The Director General

Maisons-Alfort, 7 August 2015

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

regarding the solicited request 2015-SA-0001

"Analysis of the results of the recent study published in Toxicological Sciences (Delclos *et al.*, 2014) on the toxicity of Bisphenol A (BPA) administered by gavage to Sprague Dawley rats exposed from gestation day 6 to postnatal day 90"

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

Its opinions are made public.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 7 August 2015 shall prevail.

On 23 December 2014, ANSES issued an internal request to undertake the following expert appraisal: "analysis of the results of the recent study published in Toxicological Sciences (Delclos *et al.*, 2014) on the toxicity of Bisphenol A (BPA) administered by gavage to Sprague Dawley rats exposed from gestation day 6 to postnatal day 90".

1. BACKGROUND AND PURPOSE 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[1] (R3) reprotoxic (according to Directive 67/548/EEC[2]) and/or endocrine disrupting (ED) substances found in consumer products marketed in France. This expert appraisal covered the general population, including vulnerable

[1] 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.

[2] Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances

populations and people in the workplace handling so-called 'consumer' products in the context of their professional activity (excluding manufacture, processing, distribution and disposal).

In this context, in 2013, ANSES published an Opinion "on the assessment of the risks associated with bisphenol A for human health, and on toxicological data and data on the use of bisphenols S, F, M, B, AP, AF, and BADGE" regarding the risks to human health related to bisphenol A (BPA) taking into account not only exposure related to consumer goods but also exposure related to other media (drinking water, food, house dust, air). This Opinion presented the expert appraisal work undertaken by a Working Group on Endocrine disruptors and category 3 reprotoxic substances (ED WG) set up 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 Assessment of the risks related to chemical substances in February 2013 (ANSES, 2013).

In the ANSES expert appraisal report published in March 2013 on the risks to human health related to BPA, the experts recommended monitoring the results of the work undertaken under the supervision of the US National Toxicology Program (NTP), with funding from a joint Food and Drug Administration/National Institute of Environmental Health Sciences (US FDA/NIEHS) programme, in order to revise the conclusions of the ANSES expert appraisal where appropriate. The article published by Delclos *et al.* in February 2014 entitled "*Toxicity evaluation of Bisphenol A administered by gavage to Sprague Dawley rats from gestation day 6 through postnatal day 90*" briefly summarises the results of the US FDA/NCTR 2013 study.

The US FDA/NCTR 2013 study was a large-scale animal study on continuous exposure to BPA from gestation day 6 (GD6) to postnatal day 90, when a number of animals were euthanised and tissues were harvested. It complied with the criteria of Good Laboratory Practice (GLP) and was conducted in accordance with the specific guidelines of the NTP. The study included a wide range of tested doses (2.5 – 8 – 25 – 80 – 260 – 840 – 2,700 – 100,000 – 300,000 µg/kg bw/day) administered by gavage using a stomach tube (mothers or pups after 5 days) or orally for newborns from the first day after birth. This descriptive study relied primarily on the observation of overall morphological-histological and biochemical criteria. Two positive control groups for the characterisation of oestrogenic effects were included in the study: treatment with 0.5 and 50 µg ethinyl œstradiol (EE2) per kg bw/day. Two negative control groups were also included (vehicle[3] and without treatment).

ANSES decided to issue an internal request to analyse this study, in particular to assess whether the new data of the US FDA/NCTR 2013 study were likely to influence the conclusions of the ANSES collective expert appraisal of 2013. Furthermore, this study was evaluated by EFSA (EFSA, 2015 opinion). The data of Churchwell *et al.*, 2014 supplement the US FDA/NCTR 2013 study as regarding the toxicokinetics of BPA. This publication was also submitted for evaluation by the expert rapporteurs appointed by ANSES.

