aux conditions d'exposition liées au dépôt de BPA sur les doigts à partir de tickets de caisses
- 8- aucune précision n'est fournie quant aux conditions de dépôt de 10 µL/cm<sup>2</sup> d'une solution aqueuse contenant du BPA pour assurer un dépôt homogène sur l'ensemble de la surface de l'échantillon. Un volume de 10 µL par cm<sup>2</sup> de peau d'une solution aqueuse n'assure par une répartition homogène par simple étalement
- 9- contrairement à l'étude de Zalko *et al.* (2011) une seule concentration a été testée (193 µg/mL)
- 10 -les auteurs estiment que seulement 9.3% de la dose appliquée est bio-disponible après 24 h d'exposition. Cette estimation est basée sur le pourcentage de la dose déposée qui est présente dans le liquide récepteur (8.6%) et dans la peau (0.6% hors *stratum corneum*). La quantité présente dans le *stratum corneum* (considérée comme non biodisponible par les auteurs et non prise en compte par l'EFSA) représente 34.9 % de la dose déposée. Pour Kaddard *et al.* (2008), étude réalisée avec de la peau de porc, 10 h d'exposition, 0.7 µg, 10 µg/mL, solution aqueuse et Morck *et al.* (2010) (avec de la peau humaine, 259 µg/cm<sup>2</sup>, 4000 µg/ml, solution hydro alcoolique), la proportion de BPA présente dans le derme (8.8% et 17.2 %, respectivement) est supérieure à celle présente dans l'épiderme (5.4% et 7.4 %, respectivement).
- 11 - on peut noter que le Kp calculé à partir du flux rapporté par les auteurs de 0,022 µg/cm<sup>2</sup>/h et pour une concentration de BPA en solution aqueuse de 193,6 µg/mL est de 1,1 10<sup>-4</sup> cm/h. Cette valeur de Kp est similaire à celle déduite des données de Zalko *et al.* (2011) de 0.9 10<sup>-4</sup> cm/h ou de Morck *et al.* (2010) avec des solutions hydroalcooliques.

Le rapport de l'EFSA fait état de différences majeures entre Marquet *et al.* (2011) (3% de métabolisation) et Zalko *et al.* 2010 (73% pour le porc et 27% pour l'humain). Cependant, plusieurs

raisons peuvent justifier ces différences. Les modèles sont différents et, Zalko *et al.* ont travaillé avec 5 concentrations et ont établi une courbe de saturation en fonction de la dose de BPA, pour le modèle porcin. Sur cette courbe, la vitesse de formation du glucuronide atteint un plateau aux alentours de 8 nmoles de BPA-glucuronide formé par heure, pour la plus forte concentration (qui est équivalente à 44 µg de BPA /cm<sup>2</sup>). Or, pour Marquet *et al.*, chez le rat, c'est une concentration de 200 µg/cm<sup>2</sup> de BPA qui est utilisée. On peut donc estimer être (largement) à un plateau de formation du BPA glucuronide. Pour Zalko *et al.*, on a, pour 800 nmole déposées sur l'explant, environ 8 nmole \* 24 (heures) de métabolite formé, soit 20-25% de la dose environ. Pour Marquet *et al.*, la dose déposée sur l'explant est de 1543 nmole de BPA. Dans ces conditions, 3% de métabolite représenterait environ 46 nmole de glucuronide, soit presque 2 nmoles formées par heure. Il ne s'agit donc pas d'un rapport de 3 à 72, mais d'un rapport de 2 à 8, qui est tout à fait possible compte tenu (1) de la différence d'espèce et (2) de la taille de l'explant (2,3 fois plus grand pour Zalko *et al.*).

