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Ms. Monet Vela
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Sacramento, California

April 11, 2015

Re: Nine Articles relevant to hazard identification for Bisphenol A

I have attached 9 files containing information relevant to the deliberation by the OEHHA bisphenol A panel.

1. The French l'Agence de Sécurité Sanitaire Française health effects assessment for BPA that led France to ban BPA in food contact products as of January 1 2015.

2. An article documenting the basis for the disagreement between ANSES and EFSA regarding the hazards posed by BPA.

3. An article identifying that EFSA misrepresented in its news report on BPA the actual wording of its risk assessment.

4. An article documenting conflicts of interest of members of the EFSA panel that determined that BPA posed a low health hazard.

5. An article concerning Wolfgang Dekant, a former member of EFSA, who was being paid by the Polycarbonate/BPA Global Group, a part of the American Chemistry Council, while he was playing a significant role in EFSA determination that BPA was "safe" and, in particular, the unsubstantiated position by EFSA in 2008 that babies could metabolize BPA as effectively as adults.

6. A letter by vom Saal et al (2012) concerning flaws in an article published by Justin Teeguarden in 2011 regarding the metabolism of BPA in humans.

7. A peer-reviewed publication by vom Saal and Welshons (2014) concerning the "manufactured controversy" created first in publications funded by the American Chemistry Council and then adopted by the FDA as the basis for the false prediction that biomonitoring studies reporting ng/mL levels of serum BPA were due to contamination of samples.

8. A peer-reviewed article concerning why depending on GLP studies by regulatory agencies (the FDA and EFSA) led to flawed conclusion concerning the safety of BPA.

9. An article by vom Saal and Hunt (2012) concerning the flaws in the FDAs BPA risk assessment.

Sincerely,

Frederick S. vom Saal. Ph.D.
Curators' Professor of Biology

an equal opportunity/ADA institution

Health effects of Bisphenol A

Request nos. 2009-SA-0331 and 2010-SA-0197

Collective Expert

REPORT

Expert Committee (CES) for Assessment of the risks related to chemical substances

Working Group on Endocrine disruptors and Category 3 reprotoxic substances

September 2011

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Key words

Bisphenol A, health effects, reprotoxicity, development, fertility, neurotoxicity, endocrine disruptor

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ADOPTION OF THE REPORT BY THE EXPERT COMMITTEE (CES)

This report was submitted for comments to the CES for Assessment of the risks related to chemical substances:

– on 4 November and 9 December 2010, 27 January, 10 March, 12 May and 30 June 2011

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After taking comments into account, the report was approved by the Working Group members on 28 June 2011.

It was adopted by the CES for Assessment of the risks related to chemical substances on 30 June 2011.

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ANNEXES 242

Collective expert appraisal: summary and conclusions

ANSES – Request nos. 2009-SA-0331 and 2010-SA-0197

COLLECTIVE EXPERT APPRAISAL: SUMMARY AND CONCLUSIONS

Regarding the health effects of bisphenol A (BPA)

This document firstly summarises the work of the Working Group on 'Endocrine Disruptors and Reprotoxic Substances' and secondly presents supplementary information from the Expert Committee).

The original of this document was drafted in the French language. If this agreement is translated into any other language, the French language text shall prevail.

1. OVERVIEW OF THE QUESTION

In a letter to the Agency dated 4 June 2009, the Directorate General for Health (DGS) requested an expert assessment on the health risks to consumers linked to reprotoxic substances and/or endocrine disruptors found in products and/or items on the market, including bisphenol A (BPA). In a letter to the Agency dated 18 February 2010, the Directorate General for Risk Prevention (DGPR) requested an expert assessment on BPA, taking into account all types of toxic effects, and not only reprotoxic effects and/or effects related to endocrine disruption. A Working Group (WG) was appointed by ANSES to assess the health effects of BPA by referring to past expert assessments, the preliminary results of the INSERM collective expert assessment and an analysis of scientific articles published since July 2010.

This WG met on eight occasions in order to examine BPA, which is a complex, changing and highly disputed issue. Numerous published studies refer to BPA's properties as an oestrogen-mimicking endocrine disrupting compound. However, the current data tend to indicate that BPA also acts through other modes of action for which the data in the literature are still fragmented. Furthermore, toxicological methods suited to the study of endocrine disruptors are currently under development.

Thus, an assessment of the health effects of BPA should cover not only this compound's effects on reproduction, an assessment that was already covered by the collective expert assessment undertaken by INSERM[1], but also other types of effects such as effects on the behaviour of exposed offspring, the immune system and thyroid function, which were not examined in the INSERM expert assessment.

2. ORGANISATION OF THE EXPERT APPRAISAL

Bibliographic analysis

The expert assessment undertaken by the WG on 'Endocrine disruptors and Reprotoxic substances' particularly relied on documents published by national and international expert assessment authorities (EU-RAR, 2002-2008; JRC, 2010; NTP-CERHR, 2008; Health Canada, 2008; OEHHA, 2009; AFSSA, 2010; INSERM, 2010[1]; etc.) and by 'Expert panels' such as Chapel Hill (2007). The EFSA expert assessment report published in September 2010 and the conclusions of the expert panel which met under the leadership of WHO/FAO that were published in November 2010 were also taken into consideration by the WG.

This expert appraisal takes into account research work published subsequent to the expert assessment reports that were available for the analysis (end date for the bibliographic analysis: 25 January 2011). Indeed, since the preliminary INSERM report was published in June 2010, numerous new studies have been made available and have been analysed by the experts (over 70 to date). Despite the undeniable importance of the assessment reports devoted to BPA that were already available, the experts considered that it might be necessary, given the complexity of the subject and gaps in knowledge regarding the mechanisms of action involved, to analyse some of the original papers considered as key studies for certain types of effects linked to BPA.

[1] Since the final report from the INSERM expert assessment was published in June 2011 (Collective expert assessment on Reproduction and the Environment), the Working Group's experts referred to the preliminary version of the INSERM report (June 2010). A comparative analysis of the conclusions in these two reports will be undertaken at a later time.

Particular attention was given firstly to epidemiological studies providing information that can directly be interpreted in terms of human effects, and secondly to experimental studies undertaken at low doses in animals.

The experts especially focused on studies assessing the effects of BPA at doses lower than the NOAEL[2] of 5 mg/kg/day, which was used to establish EFSA's current Tolerable Daily Intake (TDI) (0.05 mg/kg/day) (2006, confirmed in 2010).

Given that questions have recently been raised regarding non-dietary BPA exposure, including dermal exposure, it was considered relevant to take into account routes other than oral exposure. Studies using the subcutaneous route have rarely been the subject of a systematic analysis in past expert assessments. The majority of them have considered the recognised effects by the oral route of exposure, which have been deemed more representative of dietary exposure. However, the subcutaneous mode of administration can be used to control the exposure levels with greater precision and highlight effects at administered doses that are much lower than the doses that can be administered orally.

In order to guarantee the traceability of the expert appraisal process and a concerted and rigorous analysis of the documents, a publication analysis chart, which had been validated by the WG beforehand, was used by the group for its work. *Rapporteurs* and reviewers were appointed to assess each type of effect potentially linked to BPA. Each *rapporteur* or sub-group of *rapporteurs* wrote a summary corresponding to the following sub-sections:

1. Effects on the male reproductive system
2. Effects on the female reproductive system
3. Effects on the brain and behaviour
4. Effects on metabolism and the cardiovascular system
5. Effects on the thyroid
6. Effects on the immune system
7. Effects on the intestine
8. Effects on the prostate
9. Effects on the breasts.

[2] NOAEL = No Observed Adverse Effect Level

The full report was presented and discussed in the WG. The expert group's comments and conclusions are presented in this report, the drafting of which was coordinated by ANSES.

An examination of the ecotoxicological effects of BPA supplements the data produced through toxicological studies, either confirming the results reported in studies undertaken in mammals, or revealing other types of effects or other mechanisms of action for BPA.

Classification by organ or system

For each type of effect, the available data were presented by exposure period as reported in the studies (prenatal, perinatal, neonatal and postnatal exposure as well as exposure during puberty and adulthood).

For each type of effect, the WG characterised and qualified these effects in terms of:

- Recognised effects
- Suspected effects
- Controversial effects
- Effects for which no conclusion can be drawn on the basis of the available data.

All the available information regarding a health effect was thus assessed using the decision tree below. It can be interpreted as follows:

When the available information was obtained from one or more studies, each study was analysed and considered either to be of 'high-quality', having non-major methodological limitations' or having 'major methodological limitations'.

A 'high-quality' study was defined as one containing an appropriate methodology (coherence of the exposure model, confounding factors taken into account, etc.) and a sufficient number of observations.

A study was considered to have 'non-major methodological limitations' when one of the above aspects was not considered to be fully satisfactory. Nevertheless, the study could be taken into account in light of its contribution to the expert appraisal.

When a study had unacceptable shortcomings (e.g. small population size, failure to take into account relevant confounding factors in epidemiological studies, etc.), it was considered as having 'major methodological limitations'.

When the results of multiple ‘high-quality’ studies undertaken by different scientific teams:

- converged, the effect was considered to be ‘recognised’,
- diverged, the effect was considered to be ‘controversial’.

When studies having ‘non-major methodological limitations’:

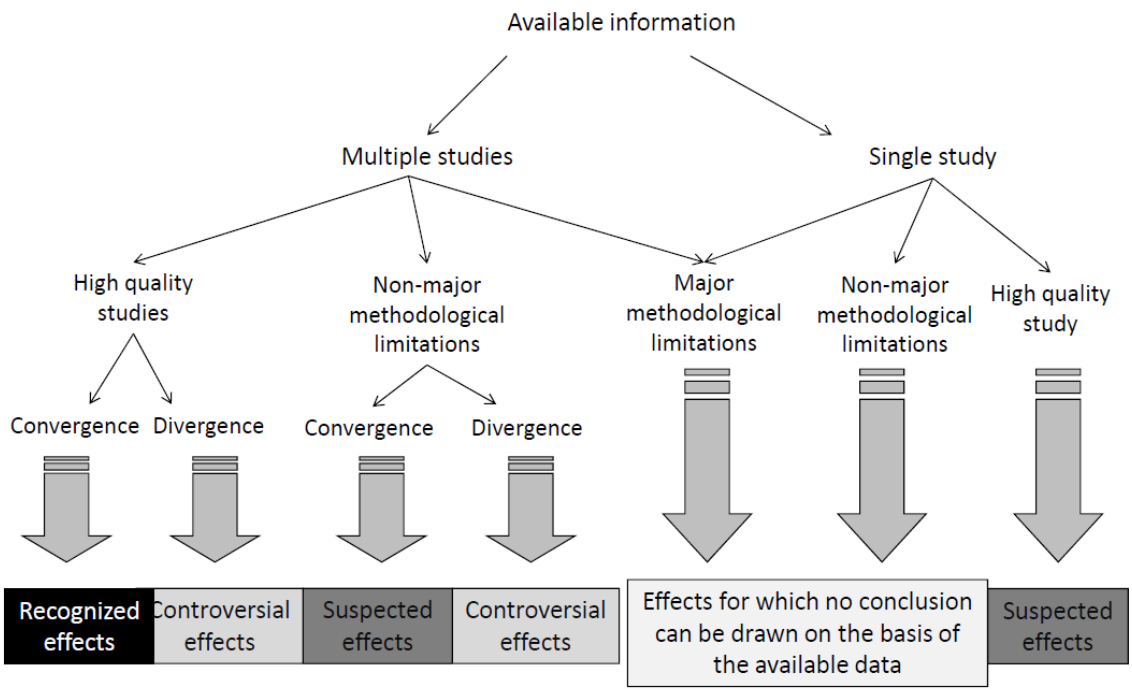
- converged, the effect was considered to be ‘suspected’,
- diverged, the effect was considered to be ‘controversial’.

Studies having ‘major methodological limitations’ were excluded as they could not be used to draw conclusions.

Lastly, when information was reported in only one study, the methodology was assessed:

- when it was 'high-quality', the effect was considered to be 'suspected',
- when it had ‘major or non-major methodological limitations’, the study was considered to be excluded and could not be used to draw conclusions regarding the effect under consideration.

The classification of effects according to this decision tree was supported by an expert judgment.



Methodological discussion regarding difficulties encountered when assessing the effects of BPA

Although there has been extensive research work on BPA, with a recent acceleration in publications, it is still difficult to assess the human toxicity of endocrine disruptors in general, and BPA in particular, due to the heterogeneity of experimental conditions and also due to the potential experimental biases that can limit the interpretation of the results of the animal studies.

Furthermore, with regard to critical stages of development, it is particularly important to take into account exposure periods for the assessment of the effects. Differences in the exposure periods examined in the available studies are an additional factor explaining why it can be difficult to interpret and/or to compare results.

The experts would like to emphasise the following points:

- The vast majority of the **epidemiological data** in humans related to BPA have methodological weaknesses limiting the scope of their conclusions. Indeed, the published studies are cross-sectional studies that are difficult to interpret, particularly in terms of the causal relationship between measured exposure to BPA and the observed health effects (e.g. failure to systematically take into account potential confounding factors, small population sizes, etc.). As a result, the HRA will essentially rely on experimental data, while epidemiology inputs may support the selection of the critical effects.

- In terms of **experimental toxicology**, the interpretation of results should take into account differences between species that may limit the transposition of data observed in animals to humans.