2. ORGANISATION OF THE EXPERT APPRAISAL

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

It falls within the sphere of competence of the Expert Committee (CES) on Substances. ANSES entrusted the expert appraisal to several expert rapporteurs in the Working Group on Endocrine

[3] Vehicle control corresponding to the negative control group that received the vehicle or diluent.

disruptors and category 3 reprotoxic substances (ED WG) with toxicological expertise, in particular experts specialising in effects on the mammary gland, the male and female reproductive systems and metabolic diseases. The methodological and scientific aspects of the work were presented to the CES on 11 June 2015. They were adopted by the CES on Substances on 30 June 2015. Each expert was mandated to assess a specific part of the US FDA/NCTR 2013 report on bisphenol A.

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.

The experts' declarations of interests are made public on ANSES's website (www.anses.fr).

The results of the expert appraisal presented below take into account the experts' comments. They deal with specific points of the US FDA/NCTR 2013 report, which was also submitted and evaluated by EFSA (EFSA 2015 opinion). The data of Churchwell *et al.*, 2014 supplementing the US FDA/NCTR 2013 study as regarding the toxicokinetics of BPA were also submitted for evaluation by the expert rapporteurs appointed by ANSES. These new data have been analysed since they are likely to influence the interpretation of the results given in the US FDA/NCTR 2013 report.

This Opinion is not intended to give a comprehensive expert assessment of the health effects of BPA. Its purpose is to compare the results of the US FDA/NCTR 2013 study with the expert appraisal work on BPA previously undertaken by ANSES.

Thus, the issues raised in the context of this expert appraisal are as follows:

- regarding the identification of the human health effects of BPA:

According to the approach for the classification of the human health effects of BPA as presented in the expert appraisal reports of ANSES (2011, 2013), the following effects were considered recognised and were used for the risk assessment of BPA.

- Effects on the mammary gland
- Effects on the female reproductive system
- Effects on metabolism and obesity
- Effects on the brain and behaviour

In light of the results of the US FDA/NCTR 2013 report, should these effects still be taken into account for the HRA of BPA?

- regarding dose-response relationships and the choice of the toxicological value to be used for the risk assessment:

In light of the results of the US FDA/NCTR 2013 study, and particularly the effects observed on the mammary gland and female reproductive system, are the critical doses selected in the ANSES expert appraisal (2013) for deriving toxicological values used for the HRA still appropriate?

- regarding the low-dose effects of BPA:

In the US FDA/NCTR 2013 study, the animals were orally exposed to a wide range of BPA doses (from 2.5 to 2,700 µg/kg/day) and were compared to two negative control groups of rats that had not been treated with BPA. However, blood levels of BPA in rats from the various groups showed that 'negative control' rats had been exposed to BPA. These results are broken down in the study by Churchwell *et al.*, 2014.

In this context, how should these observations be taken into account when interpreting the results of the US FDA/NCTR 2013 report?

3. ANALYSIS AND CONCLUSIONS OF THE CES ON SUBSTANCES AND THE WORKING GROUP ON ENDOCRINE DISRUPTORS

This study is unique in that it characterises internal exposure (conjugated and aglycone BPA) in the serum of animals. However, the reported data are difficult to interpret, in particular due to the detection of BPA in negative control animals (a predictable situation for a ubiquitous substance such as BPA that likely occurs in all such types of studies). The internal exposure data reported in this study and published by Churchwell *et al.,* 2014 thus raise questions when it comes to distinguishing between the negative control animals and the animals exposed to the lowest doses and ultimately to interpreting the effects at low dose levels. These observations suggest that the colony from which the mothers had been obtained was constantly exposed to BPA, possibly through cages, bottles and food; it cannot be ruled out that this exposure may have caused or hidden an effect. Despite this, it should be noted that background levels were very seldom monitored in all of the studies taken into account by the ED WG during its previous expert appraisal (ANSES, 2011-2013). It cannot be ruled out that this same finding may apply to these other studies.

3.1. Analytical aspects

The analytical aspects of the methodology for measuring levels of internal exposure to BPA, reported in the publication by Churchwell *et al.*, 2014, were taken into account by the experts.