### **Au sujet des critiques émises sur l'étude de Zalko *et al.* (2010):**

#### Page 231 :

- Une seule concentration est citée (9568) : 5 ont été utilisées, et c'est justement pour cela que de l'EtOH a été mis dans le véhicule.
- L'étude n'est pas une étude de passage, mais une étude de métabolisme. C'est pour cette raison qu'un système permettant d'aller au-delà de 24 heures a été évalué, le point 24 heures ayant été lui-même détaillé pour ce qui est du passage.
- Il est exact que la présence d'éthanol ait pu affecter la pénétration du BPA. En revanche, il est évident que le ratio élevé de conversion en métabolites est à prendre en considération, car si la présence d'EtOH avait joué un rôle, cela aurait été par la dénaturation des protéines, dont les enzymes de métabolisation (transférases). Ce ratio de conversion, en dehors des éléments de pénétration de la molécule à travers la peau, est donc plus vraisemblablement sous-estimé que surestimé.
- Ligne 9575 (« *percentages are unclear* ») : les données (« chiffrées figurent dans la publication (nmoles), et les % peuvent donc en être facilement calculés.
- Lignes 9576-9579 : le texte se base sur les guidelines concernant l'absorption, mais les cellules de Franz classiquement utilisées pour les études de passage ne permettent pas la survie de la peau, car un écrasement de la peau se produit et donc une nécrose en bien moins de 24h ; d'où l'utilisation d'inserts. Concernant l'intégrité de la peau, celle-ci est évaluée par mesure de la PIE (perte insensible en eau) (TEWL = transepidermal water loss, en anglais) or cela nécessite une cellule étanche (genre cellules de Franz). Dans l'étude de Zalko *et al.*, cela n'était donc pas possible. A noter que cette étude fait partie d'un travail de thèse (Jacques *et al.*) ayant fait l'objet, pour le même système, d'autres publications. En particulier, dans Jacques *et al.*, Toxicol in vitro, 2010, des données détaillées démontrent l'intégrité des explants de peau utilisés avec exactement le même protocole, et ce avec des méthodes bien plus poussées que pour Demierre *et al.* Enfin, le test d'intégrité n'est réglementairement requis que dans le cas de la peau morte (OECD 428 "Although viable skin is preferred, non-viable skin can also be used provided that the integrity of the skin can be demonstrated").
- Ligne 9580 : l'étude cible le métabolisme, et donc aucune tentative d'extrapolation quantitative à l'homme n'a été faite.
- Lignes 9581-9588 : le paragraphe est confus et peu justifié. Le calcul des conditions SINK avait été fait et était, dans l'étude de Zalko *et al.* correct. Il ne faut pas oublier que ce n'est pas de l'eau qui est dans le compartiment récepteur, mais un milieu de culture, qui dissout mieux le BPA. Concernant les systèmes statiques ou dynamiques, il n'y a aucune publication qui montre à ce jour des différences significatives entre les deux types de systèmes, qui sont d'ailleurs tout deux acceptés par les guidelines sur l'absorption percutanée. Il y a un paragraphe sur ce sujet dans le *Dermal absorption du WHO*. " *static and flow-through diffusion cells are both acceptable*. " extrait de l'OECD 428. Extrait du guideline SCCS : "The

*choice of static or flow-through conditions in the receptor cell should be made on a compound-by-compound basis, depending on its theoretical absorption properties and the objective of the study*. L'hypothèse de "re-uptake" du BPA (LL 9586-9587) n'est pas basé sur des arguments tangibles scientifiquement démontrés.

**Page 495 :**

- Le texte reprend en partie les éléments de la page 231, avec les mêmes réponses.
- L18917 : plusieurs concentrations ont été utilisées dans l'étude (bis)
- L18922 : cf réponses précédentes également (bis)
- L18923 (non-conformité / OCDE 428) : il s'agit d'un guideline concernant l'étude du passage cutanée uniquement, il n'y a pas de guideline pour le métabolisme cutané à ce jour. Il est donc injustifié de conclure à des biais méthodologiques (LL18934-35) sur la base de guidelines ne concernant pas l'étude.