- Given that BPA can have effects at very low doses (around one µg/kg bw or less), the experts emphasise that numerous **experimental variables** can explain diverging results, such as the animal model used (species and strains), the feed administered to the animals (particularly regarding its phyto-oestrogen level), the nature of the parameters examined, the inappropriate use or lack of positive controls, etc.

- The possible existence of a **non-monotonic dose-response** relationship also makes the interpretation of results complex.

- The experts emphasise the **heterogeneity of the protocols used**, particularly in terms of measurement tools, observed effects and exposure routes, which makes it difficult to interpret and/or compare the results. This suggests that a high degree of caution should be used when assigning a level of confidence to certain reported effects. The main limitations recorded by the experts were as follows:

    - some effects highlighted by certain teams have not, to date, been reproduced by others,
    - insofar as certain studies were not undertaken in an HRA context, the results cannot always be used to that end (e.g. inappropriate study model, protocol using only one exposure dose, etc.),
    - for certain types of effects, the current OECD guidelines do not allow investigation of the long-term toxicological effects after pre-, peri- or early postnatal exposure (e.g. development of a tumour in adulthood as a result of prenatal exposure).

- Regarding **toxicokinetic** data, the experts highlight diverging results between older and more recent studies, possibly depending on whether free or conjugated forms of BPA were taken into account. In fact, only current analytical techniques can distinguish between these two forms. It should also be noted that the conjugated forms are not active on hormonal receptors. Furthermore, recent studies indicate that deconjugation producing free BPA is likely to occur, particularly in the placenta and foetal tissues.

- As the kinetics of absorption, distribution, biotransformation and excretion of BPA varies according to the **route of administration and the species**, the expert group considers that it is important to carefully analyse the impact of routes of exposure depending on the animal species.

The effects of endocrine disruptors can differ depending on the **period of exposure**. The experts consider it is important to carefully characterise the influence of these periods and their concordance with windows of susceptibility, which are not always known.

3. RESULTS OF THE COLLECTIVE EXPERT APPRAISAL

Conclusions by organ or system

The conclusions are based on the results of available human and animal data, which have most often been obtained at doses lower than the NOAEL of 5 mg/kg/day that was used to calculate the TDI currently used by EFSA.

Effects on the male reproductive system

In humans:

- **The effects of BPA on the male reproductive system** are **controversial**. The experts emphasise that it is difficult to draw a conclusion on the basis of epidemiological studies since these do not totally converge, given that the populations under study are not always identical in the studies examined (fertile males, infertile males, workers).

In animals:

- Impaired **sperm production** linked to 5-week exposure to BPA during **adulthood** is **recognised**. The observed effects after oral exposure in the study by Chitra *et al.* (2003)[3], and those observed after subcutaneous exposure in the study by Herath *et al.* (2004)[4], converge for this parameter only, for **exposure occurring in adulthood**;

- Effects on the **male reproductive system** (reduced plasmatic testosterone concentrations, modified sexual behaviour), due to exposure during **puberty**, are **suspected**;

- Effects on the **male reproductive system** due to exposure during the **prenatal, neonatal and postnatal (lactation) periods of exposure** are **controversial.**

Effects on the female reproductive system

In humans:

- The effects of BPA on **oocyte maturation in women** (decrease in the number of oocytes after ovarian stimulation and alteration of the quality of collected oocytes), in a context of ART (Assisted Reproductive Technology), are **suspected** on the basis of a high-quality study (Mok-Lin *et al.*, 2010)[5] and another study having non-major methodological limitations (Fujimoto *et al.*, 2010)[6];

- There are few other epidemiological studies available and they present methodological limitations (study population size, selection of participants, statistical analyses, confounding factors, etc.). Human data should therefore be considered with the utmost caution and are in no respect conclusive as to BPA's effect on the parameters studied. The experts consider that in the current state of knowledge, on the basis of **human data related to the effects of BPA on the endometrium (endometriosis, hyperplasia), the ovaries (polycystic ovary syndrome) and the outcome of pregnancy (miscarriages and prematurity) in women, it is not possible to draw a conclusion**.

---

[3] Chitra KC, Latchoumycandane C, Mathur PP. Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. Toxicology. 2003 Mar 14;185(1-2):119-127

[4] Herath CB, Jin W, Watanabe G, Arai K, Suzuki AK, Taya K. Adverse effects of environmental toxicants, octylphenol and bisphenol A, on male reproductive functions in pubertal rats. Endocrine 2004 Nov;25(2):163-172.

[5] Mok-Lin E, Ehrlich S, Williams P., Petrozza J, Wright DL, Calafat AM, Ye X, Hauser R. Urinary bisphenol A concentrations and ovarian response among women undergoing IVF. Int J Andro. 2010 Apr; 33(2):385-393

[6] Fujimoto VY, Kim D, vom Saal FS, Lamb JD, Taylor JA, Bloom MS. Serum unconjugated bisphenol A concentrations in women may adversely influence oocyte quality during in vitro fertilization Fertil Steril. 2011 Apr;95(5):1816-9.

In animals, on the basis of converging results from studies undertaken **during development** (pre- and postnatal exposure) under various conditions and on various models, the following effects can be considered to be **recognised**:

- **Increased occurrence of ovarian cysts;**
- **Appearance of endometrial hyperplasia;**
- **Early onset of puberty after prenatal and postnatal exposure;**
- **Effects on the hypothalamic-pituitary-gonadal axis** after *in utero* or early postnatal exposure resulting in changes in sex hormone levels and the expression of these hormones' receptors.

Moreover, in animals, effects related to exposure in adulthood (e.g. number of implantation sites, histological changes in the uterine wall, morphology of the genital tract, etc.) are observed, but at doses much higher than the NOAEL used by EFSA.

Effects on the brain and behaviour

In humans:

- The experts consider that the human data that are currently available are **insufficient** to draw a conclusion as to the **effects of BPA on behaviour**.

In animals:

- The effects on **cerebral development** linked to **pre- or perinatal exposure to BPA** have been confirmed by several studies that show, in particular, changes in neural differentiation, alterations of the aminergic and glutamatergic systems, changes in oestrogen receptor α and β expression, and changes in the number of neurons sensitive to oxytocin and serotonin. These effects on neurogenesis are considered to be **recognised**;
- **Changes in maternal behaviour** (e.g. less time spent by mothers caring for their offspring) linked to **pre- or postnatal exposure to BPA** are **suspected** effects;
- Effects on **anxiety, exploratory behaviour and behavioural sexual dimorphism** (increased anxiety, reduced exploratory behaviour, behavioural feminisation of the male offspring of treated mothers) linked to **pre- or perinatal exposure to BPA** are considered to be **controversial** by the WG.

Effects on lipid and carbohydrate metabolism and the cardiovascular system

In humans:

- In the cross-sectional epidemiological study by Melzer *et al.* (2010)[7], a **correlation** was observed between the highest urinary levels of BPA and **cardiovascular diseases (coronary diseases) and diabetes**. The experts consider these effects to be **suspected**.

In animals:

- BPA increases blood lipid levels, leads to excess body weight and enhances lipogenesis. The **effects on lipogenesis** (*in vivo* and *in vitro* data), after **pre- or perinatal exposure or exposure in adulthood**, are considered to be **recognised**;

- The effects on **glucose metabolism after pre- or perinatal exposure to BPA** are considered to be controversial.

Effects on the thyroid

In humans:

- The only available study dealing with the effects of BPA on thyroid function is **not conclusive**.

In animals:

- The data on amphibian metamorphosis in response to triiodothyronine (T3) show a potential effect of BPA as a thyroid hormone antagonist (e.g. inhibition of metamorphosis). This effect is considered to be recognised in amphibians and could be due to antagonistic effects described *in vitro*. However, while the amphibian model is useful for screening and studying mechanisms of action, it alone is not sufficient to characterise the hazard to humans;

- In rodents, the two experimental studies using relatively similar approaches (developmental exposure, spontaneous oral uptake, etc.) tend to show that BPA has an effect on thyroid function over a period corresponding to the final maturation of the hypothalamic-pituitary-thyroid axis.

[7] Melzer D, Rice NE, Lewis C, Henley WE, Galloway TS. Association of urinary bisphenol A concentration with heart disease: evidence from NHANES 2003/06. PLoS One. 2010 Jan 13;5(1):e8673.

Thus, on the basis of all the experimental data (e.g. amphibians, rodents, *in-vitro* data), the **effects on thyroid function linked to neonatal exposure to BPA** are considered to be **suspected**.

Effects on the immune system

In humans:

- The only available study dealing with the effects of BPA on the immune system **does not make it possible to draw a conclusion.**

In animals:

- The induction of T-cells, and more particularly of Th2-cells, accompanied by the excess production of cytokines, is considered to be a **recognised effect**. The observed shift in immune response suggests the induction of an allergy-prone profile (proliferation and activation of Th2-cells and production of cytokines involved in allergy). It is not currently known if these observations can be extrapolated to humans.

Effects on the intestine

In humans:

- **No studies have been identified by the WG to date**.

In animals:

- The study by Braniste *et al.* (2010[8]) shows an anti-inflammatory and pro-nociceptive effect of BPA, similar to those produced by oestradiol, and a dose-dependent decrease in intestinal permeability after adult exposure in ovariectomised rats. In the female offspring of treated mothers, a pro-inflammatory effect and a decrease in intestinal permeability are observed. **The effects of BPA on inflammation and intestinal permeability are suspected.**

[8] Braniste V, Jouault A, Gaultier E, Polizzi A, Buisson-Brenac C, Leveque M, Martin PG, Theodorou V, Fioramonti J, Houdeau E. Impact of oral bisphenol A at reference doses on intestinal barrier function and sex differences after perinatal exposure in rats., PNAS 2010, 107(1):448-53.

Effects on the prostate: development and carcinogenicity

In humans:

- **No studies have been identified by the WG to date.**

In animals:

- The studies by Tyl *et al.* (2002 and 2008)[9],[10], undertaken in mice and rats over several generations, do not show an effect on prostate weight. However, other studies (Chitra *et al.*, 2003 et Herath *et al.*, 2004)[2,3] show increased ventral prostate weight in rats after exposure in adulthood only and increased prostate weight after prenatal exposure in mice (Nagel *et al.*, 1997)[11]. **The effects on prostate weight are controversial**. When a histological examination was conducted, this weight increase was associated with hyperplasia;

- In rodents, **neonatal exposure** to BPA, in induced conditions, highlighted the appearance of **prostatic intraepithelial neoplasia (PIN) lesions**, without the appearance of prostatis adenocarcinoma. The observed effects in these experimental conditions are **suspected**.

  In light of the above results, the experts consider that the **effects on the prostate in animals are controversial**.

Effects on the breasts: carcinogenicity

In humans:

- **The only available study does not make it possible to draw a conclusion** regarding the link between BPA exposure and breast cancer.

In animals (rodents), the experts consider that, on the basis of the available data:

- **Acceleration of the mammary gland's structural maturation in adulthood after pre- or perinatal exposure to BPA is a recognised effect**;

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[9] Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM, Fail PA, Chang TY, Seely JC, Joiner RL, Butala JH, Dimond SS, Cagen SZ, Shiotsuka RN, Stropp GD, Waechter JM. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicol Sci. 2002 Jul;68(1):121-46.

[10] Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG,Waechter JM Jr. Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice. Toxicol Sci. 2008 Aug;104(2):362-84.

[11] Nagel SC, vom Saal FS, Thayer KA, Dhar MG, Boechler M, Welshons WV. Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol.Environ Health Perspect. 1997 Jan;105(1):70-6.

- The **development of intraductal hyperplastic lesions** after **pre- or perinatal exposure** to BPA is a **recognised effect**;
- The **development of neoplastic lesions** (CIS: intraductal carcinomas) after **perinatal exposure** to BPA is a **suspected effect**;
- **An increase in mammary gland susceptibility to developing mammary tumours at a later period** (with co-exposure to a carcinogenic agent) after **pre- or perinatal exposure** to BPA is a **suspected effect.**

Ecotoxicological data

Ecotoxicological studies indicate that effects on reproduction and development may affect wild species and that these effects occur at concentrations that are likely to be found in the environment.

Effects considered for the risk assessment

Further to this analysis, the experts will first consider the effects found to be 'recognised' in animals (since no recognised effects have been identified in humans to date) and 'suspected' in humans when undertaking the HRA. Nevertheless, they reserve the possibility, depending on the relevance and plausibility of the effects, to also subsequently consider those effects found to be 'suspected' or 'controversial' when undertaking the HRA.

The WG will therefore take into account the following effects for the risk assessment:

- **'recognised effects in animals:**
    - Increased occurrence of ovarian cysts after pre- and postnatal exposure;
    - Hyperplastic modifications of the endometrium after pre- and postnatal exposure;
    - Early onset of puberty after pre- and postnatal exposure;
    - Altered sperm production after adult exposure;
    - Histological changes in neurogenesis after pre- or perinatal exposure;
    - Effects on lipogenesis after prenatal, perinatal or adult exposure;
    - Effects on the mammary gland: acceleration of the mammary gland's structural maturation in adulthood and development of intraductal hyperplastic lesions after pre- or perinatal exposure to BPA.

- **'suspected' effects in humans**:
    - Effects on oocyte maturation in females in infertile couples undergoing ART (Assisted Reproductive Technology);
    - Effects on cardiovascular diseases (coronary diseases) and diabetes.