Since the authors of the article reported levels of BPA in control animals, the interpretation regarding the lack of observed effects for low-dose exposure raised some questioning among the experts. The word 'contamination' is not necessarily the most appropriate to characterise this detection of residual BPA among negative controls. In the US FDA/NCTR 2013 study, as in all studies on this substance, these are in fact background levels, or baseline levels, that are difficult to eliminate below a certain limit. This is one of the known and expected characteristics of such a ubiquitous substance. The challenge raised is thus the objective characterisation of these background levels and the consequences of their interpretation. However, this study deserves credit for measuring them, which should not be negatively interpreted, considering how seldom they have been taken into account in other studies of the same type.

Moreover, the experts underline the efforts made in this study to monitor and characterise these 'background levels' of BPA. The authors provide details about the measures taken when conducting the study, the decision-making rules implemented based on this characterisation and the qualities of the analytical method. However, the management of 'blank' values is not always detailed in the article by Churchwell *et al.*, 2014.

From the experts' point of view, the analytical methodology, the quality of the data produced and the method used to report the results thus appear adequate and sound. That said, it is difficult to assess the validation data in detail due to the successive referencing of documents in which these analytical aspects are described. This generates a lack of visibility as to the uncertainty associated with each measured value, particularly for the lowest doses. The most limiting critical point of this study by Churchwell *et al.*, 2014 is the variability of the results, which directly impacts the interpretation of significant differences between the various sub-groups of animals, in particular between the negative controls and the first three doses (i.e. 2.5 - 8 and 25 µg/kg bw/day). Actual exposure levels were not compared with the values of the control groups. This is not a case of analytical limits for this study but rather of difficulties interpreting the low-concentration results in relation to the baseline levels observed in the negative controls.

In conclusion, according to the information provided in the study by Churchwell *et al.*, 2014, the difference between the concentrations measured in the negative control animals and those in the animals exposed to the three lowest doses was not statistically analysed by the authors, which limits the interpretation of toxicological effects at these low doses.

3.2. Statistical data analysis

Statistical analyses of the lesions recorded by histopathology on postnatal days 21 and 90 were undertaken with the Poly-K test (K set at 3). The Poly-K test is a method based on the Cochran-Armitage trend test that takes into account the suspected effect to increase statistical power. Following the Poly-K test, two other statistical methods were used to analyse the severity scores of the lesions: (i) a test combining the Jonckheere-Terpstra test (Jonckheere, 1954; Terpstra, 1952) and the Shirley-Williams test (Shirley, 1977; Williams, 1986) and (ii) the RTE test (nonparametric relative treatment; Brunner, Domhof, & Langer, 2002).

The Technical University of Denmark (DTU, 2015) considered that the statistical analyses proposed by Delclos *et al.* (2014) were acceptable but that the choices made were too conservative. Thus, a new analysis of the data on 'mammary gland hyperplasia' on postnatal day 90 was undertaken. Only females with a lesion severity score ≥ 2 were included in the analysis, and the 'naive control[4]' and 'vehicle control[5]' groups were grouped together. With this new analysis, 0the DTU concluded there was a statistically significant increase in the number of females with hyperplasia of the mammary tissues at the dose of 80 µg/kg bw/day of BPA, but no dose-response relationships were observed. The lack of clear dose-response relationships at low doses may be related to limited sensitivity at low doses and/or a false-positive result.

The experts consider that to increase statistical power (and thus reduce the rate of false-negatives), it would be advisable to group together the controls, when it can be assumed that the vehicle has no effect on the measured parameter. However, this statistical test strategy, i.e. undertaking seven independent statistical tests to compare the control group and each tested dose, can lead to false positives (0 to 2 false-positive results for a binomial distribution where p=0.05, n=7 and 5% alpha risk) with no correction of p values (for example, the Bonferroni correction to offset error in all statistical tests).
Thus, it is not possible to know whether the statistically significant effect at the dose of 80 µg/kg bw/day found in the new analysis undertaken by the DTU is due to a false positive or an increase in statistical power.