**Ligne 9425**

**Kaddar N, Harthe C, Dechaud H, Mappus E and Pugeat M. 2008. Cutaneous penetration of bisphenol A in pig skin. Journal of Toxicology and Environmental Health-Part a-Current Issues. 71. 471-473.**

*"The Panel consider that the study reporting is insufficient due to the omission of several methodical details including the applied surface density ( $\mu\text{g}/\text{cm}^2$ ) and the skin thickness. "*

Les autres omissions méthodologiques sont

- 1- Le nombre d'animaux utilisés n'est pas donné
- 2- Les échantillons de peau sont congelés
- 3- Pas de vérification de l'intégrité des échantillons de peaux
- 4- Le BPA est déposé en solution aqueuse, ce qui ne correspond pas aux conditions d'exposition aux tickets de caisse
- 5- Pas de précision sur la méthode de dosage du BPA contenu dans les échantillons de peau et le rendement d'extraction du BPA
- 6- Pas de mesure de la radioactivité présente dans les échantillons de peau après exposition (mais uniquement le BPA inchangé dosé par CLHP), ce qui aurait permis d'expliquer la décroissance du taux de récupération de la dose déposée en fonction du temps d'exposition (98,2 à 84,3%).

L'hypothèse des auteurs que la décroissance du taux de récupération de la dose déposée soit due à une dégradation du BPA en solution aqueuse est peu probable. On ne peut donc exclure que le taux de pénétration reportée par les auteurs soit sous estimée

*L i g n e 9 4 4 1 " As for data interpretation, the percutaneous penetration value of 4.1% is in line with the value of 8.6% reported by the high quality study of Demierre et al. (2012).*

La valeur de 4.1 % n'est pas mentionnée dans l'article dans la section *Results*, il est écrit « *the proportion found in the receptor fluid varied from 0 to 5 % over time (0-24 h) (figure 1)* »

Cette valeur de 4.1% ou 5% correspond à la quantité tel que rapportée par l'EFSA « *percutaneous absorption* »

*L i g n e 9 4 4 1 " As for data interpretation, the percutaneous penetration value of 4.1% is in line with the value of 8.6% reported by the high quality study of Demierre et al. (2012).*

Malgré les insuffisances de report méthodologiques soulignées par le Panel ce dernier retient la valeur de 4.1 % pour la comparer favorablement au taux d'absorption en 24 h de 8.6 % rapportée par

Demierre *et al.* 2012. En revanche, la différence de répartition de la quantité de BPA dans le derme et l'épiderme entre les deux auteurs n'est pas commentée. Ainsi, Demierre *et al.* indiquent que 34.9 % de la dose de BPA est présent au niveau du *stratum corneum* et 0.6 % de la dose de BPA au niveau du reste de la peau. En revanche pour Kaddar *et al.* 2010, quelque soit le temps d'exposition la quantité présente dans le derme est supérieure à la quantité présente dans l'épiderme. Demierre *et al.* et Kaddar *et al.* ont utilisé tous les deux des solutions aqueuses de BPA (193,6 µg /mL et de 10 µg/mL, respectivement), des quantités déposées qui semblent du même ordre de grandeur, de 1.83 µg/cm<sup>2</sup> et de 0.7µg/cm<sup>2</sup>), des échantillons de peau congelée (humain et porc, respectivement).

#### Ligne 9443

**Marquet F, Payan JP, Beydon D, Wathier L, Grandclaude MC and Ferrari E, 2011. In vivo and ex vivo percutaneous absorption of [14C]-bisphenol A in rats: a possible extrapolation to human absorption? Archives of Toxicology, 85, 1035-1043.**

*Ligne 9456 "experiments with 15 non-viable human skin sections".*

En raison de la forte variation entre les peaux des différents donneurs il est plus important d'inquer le nombre de donneurs (n=6) que le nombre de sections utilisées (n=15). Dans cette étude des peaux provenant de 6 donneurs ont été utilisées, chaque peau ayant fourni 2 à 3 sections différentes. L'utilisation d'un grand nombre de donneurs différents (par rapport aux publications de Demierre *et al.* (n=2) et Zalko *et al.* (n=3) a permis de mettre en évidence une forte variation des flux d'absorption du BPA entre les donneurs.