4. RECOMMENDATIONS OF THE EXPERT COMMITTEE (CES)

Methodological perspective

As a follow-up to the expert assessment work on health effects that has already been undertaken, the feasibility and relevance of undertaking a health risk assessment, taking into account all routes of exposure and uses, will be studied. To do so, the Expert Committee (CES) for "Assessment of risks linked to chemical agents" recommends the following methodological developments:

- Apply a method for rating the level of evidence for each of the effects, integrating all the human and animal data;
- Assess the effects of BPA, particularly with regard to a potential endocrine disrupting effect, taking into account the harmful nature of the effects, their severity, their significance and their reversibility;
- Undertake an in-depth analysis of studies that have highlighted non-monotonic dose-response relationships;
- Conduct an additional analysis of toxicokinetic data in order to be able to assess similarities or discrepancies (qualitative or quantitative) between animal species and humans, and thus determine the bioavailability of BPA in humans;
- Determine bioequivalent doses based on toxicokinetic data, in order to be able to use the results of studies on subcutaneous exposure in view of a route-to-route transposition for the HRA;
- Propose the development of a PBPK model to determine the active dose in the target organ in animals that can then be transposed to humans. This tool would also incorporate current and future biomonitoring data;
- Assess the relevance of using toxicity reference values (TRVs) or Tolerable Daily Intakes for substances with non-monotonic dose-response curves;

- Take into account past and future “hearings” regarding endocrine disruptors in general in order to document the uncertainties and concerns of stakeholders (civil society representatives, industries, the general public, etc.);
- Inventory identified substitutes and the available data regarding the toxicity of these substitutes.

Research recommendations

Some initial research recommendations were proposed by the experts. These will be added to after the expert assessment work has been undertaken.

- Improve knowledge of human exposure to BPA, and to do so:
    - Identify environmental sources of BPA and develop use/exposure matrices;
    - Provide information on occupational exposure and develop occupation/exposure matrices;
    - Promote the implementation of epidemiological studies with characteristics (study type, population size and recruiting method, control of the main confounding factors, etc.) that would produce results with a high level of proof regarding a causal relationship between exposure to BPA and the health effects examined;
- Acquire biological surveillance data taking the *in utero* period into account and generate precise and reliable data on BPA exposure (dietary in particular) and contamination in the population, distinguishing between the free and conjugated forms. Indeed, this type of data is lacking in general, and in France in particular, whereas it is useful to the HRA;
- Given the importance of the exposure period in the expected impact of endocrine disruptors and the late appearance of several of these effects including pre-neoplastic and/or neoplastic effects, it would be advisable to undertake an experimental study combining prenatal and/or postnatal exposure at low doses and a follow-up of effects throughout the animal's lifetime. This type of protocol would aim, for example, to combine the principles and procedures described in the current OECD guidelines for the study of developmental toxicity on the one hand and carcinogenesis on the other hand, subject to the appropriate modifications.

Maisons-Alfort, 08/09/2011

On behalf of the Expert Committee (CES) for

Assessment of risks related to chemical substances,

Michel Guerbet

Chairman

Abbreviations

ACC	Acetyl-CoA carboxylase
DNA	Deoxyribonucleic acid
AhR	Aromatic hydrocarbon receptor
AFSSA	French Food Safety Agency
AFSSET	French Agency for Environmental and Occupational Health Safety
MAP	Medically-assisted procreation
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
APV/AVPV	Anteroventral periventricular nucleus
AR	Androgen receptor
ARC	Arcuate nucleus
mRNA	Messenger ribonucleic acid
AUC	Area under the plasma concentration time curve
BADGE	Bisphenol A DiGlycidyl Ether
BfR	Federal Institute for Risk Assessment (Germany)
BKH	BKH Consulting Engineers
BMD	Benchmark dose
BPA	Bisphenol A
BPA-GA	Bisphenol A glucuronide
BPA-SG	BPA specific-gravity
BPB	Bisphenol B
BPE	Bisphenol E
BPF	Bisphenol F
GLP	Good laboratory practices
CR-SD	Sprague-Dawley (SD) rats provided by the Charles River Laboratories
C/EBP-α	CCAAT enhancer binding protein α
CFSAN	Center for Food Safety and Applied Nutrition (USA)
CIS	Carcinoma *in situ* (ductal or lobular)
LCIS	Lobular carcinoma *in situ*
CERHR	Center for the Evaluation of Risks to Human Reproduction (USA)
CES	Expert Committee
LCIS	Lobular carcinoma *in situ*

Cmax	Maximum serum concentration
DA	Dopamine
DAT	Dopamine transporter
DES	Diethylstilbestrol
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulphate
DHI	DHI Water Environment Health Consulting Engineers
DHT	Dihydrotestosterone
DIN	Ductal intraepithelial neoplasia
ADI	Acceptable daily intake
DGPR	French Directorate General for Risk Prevention
DGS	French Directorate General for Health
DMBA	Dimethylbenzanthracene
DMSO	Dimethyl sulphoxide
DOPAC	3,4-dihydroxyphenylacetic acid (metabolite of dopamine)
TDI	Tolerable daily intake
EB	Oestradiol benzoate
EC50	Effective concentration (required to induce a 50% effect)
ECHA	European Chemicals Agency
EE2	Ethinyloestradiol
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
ENU	N-ethyl-N-nitrosourea
ER	Oestrogen receptor
ER α	Oestrogen receptor α
ER β	Oestrogen receptor β
ERR γ	Oestrogen related receptor γ
HRA	Health risk assessment
FAI	Free androgen index
FAO	Food and Agriculture Organization of the United Nations
FAS	Fatty acid synthase
FDA	Food and Drug Administration (USA)

IVF	*In vitro* fertilisation
FSH	Follicle stimulating hormone
GD	Gestational day
GFP	Green fluorescent protein
GHR	Growth hormone receptor
GPCR	G-protein-coupled nonclassical membrane oestrogen receptor
GPR30	G-protein coupled receptor 30, transmembrane receptor involved in cell proliferation
ADH	Atypical ductal hyperplasia
HHG	Hypothalamo-hypophyseal-gonadal
HOMA	Homeostatic model assessment
Hpf	Hour post-fertilisation
HPLC	High-performance liquid chromatography
TH	Thyroid hormone
5-HT	5-hydroxytryptamine or serotonin
5-HIAA	5-hydroxyindoleacetic acid (urinary metabolite of serotonin)
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods (USA)
IDF	International Diabetes Federation
Ig	Immunoglobulin
BMI	Body mass index
INSERM	French National Institute for Health and Medical Research
IP	Intraperitoneal
JRC	Joint Research Centre (EU)
LC-MS/MS	Liquid chromatography coupled with tandem mass spectrometry
LH	Luteinising hormone
LIN	Lobular intraepithelial neoplasia
LOAEL	Lowest observed adverse effect level
LOQ	Limit of quantification
LPL	Lipoprotein lipase
MIF	Macrophage migration inhibitory factor
MPO	Myeloperoxidase
MR	Hippocampal mineralocorticoid receptor
NCEP-ATPIII	National Cholesterol Education Program (USA) Adult Treatment Panel III

ncm ER	Nonclassical membrane oestrogen receptor
ND	Not detectable
NE	Noradrenalin
NHANES	National Health and Nutrition Examination Survey (USA)
NMDA	N-methyl-D-aspartic acid
NMU	N-Methyl-N-nitrosourea
NOAEL	No observed adverse effect level
NS	Not significant
NTP	National Toxicology Program (USA)
OECD	Organisation for Economic Cooperation and Development
OEHHA	Office of Environmental Health Hazard Assessment (California, USA)
WHO	World Health Organization
bw	Body weight
PEC	Predicted environmental concentration
PIN	Prostatic intraepithelial neoplasia
PND	Postnatal day
PNEC	Predicted no effect concentration
PNW	Postnatal week
PPAR	Peroxisome proliferator-activated receptor
PPT	4,4',4''-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol)
PR	Progesterone receptor
PSA	Prostatic specific antigen
REACh	Registration, Evaluation, Authorisation and Restriction of Chemicals
RfD	Reference dose
RT-PCR	Reverse transcription polymerase chain reaction
SC	Subcutaneous
SCD-1	Stearoyl-CoA desaturase
SD	Sprague-Dawley rat
SHBG	Sex hormone-binding globulin
SOPK	Polycystic ovary syndrome
SREBP-1C	Sterol regulatory element-binding protein-1C
SULT	Sulphotransferase

T3	Triiodothyronine
T4	Tetraiodothyronine or thyroxine
TBG	Thyroxin-binding globulin
TEB	Terminal end bud
TNBS	Trinitrobenzene sulphonic acid
TNF	Tumour necrosis factor
TPX	Polymethylpentene
TRβ	Thyroid hormone receptor β (NR1A1)
TSH	Thyroid stimulating hormone
TTR	Transthyretin
EU	European Union
UGT	Uridine diphosphate glucuronosyl transferase
OR	Oral route
Vtg	Vitellogenin
TRV	Toxicity reference value

Glossary

Androgen, generic term for any natural or synthetic compound that stimulates or controls the development and maintenance of male characteristics in vertebrates, including the activity of accessory male sex organs and the development of male secondary sexual characteristics. The primary androgen is testosterone. All natural androgens are steroid derivatives of androstane (19-carbon tetracyclic hydrocarbon nucleus, $C_{19}H_{32}$). They are also the precursors of oestrogen, the female sex hormones.
A subset of androgens, adrenal androgens, includes any of the 19-carbon steroids synthesised by the adrenal gland, that function as weak steroids or steroid precursors, including dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEA-S) and androstenedione. Besides testosterone, other androgens include:

- dehydroepiandrosterone (DHEA)
- androstenedione
- androstanediol
- androsterone
- androstenolone
- dihydrotestosterone (DHT)

Andropause: Stopping or slowing of sexual activity in men, due to aging, and accompanied by certain general disorders (this term has no precise physiological meaning).

Antiandrogen: Substance that blocks the action of androgens. It binds to androgen receptors but without activating them.

Abnormalities in the male reproductive tract: Sexual differentiation is a complex hormone-dependent process, which determines whether a foetus will become male or remain female (the default state). This process is triggered by a series of events that must occur in a precise and coordinated manner to ensure the development of the male reproductive system and related secondary sexual characteristics. Cryptorchidism, or testicular retention in the abdomen after the age of one year, and hypospadias, an abnormality characterised by incorrect positioning of the urinary meatus, are two common abnormalities of the male reproductive tract.

Spontaneous abortion: Commonly called "miscarriage", spontaneous abortion refers to the expulsion of a nonviable foetus of less than 500 g, usually before the 20th week of pregnancy (calculated from the first day of the last menstrual period). The incidence of spontaneous abortions is estimated at 50% of all pregnancies, this assumption is based on the fact that many pregnancies are not clinically recognised.

Biomarker: Indicator signalling an event or condition in a biological system or a sample, and giving a measure of exposure (exposure biomarker), effect or susceptibility (effect biomarker). As related to biomonitoring, a biomarker is the presence of any substance, or a change in any biological structure or process that can be measured as a result of exposure. Many biomonitoring studies focus on chemical substances or their metabolites as biomarkers. (www.biomonitoringinfo.org)

Biomonitoring (biological monitoring): Continuous or repeated measurement of potentially toxic substances, their metabolites or their biochemical effects in tissues, secreta, excreta, expired air or any combination of these media or substances. Its purpose is to evaluate occupational or environmental exposure and health risks by comparison with appropriate reference values based on knowledge of the probable relationship between ambient exposure and resultant adverse health effects. (www.biomonitoringinfo.org).

Biotransformation: Biotransformation (or metabolism) is the process whereby a chemical is changed (transformed) by a chemical reaction within the body.

Testicular cancer: Testicular cancer can be broadly classified into two histological types, seminomas, which account for about 30% of all testicular tumours and non-seminomas, which are a group of cancers including choriocarcinoma, embryonal carcinoma, teratoma and yolk sac tumours. Both types may be found in the same tumour. Risk factors for testicular cancer include cryptorchidism and Klinefelter's syndrome.

Risk characterisation: Integration of hazard identification, hazard characterisation and exposure assessment into an estimation of the adverse effects likely to occur in a given population, including attendant uncertainties. (http://www.who.int/foodsafety/micro/riskassessment/en/):

- *Hazard identification*: The identification of known or potential health effects associated with a particular agent. (http://www.who.int/foodsafety/micro/riskassessment/en/);

- *Assessment of the dose-response* ***relationship***: The second of four steps in risk assessment, consisting of the analysis of the relationship between the total amount of an agent absorbed by a group of organisms and the changes developed in the group in reaction to the agent, and inferences derived from such an analysis with respect to the entire population.
(http://glossary.eea.europa.eu/EEAGlossary/D/dose-response_assessment);

- *Exposure assessment*: Quantitative and/or qualitative evaluation of the contact of a chemical with the outer boundary of the human body, which includes consideration of the intensity, frequency and duration of contact, the route of exposure (e.g. dermal, oral or respiratory), rates (chemical intake or uptake rates), the resulting amount that actually

crosses the boundary (dose) and the amount absorbed (internal dose) (IPCS, 1999) (http://www.who.int/phe/).

Climacteric: Period of life corresponding to the menopause.