[4] Naive control corresponding to the negative control group that did not receive the vehicle or diluent.
[5] Vehicle control corresponding to the negative control group that received the vehicle or diluent.

In conclusion, the new analysis undertaken by the DTU does not call into question the initial statistical analysis undertaken by the US FDA/NCTR 2013. The experts consider that the statistical analysis tools used by Delclos *et al.,* 2014 are suitable.

3.3. Effects on the mammary gland

The effects of chronic exposure to various doses of BPA (2.5 – 2,700 µg/kg bw/day and higher doses of 100,000 - 300,000 µg/kg bw/day) from gestation day 6 (GD6) were analysed for the mammary glands of male and female rats in the juvenile stage (weaning, corresponding to postnatal day 21), to view the effects of neonatal exposure, and in adulthood (postnatal day 90), i.e. in a period of genital activity.

The US FDA/NCTR 2013 study does not use the terminology or methodological criteria typically used to analyse the effects of endocrine disruptors on the mammary gland, which were also used in the studies identified by ANSES in 2013. Therefore, it is not possible to directly compare the results of this study with the critical effects taken into account in the key studies identified previously by ANSES in 2013.

Traditionally from a methodological standpoint, effects on architecture and morphometry are observed at weaning (postnatal day 21) in the 4th or 5th mammary gland using the 'Whole Mount' technique combined with a histological examination. In adulthood (postnatal day 90), the analysis preferably focuses on the histological evaluation of ductal hyperplasia and in some cases of lobulo-alveolar hyperplasia. Thus, 'Whole Mount' analysis provides a better assessment, particularly due to greater sensitivity for the quantification of structures relating to mammary development and on account of the presence of the whole gland (compared to analysis of a histological section, Davis and Fenton, 2013). This standard procedure was not considered in the US FDA/NCTR 2013 report. There are other differences regarding the definition of ductal hyperplasia. In fact, the term ductal hyperplasia used in the US FDA/NCTR 2013 study, on postnatal days 21 and 90, is defined by the authors as a "relative increase in the number of branching ducts and alveolar buds per unit area" whereas the definition considered by ANSES in 2013 is based on an increase in the number of epithelial cell layers in the ducts (Durando *et al.*, 2007; Murray *et al.,* 2007*).*

Moreover, no microscope views illustrate 'ductal hyperplasia' lesions in histological sections in the US FDA/NCTR 2013 report. This US FDA/NCTR 2013 report does not mention any proliferation analyses. Lastly, no analyses are undertaken on postnatal day 50, a key period of mammary gland development (stage of maturity that marks the end of puberty), with optimum sensitivity to carcinogens.

Thus, according to the experts, the analysis of the US FDA/NCTR 2013 study, which was limited to histological sections (with only one section/gland), does not indicate a 'ductal hyperplasia' effect, as defined by the authors, but rather changes in mammary morphogenesis.

On postnatal day 21, a trend test was undertaken in females at the doses of 2.5 to 2,700 µg/kg bw/day. This revealed statistically significant 'ductal hyperplasia' in the groups treated with the doses of 2,700 µg BPA/kg bw/day and 100,000 µg/kg bw/day with a minimum degree of severity. In the positive control group, EE2 did not have any effects. BPA did not have any effects on terminal end bud (TEB) density. Conversely, in males, an effect of EE2 was observed but no effects of BPA on ductal hyperplasia, as defined by the authors, were found.

On postnatal day 90 (young adult stage), a significant increase in the incidence of 'ductal hyperplasia' was reported in females only at the highest dose of 300,000 µg/kg bw/day compared to the negative control group ''that received the vehicle". However, the experts note that, while the incidence of ductal lesions was not increased at low doses of BPA compared to the control group,

the severity score was higher particularly at 80, 260, 2,700 and 100,000 µg/kg bw/day. These observations could be taken into account in the risk assessment of BPA.