*Ligne 9457 "The quotient of maximum percutaneous flux and vehicle concentration yielded a permeability coefficient of  $3.0 \times 10^{-5}$  cm/h which was 3.7-fold lower than in Demierre et al. (2012) but still comparable given the differences in vehicle type, surface density, and diffusion-cell design"*

Le calcul de Kp à partir de la concentration de BPA dans l'acétone a peu de sens. L'expérimentation a été réalisée en mode non occlusif. L'acétone (50 µL/cm<sup>2</sup>) a été évaporée immédiatement après le dépôt. Durant toute la durée de l'exposition, les échantillons de peau sont en contact avec du BPA solide et/ou du BPA dissous dans le film lipidique du *stratum corneum*. Une valeur de Kp ne peut être calculée que si la concentration de BPA dissoute par le sébum au niveau du *stratum corneum* est connue.

*Ligne 9467 "In addition, inter- and intra-individual variability of up to tenfold was observed in humans".*

L'utilisation d'un plus grand nombre de peaux de donneurs différents (n=6) par rapport à Zalko *et al.* (n=3 ?) et Demierre *et al.* (n=2) a permis de mettre en évidence une forte variation du flux d'absorption inter-individu. Cette variation étaye l'utilisation d'un facteur de sécurité de 10, retenu par l'EFSA, pour tenir compte de la forte variation des paramètres toxicocinétiques inter individus.

*Ligne 9468 "The skin clearance rate following exposure was estimated at 0.4 µg/cm<sup>2</sup>/h."*

C'est le seul résultat expérimental qui montre clairement que la peau constitue un réservoir pour le BPA. Près de 80 % du BPA présent dans la peau après la fin d'une exposition, la fraction non absorbée de BPA avec de l'éthanol (31 µg/cm<sup>2</sup>) est susceptible de diffuser dans le liquide récepteur.

#### Ligne 9517

**Mørck TJ, Sorda G, Bechi N, Rasmussen BS, Nielsen JB, Ietta F, Rytting E, Mathiesen L, Paulesu L and Knudsen LE, 2010. Placental transport and in vitro effects of Bisphenol A. Reprod Toxicol, 30, 131-137.**

*Ligne 9517 "Mørck et al. (2010) used a static Franz diffusion cell and analyzed non-viable human skin from breast- surgery patients according to the OECD TG 428. Full thickness skin (800–1000 µm) was used, and the skin integrity was checked by capacitance measurements. A diluted ethanol solution was used as vehicle, and <sup>14</sup>C-BPA was applied in a high concentration of 4000 mg/l, resulting in a surface density of 259 µg/cm<sup>2</sup>."*



La plupart des informations méthodologiques présentées par l'EFSA ne sont pas mentionnées dans le texte de la publication de Mork *et al.*, 2010. L'EFSA aurait utilisé les informations provenant d'une publication antérieure (Nielsen *et al.* 2009.) Seul est indiqué dans l'article: " *the skin was exposed to 17,5 mM BPA for 48 h in the donor chamber*". Aussi, dans son rapport le SCENIHR (2014) indique que "17,5 mM corresponding to 3,99 g/L, inconsistent with BPA solubility of 120-300 mg/L water at 25 °C (EFSA 2010)". Alors que l'information « *a diluted ethanol solution* » rapportée par l'EFSA explique la concentration élevée de BPA utilisée

Les autres omissions méthodologiques non mentionnées par l'EFSA sont : le nombre de donneurs, les caractéristiques des donneurs.