***Corpus luteum*:** (Latin for "yellow body") is a temporary formation inside the ovary, resulting from the transformation of a Graafian follicle (mature or dehiscent follicle) after release of the oocyte during ovulation. The function of the *corpus luteum*, which therefore forms in the second part of the menstrual cycle called the luteal phase, is to secrete progesterone under the control of a pituitary hormone, LH. The role of progesterone is to maintain the uterine lining ready to receive the embryo upon implantation. If the ovum is not fertilised, the *corpus luteum* degenerates, causing a decrease in progesterone secretion and finally the onset of menstruation corresponding to the beginning of a new cycle. If the embryo is implanted, the *corpus luteum* will be maintained and will secrete progesterone during early pregnancy. It disappears at around the third or fourth month when the placenta becomes self-sufficient.

Cryptorchidism: In the male foetus, the testicles develop in the abdominal cavity and descend into the scrotum before birth. Cryptorchidism, or undescended testicles, occurs when one or both testicles fail to move into the scrotum prior to birth, and remain in the abdominal cavity. Cryptorchidism is fairly common in premature infants, and occurs in about 3 to 4% of full-term infants. In about 65% of cases, the testicles typically descend by 9 months of age. Cryptorchidism is a risk factor for the later development of testicular cancer.

Desmoplasia: Formation and development of fibrous tissue.

Differentiation: Acquisition of a specialised or tissue-specific function by immature cells.

EC_{50}: Effective Concentration (required to induce a 50% effect), i.e. the concentration that induces 50% of the maximal effect in terms of activity (receptor binding, for example) or individual (mortality).

Endogenous: Something (i.e. chemical, hormone) originating or produced within the organism.

Endometriosis: Endometriosis is characterised by the presence of ectopic (located outside the uterus) endometrial tissue, either in the ovaries, fallopian tubes, uterine ligaments, rectovaginal septum, or pelvic peritoneum. Like the uterine endometrium, the tissue is subjected to the menstrual cycle. The etiology of endometriosis is still highly controversial.

Epidemiologic studies: Epidemiology is the study of the factors that influence population health and diseases. It seeks both to quantify the frequency of a health event and to identify its

determinants (biological, medical, environmental, socioeconomic, etc.). The ultimate goal is to identify the factors (air pollution, food, etc.) implicated in the occurrence of a health event, so as to limit or eliminate them.

Exogenous: Something (i.e. chemical, hormone) originating or produced outside of the organism.

Fecundity/Infecundity: The concept is that of a *state*, i.e. having been involved (or not) at some point in achieving a pregnancy or childbirth.

Fertility/Infertility: The concept is that of *ability*, i.e. possessing (or not) the clinical and biological factors necessary to conceive a pregnancy.

Hox genes: Morphogens involved in uterine development and differentiation, and associated with endometrial proliferation. Many xeno-oestrogens can alter the expression of these genes. In the endometrium, the Hoxa 10 and Hoxa 11 genes are particularly important for female fertility, in particular for allowing implantation.

Adrenal glands: There are two adrenal glands, one located on the apex of each kidney. They consist of a cortical and a medullary part. The latter, called the adrenal medulla, secretes epinephrine, norepinephrine and a small amount of dopamine. The outer layer of the gland, the adrenal cortex, produces three groups of corticosteroids: mineralocorticoids (aldosterone) that control fluid and electrolyte balance, glucocorticoids (cortisol) that affect carbohydrate metabolism and finally sex steroids (androgens, DHEA).

GnRH: Peptide neurohormone produced by the hypothalamus and responsible for the synthesis and secretion of FSH and LH by the anterior pituitary gland. The role of FSH and LH, in turn, is to stimulate the sex glands (gonads).

HOMA (Homeostatic Model Assessment): A measure of insulin resistance, which is associated with metabolic syndrome and type II diabetes.

Hormones: Chemical messengers secreted by endocrine glands and transported by blood to other tissues or organs where they exert a specific action. For example, insulin is a hormone that helps our body digest food. Our growth, digestion and sexual and reproductive functions are all regulated by hormones.

Pituitary gland: The pituitary gland is the body's "master" gland. It secretes many hormones that affect the functioning of other endocrine glands.

Hypospadias: Hypospadias is a relatively common congenital abnormality, characterised by an incorrectly positioned meatus. This anomaly, of varying severity, may affect up to three in 1000 newborn boys. In most cases, the urethral opening is near the tip of the penis, on the glans, in more severe cases, however, the orifice is at the midshaft or the base of the penis, and may even be located in the scrotum or the perineum (beneath the scrotum).

Hypothalamus: The hypothalamus contains vital centres for controlling the autonomic nervous system, body temperature and water and food intake, and is the centre for primitive physical and emotional behaviour. It also produces hormones for regulating pituitary secretion and two systemic hormones (e.g. vasopressin).

Metabolite: Any intermediate or product resulting from metabolism (the physical and chemical changes that take place in a given substance within an organism). (www.biomonitoringinfo.org)

Endocrine modulator: Another term used to describe modulation of the endocrine system by an exogenous substance. See also Endocrine disruptor/Hormonally active agents.

Oestrogen: Oestrogens are steroids secreted by the ovaries, placenta, adrenal cortex and testes, which determine the female secondary sexual characteristics, and control the menstrual cycle and various metabolisms.

Environmental oestrogen: Phyto-oestrogens (plant-based oestrogens found in such plants as soya, beans, grains) and the manmade chemicals that are found in the environment and have oestrogenic properties. See also Endocrine disruptor/Endocrine modulator/Hormonally active agents.

Ovaries: These double organs are the female sex glands. The ovaries produce the ova (oocytes). The ovaries are important endocrine glands that produce oestrogens and progesterone. These two hormones help regulate the ovulatory cycle leading to the maturation and ovulation of a mature egg, as well as help prepare and maintain the uterus during pregnancy. At puberty, oestrogens give rise to secondary sexual characteristics (i.e. breasts, pubic hair, etc.).

Pancreas: The islets of the pancreas produce two hormones necessary for the regulation of blood sugar levels - insulin and glucagon. The alpha cells of the islets secrete glucagon, which raises blood glucose (sugar) levels by stimulating the breakdown of liver glycogen. When blood sugar levels are too high, the beta cells of the pancreas secrete insulin which stimulates the uptake of glucose.

Parathyroids: The parathyroids are four small glands attached to the thyroid gland, which act to maintain normal levels of calcium and phosphate in the blood and thus normal function of muscles and nerves.

Prenatal exposure period: Exposure that occurs only during pregnancy.

Perinatal exposure period: Exposure that occurs during pregnancy and after birth.

Early postnatal or neonatal exposure period: Exposure that occurs during a short period after birth.

Pubertal exposure period: Period of exposure occurring at the time of puberty.

Exposure period in adulthood: Exposure period occurring only in adulthood.

Endocrine disruptor: An exogenous substance or mixture that alters the function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny. Other definitions are available (French Ministry **of Health and Sports**

The Director General for Health

EA4 No. 220

Paris, 4 June 2009

The Director General

French Agency for Environmental and Occupational Health Safety

253 avenue du Général Leclerc

94701 MAISONS-ALFORT CEDEX

MAIL RECEIVED

08 JUNE 2009

2186

Subject: Reprotoxic substances and endocrine disruptors.

Ref. /No.: 090018 *(file number to be referenced in all correspondence)*

The scientific data seem to show a fall in male fertility in industrialised countries over the past twenty or so years. These worrying changes are often attributed to chemical pollution in our environment.

The action of certain chemicals on reproduction or on the reproductive organs can be due either to a direct reprotoxic action affecting the adult, young child, embryo or foetus during its prenatal development, or to a hormone-mimetic action (oestrogen- or androgen-like) when these substances are endocrine disruptors.

In this context of concern, both for the public and the health authorities, I requested that INSERM conduct a collective expert appraisal aiming primarily to draw up, within six months, a list of the reprotoxic substances of concern, to describe the different known reprotoxic effects on development or fertility and their mechanisms, to analyse the risk factors in children and adults by identifying in particular the most susceptible periods in life in terms of exposure, and to analyse the *in vitro* and *in vivo* tests currently used, for regulatory or research purposes, to detect these effects. AFSSET has been contributing to this expert appraisal.

Based on the list of reprotoxic substances compiled as part of INSERM's expert appraisal, I would like you to pursue this expert work in your sphere of competence in order to determine whether there are currently products intended for the general public that contain such substances, to quantify their use and the associated exposure levels, and to conduct a benefit/risk assessment. AFSSA and AFSSAPS will likewise receive solicited requests relating to their respective spheres of competence.

I would also like your agency to coordinate overall the work of these organisations in order to make a general assessment of exposure and risk and, if necessary, to propose the reinforcement of certain recommendations for use. Accordingly, with respect to these products I would be grateful if you would:

- identify those containing these substances or likely to be affected;
- select those to be studied as a priority:
- analyse and if possible quantify the routes by which the general population are exposed to these substances, specifying direct and indirect sources, and including vulnerable populations and people exposed in occupational environments;
- consider substitutes.

With respect to drug residues in water, I can inform you that I have already requested an expert appraisal on this topic from AFSSA and AFSSAPS. Moreover, an action plan relating to drug residues in different environments is currently being prepared jointly by the Directorate General for Health and the Directorate for Water and Biodiversity of the MEEDDAT [Ministry of Ecology, Energy, Sustainable Development and Land Planning]. If a solicited request is needed on this theme, it will be prepared in consultation with both Ministries to ensure coherence with the abovementioned action plan.

Director General for Health

[Signature]

Pr Didier HOUSSIN

MAIL RECEIVED
22 February 2010
660

MINISTRY OF ECOLOGY,
ENERGY,
SUSTAINABLE DEVELOPMENT
AND THE SEA
responsible for Green technologies
and Negotiations on the climate

Directorate General for Risk Prevention

Paris, 18 February 2010

The Director General for Risk Prevention
to:
The Director General
French Agency for Environmental and Occupational Health Safety
253 avenue du Général Leclerc
94701 MAISONS-ALFORT CEDEX

Subject: Bisphenol A
cc: DGS, DGAL, DGT, DGCCRF, AFSSA, INERIS

Bisphenol A has been the focus of particular attention at the international level due to the regular publication in specialised journals of new studies relating to the health effects. The susceptibility of children and pregnant women and the endocrine-disrupting nature of bisphenol A have thus led to concerns being raised within the population and the scientific community.

In view of this complementary scientific information and the steps taken by the authorities in various countries (Japan, Norway, Canada), it seems appropriate to us to examine in greater depth the risks posed to health and the environment.

Bisphenol A is used to manufacture polycarbonates used in many plastics, and in the manufacture of different resins and fire retardants. In addition, bisphenol A is used, among other things, as an additive in flame retardants and as a developer in thermal papers.

In this context, I would like you to undertake work focusing on the health risks resulting from human exposure to bisphenol A via the environment. I would also be grateful if you would, in conjunction with AFSSA:

- issue a summary of the hazards posed by bisphenol A, mainly using a review of the studies published since the risk assessment report presented at European level by the United Kingdom in February 2008 in the context of Regulation (EEC) 793/93 and the transitional Annex XV dossier submitted on 1 December 2008, to review the multidisciplinary knowledge on the subject and identify any gaps; to conduct this work you may find it useful to consult the Chairman of the scientific committee of the research programme on endocrine disruptors in order to identify any scientists who may be able to help you identify;
- the uses leading to human exposure and characterise these exposure situations;
- substitutes for bisphenol A as well as the available data on the hazards posed by these substitutes.

You may also contact INERIS, who at ONEMA's request has undertaken work on data on bisphenol A production and use, discharges and fate of this substance in the environment, as well as prospects for substitution and emissions reduction.

Depending on the information gathered during this first phase, I would then like you to analyse the feasibility and relevance of conducting a health risk assessment on the different methods of exposure and based on the available data.

It seems worthwhile for your agency to focus on this compound while work on endocrine disruptors is undertaken at the national level with INSERM.

In light of this work, you should if necessary formulate recommendations, particularly on the inclusion of bisphenol A in the scope of Regulation (EC) no. 1907/2006 (REACh Regulation). An analysis of the most relevant procedure (inclusion in the first EU action plan for assessment, preparation of a dossier for restriction, harmonised classification and labelling or identification of a substance of very high concern with a view to its inclusion in Annex XIV) should in particular be conducted, taking into account the management tools stipulated by other sector regulations.

Finally, please produce a review of current research on bisphenol A or its substitutes and make the necessary recommendations concerning the areas to investigate as a priority.

Director General for Risk Prevention

[Signature]

Laurent Michel

Annex 2).

Phyto-oestrogen: Plant based oestrogens found in such plants as soya, beans, grains.

Persistent organic pollutants (POPs): Chemical substances that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to human health and the environment. This group of priority pollutants consists of pesticides (such as DDT), industrial chemicals (such as polychlorinated biphenyls, PCBs) and unintentional by-products of industrial processes (such as dioxins and furans).

Polymorphism: Variation in DNA sequence among individuals that may explain phenotypic differences.

Non-persistent chemical: A substance that is readily removed from an environment through physical, chemical or biological processes.

Precocious puberty: Premature development of body characteristics that normally occur during puberty (the period in life at which rapid physical and physiologic changes occur, including development of reproductive capability). Puberty normally occurs between the ages of 13 and 15 in boys and between 9 and 16 in girls.

Sex ratio: The sex ratio is defined as the number of live male births divided by the total number of births for a given period of time.