In females, an adenocarcinoma was reported at 2.5 µg BPA/kg bw/day. This observation is consistent with the results of the study by Acevedo *et al.* 2013. However, in order for it to be considered as the possible result of an effect of BPA, it is necessary to determine the incidence of adenocarcinomas in Sprague-Dawley animals in the historical control group for the strain used.

In this study, treatment with EE2 in males led to mammary, ductal (increased incidence and severity) and alveolar hyperplasia, at the two EE2 doses (compared to the control group) of 0.5 and 5 µg EE2/kg bw/day. Furthermore, the effects of BPA observed at the doses of 2.5 and 840 µg BPA/kg bw/day showed a significant incidence of ductal hyperplasia.

Conclusion of the expert appraisal
The aim of the US FDA/NCTR 2013 study was to characterise the effects of BPA, particularly at low doses, on certain morphological parameters of the mammary gland on postnatal days 21 and 90. The large number of BPA doses, and internal dosimetry of BPA, are the fundamental points of this study.

However, there are a number of methodological uncertainties (which, in particular, limit the sensitivity of this study: lack of a Whole Mount analysis, no sampling on postnatal day 50, no microscope views, etc.) that make it difficult to assess the doses from which BPA causes hyperplastic ductal lesions.

3.4. Effects on the female reproductive system

This descriptive study relied primarily on the use of overall morphological-histological criteria. Endometrial (in particular hyperplasia, metaplasia, cysts) and ovarian (cysts, follicle count) abnormalities were screened for through histological evaluation. Hormone levels were also recorded from a single blood sample on postnatal day 80 in females in oestrus. The time of vaginal opening was determined. Oestrous cycles were monitored based on daily cytological analyses of vaginal smears from postnatal day 69 to postnatal day 90, and from postnatal day 150 to postnatal day 170 (5 cycles/animal on average). These effects were systematically observed only at the two highest doses (100,000 and 300,000 µg/kg bw/day) and overall were similar to those observed with EE2.

However, a trend test suggested a statistically significant treatment effect for all of the lowest doses (from 2.5 to 2,700 µg/kg bw/day) for oestrous cycle abnormalities, in particular extended dioestrus between postnatal days 69 and 90. Occasionally, the incidence of certain diseases was higher in isolated groups (e.g. a significant increase in the incidence of cystic endometrial hyperplasia in the group treated with 8 µg/kg bw/day).

The morphological-anatomical criteria used in the US FDA/NCTR 2013 study to characterise abnormalities of the female genital system are general and less specific than those described in the studies taken into account in ANSES's previous expert appraisal, which included for example an in-depth morphometric analysis confirmed by the expression of apoptosis and/or cell proliferation biomarkers.

Conclusion of the expert appraisal
On account of methodological uncertainties, the use of less specific criteria than in the key studies previously identified by the experts, and uncertainties related to levels of BPA in untreated animals and/or exposure in F0 dams, the experts consider that as things stand, the US FDA/NCTR 2013

study does not call into question the experts' conclusions regarding the effects of BPA on the female reproductive system (ANSES 2011, 2013).

3.5. Effects on metabolism and obesity

Although the US FDA/NCTR 2013 study did not specifically deal with metabolism and obesity as such, the following parameters were studied: weight changes of the exposed animals, levels of thyroid hormones (on postnatal days 15 and 90), cholesterol, serum triglycerides, glucose, insulin and leptin. The US FDA/NCTR 2013 study concludes that BPA has no effects on the assessed parameters for doses ranging from 80 µg/kg bw/day to 2,700 µg/kg bw/day, with the exception of an increase in AST (aspartate aminotransferase) in females on postnatal day 90 for the dose of 2,700 µg/kg bw/day; this effect was not found at higher doses (100,000 and 300,000 µg/kg bw/day).