D'après les précisions méthodologiques fournies, par l'EFSA, on peut estimer une valeur de Kp égale à  $1.75 \cdot 10^{-4}$  cm/h (13% dans le liquide récepteur en 48 h /  $48 \cdot 259 \mu\text{g}/\text{cm}^2/4000 \mu\text{g}/\text{mL}$ ). Cette valeur est très comparable à celles obtenues par Demierre *et al.* ( $1.1 \cdot 10^{-4}$  cm/h) et Zalko *et al.* ( $0.9 \cdot 10^{-4}$  cm/h).

La quantité dans le derme est 3 fois supérieure à l'épiderme contrairement à Demierre *et al.*, 2012.

*Ligne 9526 "Percutaneous penetration was 13.0%." C'est l'absorption qui est de 13 %, quantité dans le liquide récepteur. La quantité pénétrée est de  $13+17,2 = 30,2$  % en excluant la fraction présente dans l'épiderme.*

#### Ligne 9560

**Zalko D, Jacques C, Duplan H, Bruel S and Perdu E, 2011. Viable skin efficiently absorbs and metabolizes bisphenol A. Chemosphere, 82, 424-430.**

*Ligne 9576 "The Panel noted several methodical flaws in the first experimental phase, e.g., use of cell culture inserts as diffusion cells, missing skin integrity check, exposure times largely exceeding 24 h, 33% ethanol solution as vehicle, which negatively impact the reliability of these estimates for in vitro skin absorption."*

L'ensemble des remarques faites par le Panel ne semblent pas avoir eu d'impact sur la détermination du flux d'absorption du BPA. Le Kp calculé à partir des données expérimentales rapportées par Zalko *et al.* (2011) est de  $0.9 \cdot 10^{-4}$  cm/h (peau humaine, fraîche, 45.4 % de la dose dans le liquide récepteur, absorption qui croît linéairement avec la dose cf. figure 3),  $2.75 \mu\text{g}/\text{cm}^2$  déposée sous forme d'une solution hydro-alcoolique à 33 % ayant une concentration de 50 nmole de BPA pour 60  $\mu\text{L}$  : 190  $\mu\text{g}/\text{mL}$ ). This Kp value is the same as the value obtained with Demierre *et al.* 2012 qui ont utilisée une solution aqueuse de BPA ( $1.1 \cdot 10^{-4}$  cm/h) ou de Morck *et al.* (2010) qui ont utilisé également une solution hydro-alcoolique ( $1.75 \cdot 10^{-4}$  cm/h).

Ainsi, la remarque de l'EFSA concernant l'utilisation d'une solution hydroalcoolique "33% ethanol solution as vehicle, which negatively impact the reliability of these estimates for in vitro skin absorption." ne semble pas être fondée dans le cas du BPA

Un résultat important, non mentionné par l'EFSA, a été rapporté par Zalko *et al.* Pour 5 solutions hydroalcooliques de BPA, l'absorption percutanée ainsi que la quantité présente dans la peau de porc à la fin de l'expérimentation est proportionnelle à la dose déposée ( $2.75 \mu\text{g}/\text{cm}^2$  à  $44 \mu\text{g}/\text{cm}^2$ ). La similitude de comportement d'une solution de BPA à 190  $\mu\text{g}/\text{mL}$  avec de la peau de porc et de la peau humaine plaide en faveur d'une transposition des résultats entre ces deux espèces.

## ANNEE 6 BMD MODELLING

Tableau 2 : Exemple de choix du niveau de réponse (BMR) en fonction du calcul des intervalles de confiance.

Parameter	Male F0	F1 males	Female F0	Female F1
Mean	0.380200	0.361100	0.306300	0.321700
SD	0.041158	0.052655	0.047893	0.038564
SEM	0.005500	0.007100	0.006400	0.005200
N	56	55	56	55
90% CI	0.370998 to 0.389402	0.349218 to 0.372982	0.295593 to 0.317007	0.312997 to 0.330403
95% CI	0.369178 to 0.391222	0.346865 to 0.375335	0.293474 to 0.319126	0.311275 to 0.332125
99% CI	0.365525 to <b>0.394875</b>	0.342143 to <b>0.380057</b>	0.289223 to <b>0.323377</b>	0.307816 to <b>0.335584</b>
BMR correspondant (IC99%- mean)/mean	3.8%	5%	5.5%	4.6%