Testicular dysgenesis syndrome: Skakkebaek and colleagues in Copenhagen have suggested that testicular cancer, poor sperm quality, hypospadias and cryptorchidism are interdependent and are the result of disrupted testicular development *in utero*. This concept, called **testicular dysgenesis syndrome**, or TDS (Skakkebaek *et al.*) is based on the juxtaposition of clinical, epidemiological and scientific data indicating the foetal origin of testicular cancer, the link between genital defects among newborn boys and reproductive disorders in adulthood, and on observations from experimental studies in animals (Skakkebaek *et al.*, 2001). In particular, the juxtaposition of epidemiological data from Denmark and its neighbour Finland shows a parallel between the two countries for all the anomalies involved, with the highest frequencies of hypospadias, cryptorchidism, testicular cancer and poorest sperm quality being observed in Denmark. All these data suggest that the prenatal period is the most vulnerable phase during which an abnormality in testicular differentiation would lead to permanent adverse effects. This abnormal testicular development could be the result of genetic defects or polymorphisms, exposure to harmful environmental factors, lifestyle-related factors, impaired foetal growth and possibly several of these factors at once.

Metabolic syndrome (defined according to the NCEP-ATPIII (National Cholesterol Education Program Adult Treatment Panel III) and the IDF (International Diabetes Federation):

Diagnosis, as defined by the NCEP-ATPIII (William & Wilkins 2002), requires a combination of at least three of the following criteria:

- *Abdominal obesity (visceral)*: waist circumference greater than 102 cm (men) or greater than 88 cm (women);
- *Hypertriglyceridemia*: triglycerides greater than or equal to 1.7 mmol/L (1.50 g/L);
- *Reduced HDL cholesterol:* less than 1.03 mmol/L (0.40 g/L) [men] or less than 1.29 mmol/L (0.50 g/L) [women];
- *Elevated blood pressure*: blood pressure greater than or equal to 130/85 mm Hg or use of medication for hypertension;
- *Fasting glucose:* greater than or equal to 6.1 mmol/L (1.10 g/L).

The diagnosis as defined by the IDF requires as a mandatory criterion visceral obesity (central) defined as waist circumference greater than or equal to 94 cm (men) or greater than or equal to 80 cm (women) plus any two of the following clinical or biological signs:

- *Hypertriglyceridemia:* triglycerides greater than or equal to 1.7 mmol/L (1.50 g/L) or specific treatment for this lipid abnormality;
- *Reduced HDL cholesterol*: less than 1.03 mmol/L (0.40 g/L) [men] or less than 1.29 mmol/L (0.50 g/L) [women] or specific treatment for this lipid abnormality;
- *Raised blood pressure:* systolic blood pressure greater than or equal to 130 mm Hg or diastolic blood pressure greater than or equal to 85 mm Hg or treatment of previously diagnosed hypertension;
- *Fasting glucose:* greater than or equal to 5.6 mmol/L (1.0 g/L) or previously diagnosed type 2 diabetes (IDF, 2011).

Polycystic ovary syndrome: a hormonal disorder characterised by an unusual increase in androgens (male hormones) in the ovaries, which affects the maturation of ova; instead of being released at the time of ovulation, the ova develop into cysts that accumulate in the ovaries.

Endocrine system: A network of glands distributed throughout the body forms the endocrine system. These glands produce hormones that are released into the circulation and distributed to distant target sites via the blood. Hormones produced by these glands act as chemical messengers to control body functions such as growth, metabolism, sexual development and egg and sperm production.

Testes: These double organs are the male sex glands. The testes in males produce the male germ cells, spermatozoa or simply, sperm. The testes are also important endocrine glands that

produce male sex hormones such as testosterone and other androgens. These hormones are important for the regulation of spermatogenesis (production of sperm), sexual differentiation of the male foetus, and the development at puberty of secondary sexual characteristics such as hair growth, voice changes, etc.

Thyroid: The thyroid gland consists of two lobes connected by an isthmus beside the larynx (voice box). The thyroid gland produces thyroid hormones T3 (triiodothyronine) and T4 (thyroxine or tetraiodothyronine) which regulate the metabolism of all cells in the body. Disorders of the thyroid gland are characterised by the inability to produce or release sufficient thyroid hormones (hypothyroidism) or the overactivity of the thyroid gland (hyperthyroidism).

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1. Context, purpose and procedure for responding to the request

1.1. Context

Bisphenol A or BPA (4,4'-dihydroxy-2,2-diphenylpropane, CAS no. 80-05-7) is a phenolic chemical that has been used for over 50 years, mainly as a monomer in the manufacture of polycarbonate plastics and epoxy resins. It is also used as a component of polyester, polysulphone and polyacrylate resins and plays a role in the synthesis of flame retardants. Polycarbonates are used in the composition of a large number of common objects (CDs, glasses, plastic bottles, baby bottles), while epoxy resins are mainly used to make tin can linings and dental amalgam. BPA is also used as a developer in thermal papers.

In 2006, global production of bisphenol A came to approximately 3.8 million tonnes, two thirds of which was used for the manufacture of polycarbonate and one third for resins.

BPA has been listed as a Category 1 endocrine disruptor (EC, 2002; DHI, 2007).

Many studies have been devoted to characterising the toxicity and endocrine effects of BPA in animals. Some of these studies were conducted in accordance with the regulatory guidelines of the OECD, particularly with regard to a sufficient number of animals and doses tested. Other studies have also been conducted by research institutes and university laboratories, often based on a smaller number of animals and doses. The results and conclusions of these studies however, are not consensual, whether in terms of the nature of the observed effects, the time of their occurrence or the dose levels at which they are produced.

Consequently, the potential link between BPA exposure and certain diseases such as prostate or breast cancer, obesity, diabetes, thyroid dysfunction, or behavioural and reproductive disorders, remains a topic of discussion.

BPA is now, and has been for some years, the subject of particular attention at the international level. In view of the concerns raised by this substance, on 11 March 2010, Canada became the first country in the world to ban baby bottles containing BPA. In the United States, several states have prohibited BPA in baby bottles and the leading six manufacturers of baby bottles have already ceased all marketing of products containing BPA. In France, following its recent expert appraisal in 2010, AFSSA (now ANSES) had also reported “warning signs” identified in the literature. The Act "to suspend the marketing of baby bottles manufactured with bisphenol A" was published in the French Official Journal on 1 July 2010.

1.2. Purpose of the request

In a letter to the Agency dated 4 June 2009, the Directorate General for Health (DGS) requested an expert assessment on the health risks to consumers linked to reprotoxic substances and/or endocrine disruptors found in products and/or items on the market, including bisphenol A (BPA).

In a letter to ANSES dated 18 February 2010, the Directorate General for Risk Prevention (DGPR) requested an assessment of the health risks resulting from human exposure to BPA via the environment. ANSES was therefore asked to:

- summarise the effects on human health,
- identify practices leading to human exposure,
- characterise exposure,
- assess the feasibility and relevance of conducting a health risk assessment.

ANSES was also asked to:

- review current research on BPA and its substitutes,
- identify these substitutes and the possible associated dangers,
- make recommendations regarding the inclusion of bisphenol A in the scope of Regulation (EC) no. 1907/2006 (REACh).

1.3. Response: measures deployed and organisation

ANSES entrusted the examination of these requests to the Expert Committee (CES) for Assessment of the risks related to chemical substances. The CES mandated the Working Group (WG) on Endocrine Disruptors and Category 3 Reprotoxic Substances to conduct the expert appraisal work.

The methodological and scientific aspects of the expert work on the health effects were regularly submitted by the WG to the CES. The report produced by the WG takes account of observations and additional information supplied by the members of the CES.

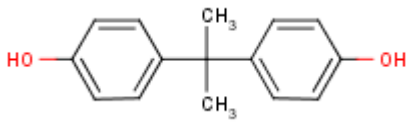
This work was therefore conducted by a group of experts with complementary skills.

It was carried out in accordance with the French Standard NF X 50-110 “Quality in Expertise Activities” to ensure compliance with the following points: competence, independence and transparency, while at the same time ensuring traceability.

2. Information on bisphenol A

2.1. Identification of the substance

Table I: Identification of bisphenol A (ANSES, 2011)

Chemical Abstracts Service registry number (CAS No.)	80-05-7
EINECS (European INventory of Existing Commercial chemical Substances) number	201-245-8
Chemical name (ESIS: European chemical Substances Information System)	4,4'-isopropylidenediphenol
IUPAC (International Union of Pure and Applied Chemistry) name	2,2-bis(4-hydroxyphenyl)propane (also called 4,4'-isopropylidenediphenol or bisphenol A)
Some synonyms (ChemiDplus, EU RAR, 2008)	Bisphenol A or BPA (common abbreviation) p,p'-Isopropylidene-bisphenol p,p'-Isopropylidene-di-phenol 4,4'-Isopropylidene bisphenol 4,4'-(1-methylethylidene)bisphenol
Chemical sub-group	Phenols
Empirical formula	$C_{15}H_{16}O_2$
Chemical structure	

2.2. Purity

The purity of bisphenol A ranges from 99-99.8%, depending on the manufacturer. The impurities are phenol (< 0.06%) and the ortho- and para-isomers of bisphenol A (< 0.2%) (EC, 2010a).

2.3. Physico-chemical properties

The physico-chemical properties of BPA are given in Table II.

Table II: Physico-chemical properties of bisphenol A (ANSES, 2011)

Physical and chemical constants	Value	Experimental or modelled values
Physical form (at ambient T°)	White solid that can be in the form of powder, flakes or crystals, with a slight phenolic odour	Experimental value
Molecular weight (g/mol)	228.3	Not specified
Boiling point (°C)	250-252°C at 1.7 kPa 360°C at 101.3 kPa	Experimental value
Melting point (°C)	150 to 157°C	Experimental value
Closed cup flash point (°C)	207 to 227°C	Not specified
Lower Explosive Limit (LEL)	12 g/m^3 with oxygen > 5%	Not specified
Upper Explosive Limit (UEL)	Not specified	Not specified
Vapour pressure (Pa)	$5.3 \cdot 10^{-6}$ at 25°C	Experimental value
Vapour density	Not specified	Not specified
Liquid density	1.1 to 1.2	Not specified
Conversion factor	1 ppm = 9.3366 mg/m^3 At 25°C and 1 atm	Not specified
Water solubility (mg/L)	120 to 300 at 25°C	Experimental value
Log Kow	3.32 to 3.4	Experimental value
Koc (L/kg)	715	Modelled value

2.4. Environmental fate

A short atmospheric half-life of 0.2 days has been calculated for the reaction of BPA with hydroxyl radicals. BPA released into the atmosphere should mainly be found in the particulate phase (Health Canada, 2008).

Bisphenol A is easily biodegradable in natural surface waters and the soil (EC, 2010a). According to the available data, BPA is not persistent under aerobic conditions. However, in conditions of no or low oxygen, it has been found not to degrade or to degrade only slowly (Health Canada, 2008).

BPA has low to moderate mobility in soil.

As a moderately hydrophobic substance with some water solubility, BPA can be expected to partition to organic phases such as sediments and soils; however a significant fraction will also likely be present in the dissolved phase (Health Canada, 2008).

2.5. European regulations[12]

According to Directive 67/548/EEC (30th ATP: Directive 2008/58/EC of 21 August 2008), bisphenol A is classified:

- Toxic for reproduction, category 3; R62
- Irritant: R 37-41
- Sensitiser: R 43
- Hazardous for the environment; R52

According to the CLP Regulation (Commission Regulation (EC) no. 790/2009 of 10 August 2009 amending Regulation (EC) no. 1272/2008), bisphenol A is classified for its:

- Toxicity for reproduction, category 2, H 361f - Specific target organ toxicity
- Single exposure, category 3: respiratory tract irritation; H 335
- Causes serious eye damage, eye irritation category 1; H 318
- Skin sensitivity, category 1: H 317

BPA is not listed in Annex I to Regulation (EC) No 689/2008 of the European Parliament and of the Council of 17 June 2008 concerning the export and import of dangerous chemicals.

BPA is authorised in food contact materials in the European Union in accordance with Commission Regulation (EU) No. 10/2011 of 14 January 2011. In this context, BPA is authorised for the manufacture of food contact materials with a specific migration limit of 0.6 mg/kg in food.

[12] Regulations, and in particular international regulations, will be described in more detail in the final report of the bisphenol A risk assessment.

- **REACh Regulation (EC) no. 1907/2006 (ANSES, 2011)**

Bisphenol A falls within the scope of Regulation (EC) No. 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (ECHA 2007).

All companies manufacturing, importing and/or using chemicals are affected by this regulation which aims to assess and control chemical substances manufactured, imported and used in the European market at a rate of over one tonne per year, in order to reduce the risks to health and the environment associated with their use. The REACh procedure included a substance pre-registration phase from 1 June 2008 to 1 December 2008 for all substances placed on the market in the EU before 19 September 1981. Bisphenol A is on the list of substances pre-registered by manufacturers and importers that was published by ECHA in January 2009 (ECHA 2009).

After the pre-registration phase, importing manufacturers were required to register substances with ECHA before 1 December 2010 if they were:

- produced or imported in quantities greater than or equal to 1000 tonnes/year,
- Category 1 and 2 CMRs produced or imported in quantities greater than 1 tonne/year,
- R50/53 substances produced or imported in quantities greater than 100 tons/year.

As of 30 November 2010, 4300 substances had been registered with the European Chemicals Agency (ECHA), which received a total of 24,675 dossiers. Regarding bisphenol A, at least one complete dossier (under Annex X of REACh) was submitted in the first phase of registration of substances above 1000 tonnes.