Two of the studied doses are same as those in the study by Miyawaki *et al.*, 2007 (260 and 2,600 µg/kg bw/day), used as a key study in the ANSES expert appraisal (2013). But these two studies differ on many methodological points, which may explain why BPA had no effects on metabolism at these two doses. These differences involve the animal model (rats in the US FDA/NCTR 2013 study and mice in the study by Miyawaki *et al.*, 2007), the exposure route, the administration mode and vehicle used (gavage *versus* drinking water), the exposure period (post-coitum day 6 to postnatal day 90 *versus* post-coitum day 6 to postnatal day 30), age of examination (adult *versus* juvenile stage), and diet (standard diet *versus* high-fat diet (30% kcal)). Furthermore, the animals were subject to fasting from the day before the study for Miyawaki *et al.*, 2007, whereas this indication is not given in Delclos *et al.*, 2014.

Not all of these differences in terms of protocol were found in the recent study by Wei *et al.*, 2014. In this study (single dose – 50 µg/kg bw/day), the authors used Wistar rats with gavage, and for part of the study, the animals were fed a standard diet. However, in the study by Wei *et al.*, 2014, gavage was used only for mothers during gestation and lactation (early exposure) and was not continued through to adulthood (postnatal day 90). Moreover, the animals were studied at a more mature stage (on postnatal day 189, not postnatal day 90).
The study by Wei *et al.*, 2014 concluded that BPA had an obesogenic effect affecting hepatic homeostasis and insulin levels; these effects were exacerbated when animals were fed a fatty diet *versus* a standard diet after weaning.

Conclusion of the expert appraisal

Despite a large number of animals and doses in the US FDA/NCTR 2013 study, it is not possible to conclude as to metabolic effects in the exposed animals based on the results. As things stand, the US FDA/NCTR 2013 study does not call into question the experts' conclusions regarding the effects of BPA on metabolism and obesity.

3.6. Effects on the brain and behaviour

No specific parameters relating to effects on the brain and behaviour were included in the US FDA/NCTR 2013 study.

3.7. Other types of effects

3.7.1.Effects on the male reproductive system

ANSES's previous collective expert appraisal (2013) concluded that the data in the literature regarding the effects of BPA on male reproductive development and functions were diverging and ANSES did not identify any critical effects related to male reproductive function for the HRA.

New data are provided in the US FDA/NCTR 2013 report. A possible impact of low doses of BPA on spermatogenesis was observed (decrease in relative testicular weight at 260 µg/kg bw/day, seminiferous tubule 'giant' cells in all the groups treated with BPA whereas no negative control animals had this type of cell. These cells were also observed in the group treated with 5 µg/kg bw/day of EE2, but not in the group treated with 0.5 µg/kg bw/day of EE2). However, due to the methodology used, it is not possible to rule out or confirm an effect on spermatogenesis. The detection of such an effect requires an in-depth morphometric analysis of testicular histology, which was not undertaken in this study.

Furthermore, the US FDA/NCTR 2013 study does not provide any new data regarding the low-dose effects of BPA on anogenital distance at birth or in adults, on the weight of reproductive organs or on sperm characteristics.

This study shows that BPA had no effect on age at the onset of puberty (assessed by preputial separation) but does not indicate whether it had an impact on delayed testicular descent due to a one-off effect at the dose of 260 µg/kg bw/day.

Although the US FDA/NCTR 2013 report concludes that BPA has no effect on testosterone levels in adults, it is noted that the trend test is statistically significant. Thus, this report does not contradict the conclusions of the many previous studies showing an anti-androgen effect of BPA.

Conclusion of the expert appraisal

The results given in the US FDA/NCTR 2013 study do not indicate whether or not low doses of BPA have an effect on male reproductive functions, other than showing that BPA does not affect age at puberty.

As things stand, the US FDA/NCTR 2013 study does not call into question the experts' conclusions regarding the effects of BPA on the male reproductive system.

3.7.2.Renal effects

In its most recent opinion, EFSA (EFSA, 2015) proposed a temporary Tolerable Daily Intake (t-TDI) of 4 µg/kg bw/day. This temporary TDI was based on the results of the study by Tyl *et al.* (2002, 2008), and particularly on the renal effects of BPA (increase in relative kidney weight in mice).