Mean, moyenne arithmétique du poids du rein gauche en g de l'étude de Tyl et al 2008 chez les animaux témoins

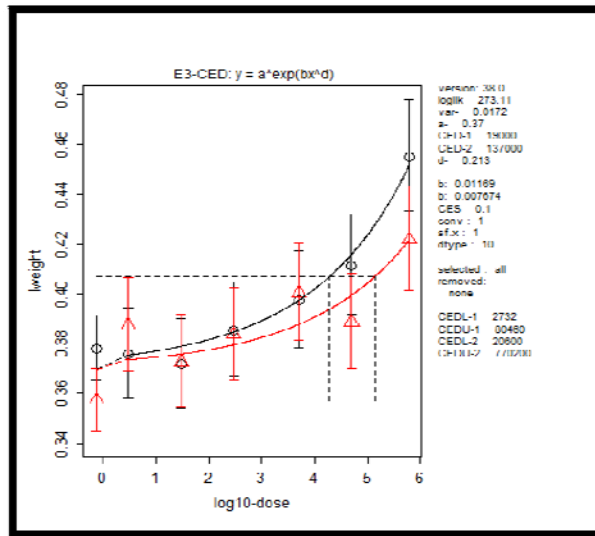
SD, écart type ; SEM, erreur standard à la moyenné

N, nombre d'animaux

CI, Intervalle de confiance

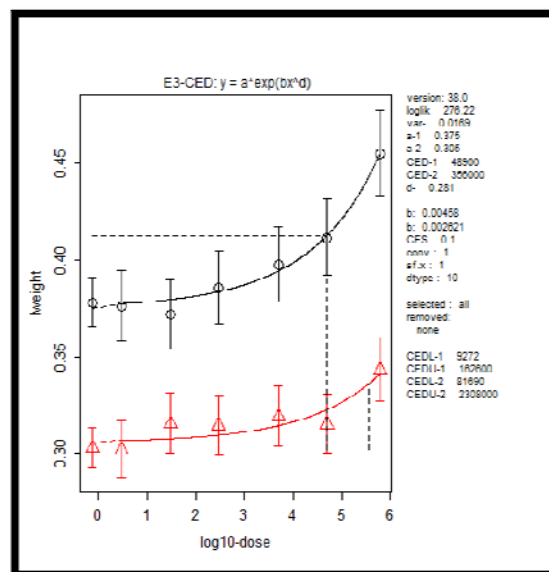
Le même calcul de BMR en prenant l'IC 95 % aboutit à des BMR < à 5%. Un BMR de 5% tel que préconisé par l'EFSA se justifie donc, si l'on considère comme niveau de réponse jugé comme anormale, toute valeur de poids dépassant la limite supérieure de l'intervalle de confiance du groupe témoins.

Exemple : comparaison des couples BMD et BMDL en fonction de la covariable (femelle F0 ou male F1)



Détermination de la BMD (CED) et BMDL(CEDL) pour un BMR de 10% avec comme covariable les males F1 : en noir F0, en rouge F1

La BMDL est de 2732  $\mu\text{g}/\text{kg}/\text{j}$ , la BMD est de 19000  $\mu\text{g}/\text{kg}/\text{j}$ . Soit des résultats comparables à ceux réalisés par l'EFSA et avec un ratio BMD/BMDL <7.



Détermination de la BMD (CED) et BMDL(CEDL) pour un BMR de 10% avec comme covariable les femelles F0 : en noir male, en rouge femelle

La BMDL est de 9272  $\mu\text{g}/\text{kg}/\text{j}$ , la BMD est de 48900  $\mu\text{g}/\text{kg}/\text{j}$ , soit des résultats comparables à ceux rapportés par l'EFSA et avec un ratio BMD/BMDL <6.