NB: An Annex XV dossier for BPA was submitted to the European Chemicals Agency in 2009 as part of the transitional measures provided for in Article 136(3) of REACh[13]. In this dossier, the rapporteur only examined the scenarios that led to a conclusion iii for human exposure in the Risk Assessment Report (RAR).

2.6. Production and uses

BPA is primarily used as a monomer for the manufacture of polycarbonates, polymers used in the plastics industry to manufacture a large number of common items such as DVDs, spectacles, plastic bottles, and car roofs and headlamps. Bisphenol A is also used as a reagent in the

[13] http://echa.europa.eu/doc/trd_substances/4_4_isopropylidene_diphenol_bisphenol_a/ann_xv_trd/trd_uk_bisphenol_a.pdf*

manufacture of resins, the most common being epoxy resins. These are found mainly in coatings for containers used in the marine environment, and in the linings of tins and cans.

There are other less widespread uses of bisphenol A. It acts as a developer in thermal papers, which are used in cash register receipts, for instance. BPA is also used in the manufacture of two flame retardants: tetrabromobisphenol A and bisphenol A-bis (diphenyl phosphate).

In addition, bisphenol A is used as an antioxidant in the automotive sector. It is also an additive in the manufacture of polyamides, a reagent in the synthesis of ethoxylated bisphenol A and a precursor in the synthesis of benzoxazines. Lastly, other uses have been identified outside of France, including as a component of heat transfer fluids and lubricants, a treatment agent for resurfacing concrete, and in formulations of fungicides.

For more information, please refer to the detailed report on the uses of BPA (ANSES, 2011).

3. General considerations

3.1. Mechanisms of action

Knowledge of BPA's mechanisms of action is an important element to consider in order to be able to transpose to humans the effects observed in animals. BPA is known as a weak agonist of oestrogen receptors α and β (ERα and ERβ). We can be certain that not all BPA's mechanisms of action are yet known. However, a growing number of *in vitro* or molecular studies suggest that interpretation of BPA's toxicological effects cannot be limited to a classical oestrogenic mechanism (NTP-CERHR, 2008). BPA may also interact with other cellular receptors such as the androgen receptor AR and cause a moderate anti-androgenic effect, and the aromatic hydrocarbon receptor (AhR), the transmembrane oestrogen receptor, the thyroid hormone (TH) receptors, as well as the transmembrane receptor GPR30 which is involved in cell proliferation (Bonaccorsi *et al.*, 2008; INSERM, 2011; Iordanidou *et al.*, 2010). In addition, BPA diglycidyl ether (BADGE) and BPA are capable of inducing expression of the nuclear receptor involved in the proliferation of PPAR γ (Bishop-Bailey *et al.*, 2000; Kwintkiewicz *et al.*, 2010). Most recently, BPA was also identified as an oestrogen-related receptor γ (ERR γ) ligand, whose natural ligands and specific physiological functions are unknown. Consequently, any interpretation of BPA's effects only in terms of an oestrogen-mimicking effect would be simplistic. The involvement of several of these systems during exposure to BPA could explain some effects observed at low doses, due to a possible synergy of action, but also the non-monotonic dose-response relationships reported in some studies. Indeed, it is easy to imagine that strong responses to low doses on a given hormonal pathway may trigger feedback phenomena, well known for some hormones, and that at higher doses of BPA, the effects observed are lower. Finally, mechanisms of action other than those via links with hormone receptors are also mentioned, such as the activation of expression of certain genes at embryo level, or the modulation of second messenger systems.

3.2. Study models

3.2.1. Epidemiological studies

Epidemiological studies provide very valuable data for highlighting associations between exposure to a substance and the presence of health effects, as they make transpositions from animals to humans unnecessary. However, the epidemiological data identified for this study have many limitations, meaning that it is difficult to use them to determine an association between health effects and exposure to BPA.

First, many studies are hampered by classic methodological biases (sample size too small, selection of exposed population and controls, method of data collection, etc.).

Second, many epidemiological studies are cross-sectional studies that include a single sample as a measure of exposure. In general, cross-sectional studies are rarely suitable for studying effects requiring a long latent period: extrapolating from a single exposure measurement taken at a period contemporary with the study may not be representative of the exposure that led to the initiation of the disease, mainly because of the changing uses of and exposure to the substance. However, in this specific case, BPA is a ubiquitous substance with recurrent exposure and a short half-life compared to other environmental contaminants. Obtaining a single sample to assess the mean internal exposure levels in a population may therefore in some cases (e.g. adequate sample size and random sampling throughout the day) be legitimate (Ye *et al.*, 2011). Moreover, cross-sectional studies may be appropriate in the following two cases:

- The study of a link between exposure at time *t* and a short-term effect (for example, the association between a single measurement of BPA in plasma and plasma levels of a hormone, in the case in which BPA induces a change in hormone secretion, synthesis, transport and metabolism).
- The study of an effect resulting from exposure during a known and well-defined window of susceptibility, in order to characterise exposure at the most relevant period with respect to the intended effect.

The limitations of epidemiological studies should therefore be analysed on a case-by-case basis, taking into account the exposure period with regard to the critical phases of development, and the dosing period for BPA, in particular for assessing deferred effects due to exposure during development.

Finally, some studies were excluded because of known bias in selection of the study population, bias related to the exposure assessment (blood samples stored in plastic tubes containing BPA) or inaccuracies in measuring the health effect (self-questionnaire).

For this study, 29 epidemiological studies were identified and assessed. Eleven studies were not selected for the characterisation of health effects because they had major methodological limitations. The analysis of these studies is described in Section **Erreur ! Source du renvoi introuvable.**.

3.2.2. Experimental studies

Many parameters can influence the results of experimental studies. Taking them into consideration is particularly important since the doses administered in studies are low, in some cases close to the contamination levels making up the environmental background, and the observed effects are also sensitive and subject to wide variability. This is largely true of the study of toxicity of endocrine disruptors and BPA in particular. Failing to consider these parameters in the study protocol can, in some cases, lead to bias in the results observed. The main parameters are therefore detailed in the Annex to AFSSA's Opinion of 29 January 2010 (AFSSA, 2010a).

Most of the expert appraisals conducted by other national or international bodies also address this issue. Thus, Health Canada in its 2008 report considers that the divergent results on exposure to BPA at low doses could be explained by a number of experimental variables (Health Canada, 2008). For example, those parameters include the choice of animal species, the strains used, the variability related to tissues, food (especially the level of oestrogenic contaminants), the inappropriate use or lack of positive controls and the consideration of exposure-related effects that present a non-monotonic dose-response curve (Richter *et al.*, 2007; vom Saal and Hughes, 2005) (vom Saal *et al.*, 2005). In addition, the period of exposure to BPA with regard to the critical phases of development is an important consideration, especially for the assessment of deferred effects resulting from exposure during development. Moreover, the nature of the effects is such that it is difficult to characterise the degree of potential 'harmfulness' and, therefore, to determine their importance in a human health risk assessment.

As part of this ANSES expert appraisal, depending on whether or not some of these biases were taken into consideration when analysing the studies, they may or may not have been used to assess the toxicity of BPA. Thus, the Working Group experts considered the following points:

- Choice of animal species and strain,
- Sample size,
- Presence or absence of one or more positive control groups,
- Nature of cages and containers (feeding bottles, etc.),
- Composition of the litter, diet and water quality,
- Route of exposure and method of administration.

The assessment of some parameters such as route of exposure, method of BPA administration (gavage, infusion, injection, etc.), period of exposure *(in utero,* during lactation, in adulthood, etc.), or co-exposure to oestrogen-mimicking substances is important for interpreting the study results. They are not, however, strictly speaking, major methodological limitations that could jeopardise the quality of the study. Studies whose methods have non-major methodological limitations have not therefore been excluded *a priori.* The working group considered that treated and untreated batches were subject to the same co-exposures. The risk here is not the demonstration of an effect that does not exist, but rather a loss of power for the study. Although weighted, these studies with "non-major methodological limitations" add to the body of evidence formed by all the studies, which is constructed using a method detailed in Section **Erreur ! Source du renvoi introuvable.**. However, studies conducted without a negative control were considered as having major methodological limitations and were not selected for the health effect assessment.

In addition, in most experimental studies, internal exposure data appear only rarely, which is a major hindrance to judging the relevance of experimental exposure schemes with regard to the level of contamination of human populations.

3.2.2.1. Choice of laboratory animals: species, strain and origin

It has been proven that different animal species have varying sensitivity to hormone-mimetic compounds. In addition, the sensitivity of strains can vary within the same species; for this reason the NTP stated in 2001 that because of clearly demonstrated differences in sensitivity between species and strains, selection of the animal model should be based on the ability to respond to compounds with an endocrine activity (i.e. the response to positive controls) and not on convenience and habit (NTP, 2001).

As with many chemicals, most experimental studies are performed in rodents. According to Chapel Hill, the Sprague-Dawley (SD) rat marketed by Charles River Laboratories (CR-SD) may have lost

susceptibility to exogenous oestrogens (Richter *et al.*, 2007). However, this observation should be modulated depending on the parameter analysed (EDMVS, 2003), moreover other authors have shown effects at low oestrogen doses using the SD rat strain: for example, in a four-generation study associating exposures with different windows of development and a chronic toxicity study, mammary hyperplasia in males was induced at 0.2 mg/kg bw/day of EE2 (Latendresse *et al.*, 2009).

According to Richter *et al.* the CR-SD strain was developed from the Sprague Dawley strain by the Charles River laboratory in 1950 (Richter *et al.*, 2007). This colony continuously underwent selective breeding based on rapidity of postnatal growth and large litter size. Then in 1991 and 1997, new colonies were established from selected animals (vom Saal and Hughes, 2005). Spearow *et al.* observed that rodents selected for their high fertility and high growth rate, such as the CD-1 mouse, were more oestrogen-resistant (Spearow *et al.*, 1999) and this observation is consistent with the loss of oestrogen sensitivity in CR-SD rats reported by Chapel Hill. Similarly, according to the NTP in 2008, *"it is evident that the SD rat and other rat strains are less sensitive to the effects of estrogens than the F344 rat. However for some traits, the reverse is true"*.

3.2.2.2. Sample size

Sample size is a factor in the classification of studies. Low numbers can lead to the power of the study being reduced, and thus a failure to demonstrate existing effects. However, there is no required minimum sample size for studies, because it depends on the incidence of the effect sought in the control group and its variability. According to the NTP-CERHR, a sample size of 6 animals per experiment and per dose seems reasonable for effects with a low degree of variability (e.g. body weight), but is not sufficient for effects with high inter-individual variability (rate of circulating hormones, etc.) (NTP-CERHR, 2008).

3.2.2.3. Positive control

The opinion of the NTP-CERHR is consistent with the view of several panels of scientists on the fact that the use of a positive control group can be very useful in evaluating the sensitivity and performance of an experimental model (NTP-CERHR, 2008). According to the expert panel that met at Chapel Hill, a study without a positive control should be considered uninterpretable (Richter *et al.*, 2007), while the NTP considers that the positive control is not essential in animal studies, especially when using animal models that are well known for the characterisation of certain effects (NTP-CERHR, 2008). In contrast, both panels agree that a study showing no effect in the treated groups and no significant effect in the positive control is not admissible.

According to the NTP, the substances most commonly used as positive controls are diethylstilbestrol (DES), ethinyloestradiol (EE2), 17 β-oestradiol and oestradiol benzoate (NTP-CERHR, 2008). These are the substances that initially led to BPA being considered as an oestrogen-mimicking substance. However, 17 β-oestradiol cannot be used as a positive control for

studies using the oral route because only 3% of the dose is absorbed (vom Saal and Welshons, 2006).

In previous assessments of the effects of BPA, studies which resulted in no response being observed for the positive control generally counted less for evaluating the effects of bisphenol A. In addition, although natural or synthetic oestrogens are used as positive controls for BPA, a growing number of *in vitro* or molecular studies suggest that interpretation of BPA's toxicological effects cannot be limited to a classical oestrogenic mechanism (NTP-CERHR, 2008). INSERM states that BPA is a weak agonist of oestrogen that can bind to the nuclear receptors ERα and ERβ, but that it is also capable of binding to other nuclear receptors such as the androgen receptor AR and causing a moderate anti-androgenic effect (INSERM, 2011). In addition, BPA diglycidyl ether (BADGE) and BPA are capable of inducing expression of the nuclear receptor involved in the proliferation of PPAR γ (Bishop-Bailey *et al.*, 2000; Kwintkiewicz *et al.*, 2010). Most recently, BPA was also identified as an oestrogen-related receptor γ (ERR γ) ligand, whose specific physiological functions are unknown. Finally, BPA also has the property of binding to membrane forms of oestrogen, androgen or thyroid hormone receptors (Bonaccorsi *et al.*, 2008; Iordanidou *et al.*, 2010) as well as the transmembrane receptor GPR30 which is involved in cell proliferation (INSERM, 2011). Under these conditions, the positive controls selected do not necessarily cover all these binding possibilities and, therefore, all the effects that may arise.

In view of this, the working group chose not to immediately rule out studies that did not use a positive control. Firstly, because BPA's mechanism of action has not been clearly identified, and therefore the relevance of a solely oestrogen-mimicking positive control (and similar to oestradiol, DES, EE2 or oestradiol benzoate) is questionable. Secondly, if an effect is observed with BPA in the absence of a positive control, it can be considered. However, if no effect is observed and there is no positive control, the study may be excluded. The choice of a positive control implies that strong assumptions be made *a priori* concerning BPA's potential mechanism of action. Therefore, a study showing a lack of response with a positive control was considered acceptable when the mechanism of action is unknown.