In the US FDA/NCTR 2013 study, an increase in the incidence of tubular cysts was observed in F1 females at all doses above 25 µg/kg bw/day on postnatal day 90. However, the study does not indicate whether there was an effect related to treatment with low doses. At high doses, a dose-dependent effect was found, at 100,000 and 300,000 µg/kg bw (with respective incidence levels for tubular cysts of 8/21 and 12/19, *versus* 6/20 in the control animals).

At biochemical level, an upward trend for creatinine at the lowest doses in the BPA range used was reported only in males, with a significant increase in creatinine at the highest dose of BPA.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety agrees with the observations made by the rapporteurs of the Working Group on Endocrine disruptors and category 3 reprotoxic substances, further to the analysis of the US FDA/NCTR 2013 report on BPA.

The US FDA/NCTR 2013 study has remarkable characteristics in particular due to the use of a large number of animals and doses and on account of the efforts made to define exposure. This study raises methodological issues described by the experts for each type of effect. In particular, the issue of effects observed at low doses making it difficult to distinguish between exposure and environmental background levels is a methodological point raised by the experts due to the ubiquitous nature of BPA.

Further to their analysis, the experts consider that the data reported in the study and its initial results pertaining to the effects taken into account in the ANSES collective expert appraisal (ANSES, 2013) do not call into question the selected NOAELs/LOAELs (see Table 1 – Annex 1) or the Agency's conclusions in 2013. As things stand, there is no need, further to the analysis undertaken by the experts, to change the NOAEL value of 25 µg/kg bw/day proposed by ANSES in 2013 (based on the study by Moral *et al.*, 2008). The long-term exposure results (2-year study) expected following the US FDA/NCTR 2013 study should provide useful information improving the interpretation of this study's results, such as the occurrence (or not) of ductal carcinoma *in-situ* lesions and mammary gland adenocarcinomas.

The French Agency for Food, Environmental and Occupational Health & Safety will therefore continue to actively monitor the literature on BPA with the aim of updating the expert appraisal report where appropriate.

Marc Mortureux

KEYWORDS

Bisphenol A, risk assessment, US FDA/NCTR 2013 study, Delclos *et al.*, 2014.

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ANNEX

Annex 1

Table 8: NOAELs / LOAELs selected for the HRA of BPA

Critical effects	Study reference	Animal population	Time of exposure	Route of exposure	Type of effect	LOAEL/ NOAEL	Population to consider in the HRA
Brain and behaviour							
	Xu *et al*., 2010a	ICR mice	GD7- PND21	Oral (gavage)	**Decreased expression of NMDA receptors in conjunction with alteration of spatial memory and of learning functions**	NOAEL 50 µg/kg bw/d	Pregnant women and offspring
Female reproductive system							
	Signorile *et al*., 2010	Balb-C mice	GD1-PND7	Sub-cutaneous	**Increase in the occurrence of ovarian cysts**	LOAEL 100 µg/kg bw/d	Pregnant women and offspring
					Endometrial hyperplasia	NOAEL 100 µg/kg bw/d LOAEL 1000 µg/kg bw/d	Pregnant women and offspring
	Rubin *et al*., 2001	Sprague Dawley rats	GD6 – weaning of the pups	Oral (drinking water)	**Disruption of ovarian cycles**	NOAEL 100 µg/kg bw/d LOAEL 1.2 mg/kg bw/d	Pregnant women and offspring
Metabolism and obesity							
	Miyawaki *et al*. (2007)	Pregnant ICR mice	Treatment of the dams from GD10 up to weaning of the pups, then treatment of the pups from weaning up to PND30	Oral (drinking water)	**Increase in body weight and cholesterolaemia in females from 0.26 mg/kg bw/d**	LOAEL 0.26 mg/kg bw/d	Pregnant women and offspring

Table 1: Summary table of NOAELs/LOAELs selected based on all of the studies identified as key studies (extracted from the BPA HRA - ANSES, 2013, page 92/282)