3.2.2.4. Uncontrolled exposure

The AFSSA report summarises the consequences of "accidental" co-exposure in experimental studies that can lead to bias (AFSSA, 2010a). Indeed, cages, litter, food and water can cause uncontrolled exposure to BPA and other endocrine disruptors and thus modulate oestrogenic activity. In addition, studies use exposure to BPA at increasingly low doses which are thus ever closer to the background levels.

The study by Howdeshell *et al.* demonstrated BPA's transfer potential from the wall of the polycarbonate or polysulphone cage (Howdeshell *et al.*, 2003). The authors concluded that the animals are subjected to chronic exposure to bisphenol A, which may occur by contact or by licking the walls. Oestrogenic activity was measured *in vitro* by an "E-screen" assay, *in vivo* by a uterotrophic assay and the concentrations of BPA released in the cage were quantified by GC/MS.

Similarly, AFSSA mentions the possibility of oestrogenic contamination depending on the nature and quality of litter, but to date, very few studies have taken into account the contribution of litter to the total oestrogenic load the animals are subjected to (AFSSA, 2010a).

Finally, AFSSA summarises several articles indicating that the presence of phyto-oestrogens in the diet has an impact on the oestrogenic response (AFSSA, 2010a). Owens *et al.* show that the use of foods with a phyto-oestrogen content lower than 325-375 mg/kg bw/day does not affect the response to BPA of the OECD uterotrophic assay (Owens *et al.*, 2003). However, a uterotrophic effect was measured for phyto-oestrogen concentrations in excess of 600 mg/kg bw/day. In a review of the literature, Jensen *et al.* qualify these findings by stating that the sensitivity to the presence of phyto-oestrogens depends on the toxicological targets (Jensen and Ritskes-Hoitinga, 2007). While in many studies, the thresholds above which responses are influenced by phyto-oestrogens are between 300 and 400 mg/kg of food, some studies show that certain toxicological targets such as behaviour or development of hormone-dependent cancers can be affected by significantly lower levels. The presence of phyto-oestrogens may have significant effects on the reproductive system (daily weight gain, anogenital distance and vaginal opening) (Thigpen *et al.*, 2007), age of puberty (Thigpen *et al.*, 2003), feeding behaviour, body fat, serum parameters associated with metabolism (Lephart *et al.*, 2004) and social behaviour of adult male rats (Hartley *et al.*, 2003). The effects on reproduction and development may instead be exacerbated by a diet devoid of oestrogen, in laboratory animals subjected for several generations to diets rich in phyto-oestrogens. Ruhlen *et al.* explain that these laboratory animals develop an adaptive process that results in an oestrogenisation syndrome, when a diet rich in phyto-oestrogens is stopped (Ruhlen *et al.*, 2008).

According to the report of the panel of experts that met at Chapel Hill, even soy-free diets may contain phyto-oestrogens, so it is recommended to use the same batch of food throughout the study (Richter *et al.*, 2007). Vom Saal and Hughes therefore recommend developing a standard diet appropriate for studies involving toxicological targets that are sensitive to oestrogenic substances (vom Saal et Hughes, 2005).

Concerning the drinking water provided to laboratory animals, this is most frequently tap water. However, it may contain chemical contaminants at trace levels, some of which may have a hormone-like activity. Nevertheless, all the data in the literature, when referring to BPA that may be present in drinking water intended for human consumption, mention concentrations of the order of a nanogram per litre. Furthermore, it is important to check whether the studies indicate the nature of the container that was used to dispense the drinking water.

Some studies have assessed the oestrogenicity of the cage, litter and food after successive extractions with organic solvents and optional purification on a Sep-Pak C18 cartridge, according to a previously published method. The extracts are ultimately suspended in the culture medium and their oestrogenicity measured by the E-Screen assay based on the MCF-7 breast cancer cell line's ability to proliferate in the presence of oestrogen (Soto *et al.*, 1992). Under these conditions, the oestrogenicity of the animal feed was estimated at less than 20 femtomoles of oestradiol equivalent per gram.

It should be noted that the E-Screen test, which is based on cell proliferation, is not recommended by the ICCVAM (Interagency Coordinating Committee for the Validation of Alternative Methods) since this proliferation may be due to mechanisms other than those strictly associated with the transcription of oestrogen response genes (ICCVAM, 2003). In addition to the E-Screen test, other bioassays, such as those based on the ability of genetically modified cell lines or yeasts to express one or other of the oestrogen receptors in response to oestrogens, are commonly used to measure the oestrogenic activity of materials, of feed matrices or of water (Ankley *et al.*, 1998; ICCVAM, 2003; Mueller 2004; OECD, 2009).

3.2.2.5. Administration route, method and vector

- **Oral administration**

According to AFSSA, studies in relation to food contamination favour exposures *per os*, either by using gastric tubes for gavage, or by directly depositing the test compounds in the oral cavity using a micropipette (AFSSA, 2010a) (Palanza *et al.*, 2002).

The oral administration routes most widely used are gavage and dispersion in feed or drinking water. Administration by gavage offers greater accuracy of the administered doses than administration in feed and drinking water. On the other hand, it causes stress to the animal and does not offer the same kinetics as the other two methods of administration. Indeed, the dose of BPA is administered in one go, thereby inducing a plasma concentration peak of the substance. Administration in the drinking water and feed gives more linear kinetics, since the animal has the feed and water at will throughout the day, but the doses given are not as accurate. The feed is weighed before each administration, and the water bottles are graduated in order to evaluate the amount consumed. However, the feed can be spilled in the cage and the feed distribution is collective for all the animals in a single cage, which gives only an average consumption per animal.

Moreover, according to AFSSA, the vehicle used to solubilise and administer the test substances can modify the absorption or introduce compounds which are themselves active on the targets studied (AFSSA, 2010a). Thus, protocols using olive oil, which is rich in polyphenols, introduce a possible risk of interaction between these polyphenols and endocrine disruptors tested at low dose.

- **Subcutaneous administration**

Numerous studies use the subcutaneous route to administer BPA, often diluted in DMSO (dimethyl sulphoxide). This may involve subcutaneous injection or a slow diffusion system such as implanted miniature pumps or a capillary system (permeable or with small pores throughout).

When BPA is administered by subcutaneous injection the daily dose can be controlled with greater accuracy. The dose administered can be corrected according to the modification of body weight during the study for long-term studies or exposures during gestation. The use of an osmotic pump or of a diffusion system facilitates repeated exposure studies and limits the stress on the animal subjected to repeated and invasive administrations, as well as making it possible to reproduce a linear exposure scheme, i.e. a scheme without an absorption peak. However, this method of administration has certain limits. Adapting doses according to changes in body weight during long-term exposures or during gestation is incompatible with the use of diffusion pumps. However, the age of the animal and its growth curve are important factors.

Subcutaneous administration bypasses the digestive barrier, intestinal and/or skin metabolism and the hepatic first-pass effect. In addition, transfer from the subcutaneous compartment to the bloodstream can also be influenced by the vector in which the substance tested was administered. According to the NTP, DMSO alone can cause a biological activity and is known to facilitate cell diffusion through the formation of channels (Zafar *et al.*, 2010). However, the NTP concludes that the impact of the use of high concentrations of DMSO is uncertain, and that this effect is probably weak at the amounts described in subcutaneous studies. Certain studies replace the use of DMSO with a 10:90 ethanol/sesame oil mixture in order to cause less skin irritation (Adewale *et al.*, 2009; Patisaul *et al.*, 2006). Moreover, when exposure is via the implantation of subcutaneous minipumps, some authors use pure DMSO as solvent. This practice is strongly advised against by the manufacturer of these pumps, which recommends a maximum concentration of 50% DMSO, otherwise the implant may dissolve, leading to tissue inflammation and oedema (NTP-CERHR, 2008).

Pottenger *et al.* used a kinetic approach to study exposure routes such as the oral, peritoneal or subcutaneous route (Pottenger *et al.*, 2000). The authors report a substantial difference in the pharmacokinetic parameters (bioavailability and metabolism) according to the exposure routes used. They warn against transposing the effects observed during subcutaneous exposure in particular, and recommend making this comparison with great care. Tominaga *et al.* also studied the impact of exposure routes (oral and subcutaneous) on BPA toxicokinetic parameters after

administration at doses of 10 mg/kg and 100 mg/kg, in rats, chimpanzees and *Cynomolgus* monkeys (Tominaga *et al.*, 2006). Notable differences in kinetics were observed depending on the species and the routes of administration used. Thus, according to the NTP-CERHR in 2008, the main difference between oral and subcutaneous administration lies in the absence of a hepatic first-pass effect with subcutaneous administration (NTP-CERHR, 2008). BPA is known to undergo a strong hepatic first-pass effect. However, in rodents as in humans, hepatic metabolism in newborns is limited, consequently reducing the hepatic first-pass effect. The higher the doses, the greater this difference. Consequently, according to this report, the effects obtained with the subcutaneous studies are relevant only if the exposure took place during the neonatal or juvenile period. Studies with subcutaneous administration in which the exposure took place in adults were only considered to be informative during the identification of the biological effects due to BPA.

To date, studies using subcutaneous exposure routes have not been taken into account in assessing the health risks arising from exposure through food, owing to the pharmacokinetic differences between the two routes of administration. Human exposures to BPA via routes other than the oral route, such as the cutaneous route (thermal papers, etc.), and quantitative studies of BPA penetration through the skin, have also recently been reported. Moreover, biomonitoring studies indicate urinary concentrations that are very much higher than those anticipated on the basis of the current food contamination data. One of the hypotheses put forward to explain this difference is that it could be due to the underestimation of an exposure route such as the cutaneous route.

Dose bioequivalences could be established on the basis of robust toxicokinetic data, in order to be able to use the results of subcutaneous studies as part of a health risk assessment.

The working group identified and examined 17 studies which had recourse to subcutaneous exposure. These studies are recent since they were published between 2002 and 2010, eight of them having been published between 2009 and 2010. These studies cover prenatal or perinatal exposures and correspond to administered doses ranging between 0.1 and 97 000 µg of BPA/kg bw. Effects were observed at administered doses of less than 5000 µg of BPA/kg bw/d. The species used in the studies are predominantly rodents (nine studies carried out in rats, in particular on the Holtzman, Wistar and Sprague-Dawley strains) and seven in mice (CD-1, ICR/Jcl, BALBC). One study was carried out in Suffolk sheep. Ten studies relate to reprotoxicity and development. Given the types of effects observed and the low doses administered, the endocrine disruptor working group decided to examine closely the studies based on subcutaneous administration and to assess to what extent the results obtained could be extrapolated to other routes, in particular exposure via the oral route.

- **Other routes of administration**

The other routes of administration used are anecdotal. One study uses the intracerebral route (Matsuda *et al.*, 2010). This route is not representative of a human route of exposure, but is part of experimental protocols aimed at demonstrating mechanisms of action. In the context of risk assessment, it cannot be included in a characterisation of the effects of BPA.

Finally, some studies use the intraperitoneal route (Pottenger *et al.*, 2000), intravenous route (Kurebayashi *et al.,* 2002) or respiratory route, but these concern pharmacokinetic studies aimed at comparing the bioavailability of various routes. One study in ewes via the intravenous route is reported, aimed at determining the effectiveness of BPA as an oestrogen-mimicking substance that inhibits pulsed secretion of LH (Collet *et al.*, 2010).

3.2.2.6. Exposure doses

The recent data of Taylor *et al.* on animal models suggests that an external dose of 400 µg/kg bw/d (eight times the current TDI) given orally would be required to reproduce the plasma concentrations commonly described in humans (of about 1 ng/mL) (Taylor *et al.*, 2011). Most of this report is based on studies in which the administered doses are of this order of magnitude, or lower and/or below the NOAEL (5 mg/kg bw/d; orally).

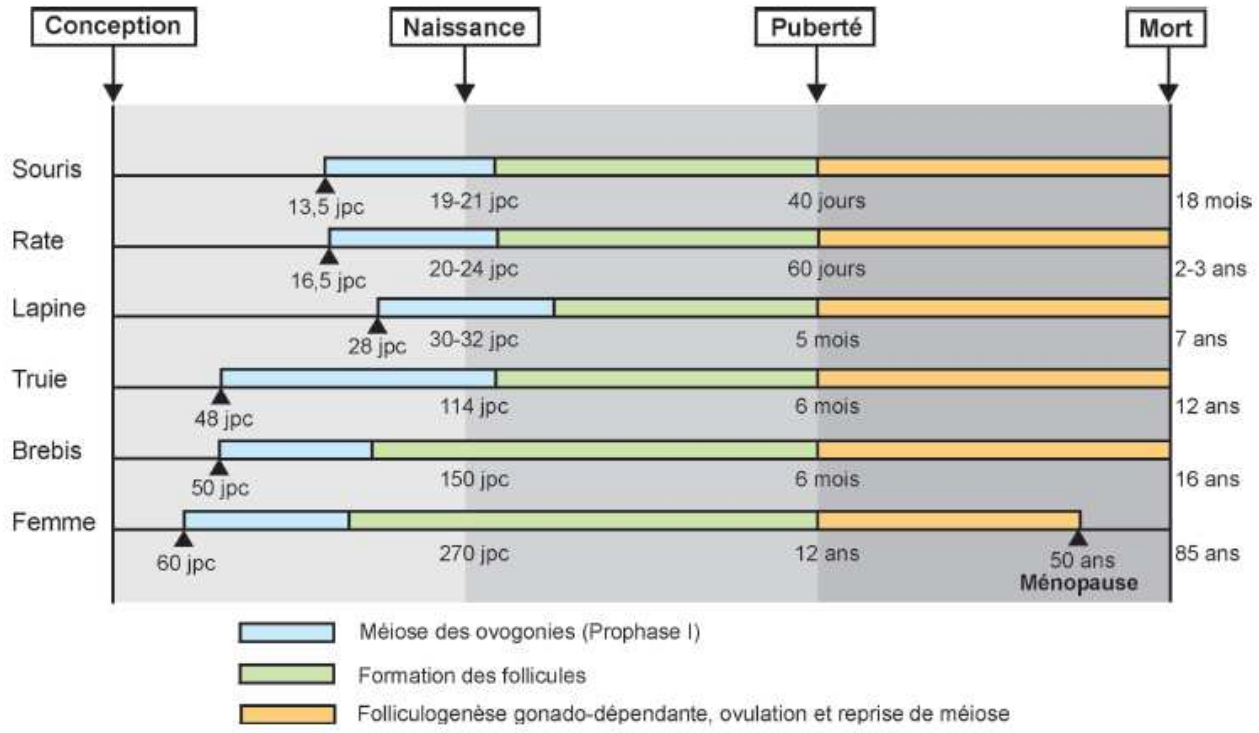
3.3. Transposition to humans

The major differences between humans and animals in terms of kinetics make it difficult to transpose to humans the effects observed in animals. The BPA biotransformation pathways are also different in nature and proportions according to species. The data collected in humans show that BPA glucoronide is the major metabolite, whereas BPA sulphate is more rarely identified and quantified. While glucuronic acid conjugation is the major pathway in rodents, the aglycone is not exclusively unchanged BPA, but part of it is hydroxylated BPA (Zalko *et al.*, 2003). Several other metabolites have also been identified, such as BPA diglucuronide, or methoxylated conjugates (Zalko *et al.*, 2003). Furthermore, the BPA metabolisation enzymes differ between animals and humans. Indeed, in rats, the 2B1 isoform of UDP-glucuronosyl transferase (UGT2B1) is mainly responsible for BPA glucuronidation (Yokota *et al.*, 1999). In humans, it is mainly UGT2B15 and 2B7 that are responsible for this glucuronide conjugation (Hanioka *et al.*, 2008). Finally, extrapolation of the pharmacokinetic data from animals to humans is unreliable owing to the various inter-species differences with regard to the existence or not of an enterohepatic cycle in the glucuronide-conjugated BPA elimination process (INSERM, 2011).

Rodents are born in a relatively immature state compared with humans, and their development continues after birth. In order to induce similar developmental effects, the exposure must be carried out in the neonatal period in rodents and the prenatal period in humans. The newborn rodent would be more vulnerable to this exposure than the human foetus, which is partially protected by the placental barrier. For example, prostate differentiation occurs around the time of birth in rodents (predominantly after birth), whereas it takes place during intrauterine life in humans. Other major differences are also noted in terms of maturation of the central nervous system (CNS) and thyroid function (Howdeshell 2002).

Moreover, the same effect can be initiated by different mechanisms of action which will not necessarily be disrupted by the same factors. For example, the masculinisation of the hypothalamic-pituitary-gonadal (HPG) axis occurs around the time of birth in the male rodent and is partially mediated by oestradiol produced locally in the brain from circulating testosterone. In humans, on the other hand, this developmental stage is initiated in the 3rd trimester of pregnancy and is brought about essentially by androgens, without oestrogens being involved.

Finally, the results must be extrapolated over time in order to adjust for the differences in longevity: the earliest stages of spermatogenesis in rodents are initiated shortly after birth and come to an end at six to eight weeks, whereas these events occur around the age of 12 to 15 in boys. In the same way, the maturation of the organs forming the HP axis, which regulate the oestral cycle, is complete at 15 days in rodents whereas this event occurs at the age of 10 to 12 in girls. Figure 1 lists the periods of ovarian differentiation in various mammals and Figure 2 represents the principal periods of development of the male genital tract in humans and rats, in relation to the level of testosterone production (INSERM, 2011).



* jpc: days post-conception

Figure 1: Comparison of ovarian differentiation periods in various mammals (INSERM, 2011)

FRANCAIS	ANGLAIS
conception	conception
naissance	birth
puberté	puberty
mort	death
souris	[female] mouse
rate	[female] rat
lapine	[female] rabbit
truie	sow
brebis	ewe
femme	woman
mois	months
ans	years

ménopause	menopause
méiose des ovogonies (prophase I)	meiosis in oogonia (prophase I)
formation des follicules	follicle formation
folliculogenèse gonado-dépendante, ovulation et reprise de méiose	gonad-dependent folliculogenesis, ovulation and resumption of meiosis

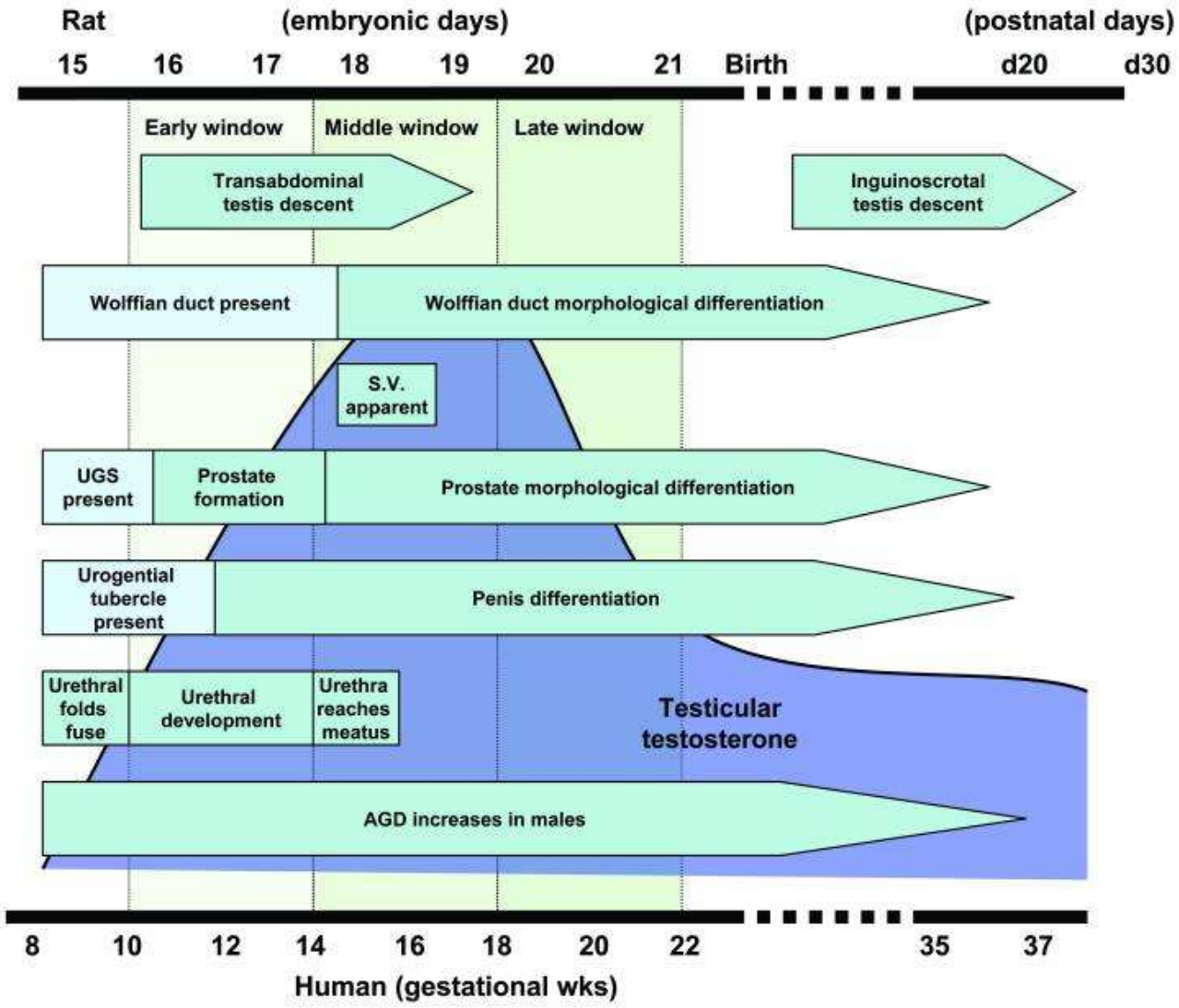


Figure 2: Diagram showing the main developmental periods of the male genital tract in humans and rats in relation to the level of testosterone production (INSERM, 2011) and according to (Welsh *et al.*, 2008)

Consequently, when transposing to humans the results obtained in animals, it is important to consider, as far as possible, the differences in periods of development influencing sexual differentiation and also to consider the role of the various hormones involved in this process.

4. Working method

As indicated in the request letter from the DGPR, the expert appraisal relied on prior work undertaken by expert assessment authorities, and particularly the European Risk Assessment Report prepared in 2008 by the United Kingdom, the preliminary INSERM collective expert assessment report on BPA published in July 2010, and the expert appraisal work undertaken by AFSSA in 2010. A hearing with INSERM representatives and the AFSSA unit in charge of assessing food risks related to BPA was held before the WG. Moreover, the EFSA expert assessment report published in September 2010 and the report by the expert panel which met under the leadership of the FAO/WHO that was published in November 2010 were forwarded and presented to the WG.

In addition to the expert assessment reports on BPA that had recently been published, the experts were invited to analyse original papers considered as key studies for certain types of effects linked to BPA. Furthermore, particular attention was paid to epidemiological studies likely to contain information that could be interpreted in terms of human effects and experimental studies using the subcutaneous route of exposure. In fact, the latter type of study has not undergone systematic analysis in past expert assessments, the majority of which have considered only recognised effects by the oral route of exposure, which have been deemed more representative of dietary exposure. And yet, given that questions have recently been raised regarding non-dietary BPA exposure, including dermal exposure, and that this mode of administration can highlight effects at doses much lower than those that can be administered orally, it is relevant to take these studies into account. Expert *rapporteurs* were thus appointed in the group to assess epidemiological studies and studies using the subcutaneous route of exposure.

Lastly, new articles published from 2010 (when the preliminary INSERM report was published) to January 2011 (bibliography end date) were listed by ANSES and sent to the experts. In addition, some articles published after January 2011 were included when they were likely to influence the expert appraisal's results. Studies assessing the effects of BPA at doses lower than the NOAEL of 5 mg/kg bw/day, which was used to establish the current TDI, were assessed as a priority by the experts.

Summaries of Opinions and assessments undertaken by European and international authorities regarding the effects of BPA at doses higher than the NOAEL of 5 mg/kg bw/day are available in the Annex of this expert appraisal report (e.g. developmental effects other than effects on the male and female reproductive systems, chronic effects, carcinogenic effects, genotoxicity).

In order to guarantee the expert appraisal's traceability, expert *rapporteurs* were appointed, often in groups of two or three, to assess various types of effects linked to BPA which generally correspond to the following sub-sections of Section 6 in this document:

1. Effects on the male reproductive system
2. Effects on the female reproductive system
3. Effects on the brain and behaviour
4. Effects on metabolism and the cardiovascular system
5. Effects on the thyroid
6. Effects on the immune system
7. Effects on the intestine
8. Effects on the prostate
9. Effects on the breasts

The expert *rapporteurs* were thus asked to write a report on their section, referring to the conclusions of the main documents published by national and international expert assessment authorities (AFSSA, 2010a; Aschberger *et al.*, 2010; EC, 2010b; INSERM, 2011; NTP-CERHR, 2008; OEHHA, 2009; Health Canada, 2008; etc.) and by 'Expert Panels' such as the one that met in Chapel Hill in 2007 (vom Saal *et al.*, 2007). The EFSA expert assessment report published in September 2010 and the conclusions of the expert panel which met under the leadership of the FAO/WHO that were published in November 2010 (EFSA, 2010; FAO/WHO, 2010) were also taken into consideration by the WG. These conclusions have been used by ANSES to introduce the various Sections (grey box) and do not bind the WG's experts. The experts also referred to recent articles that were forwarded to them on a regular basis by ANSES. These various reports and articles were sent to the entire group and discussed in a work session. For each type of effect, the available data were presented by exposure period: gestational or *in utero*, prenatal, perinatal, neonatal, postnatal exposure or exposure during puberty or adulthood. The term 'exposure' does not provide information on the number of administrations (e.g. single or repeated).

For those articles considered significant by the experts in providing information about the health effects of BPA, particularly at low doses for which there is currently no consensus in the international scientific community, a publication analysis chart was used by the experts with the support of ANSES. The items on this chart were discussed in the WG; they list the important points to be specified when analysing articles, considering the limiting factors likely to interfere with the interpretation of results that were discussed in the previous Section.

For each type of effect and on the basis of the expert *rapporteurs*' conclusions, the WG was invited to determine the nature of the observed effects and characterise them as:

- Recognised effects
- Suspected effects
- Controversial effects
- Effects for which no conclusion can be drawn on the basis of the available data.

In order to qualify the health effects of BPA, the WG proposed the following decision tree (Figure 3):

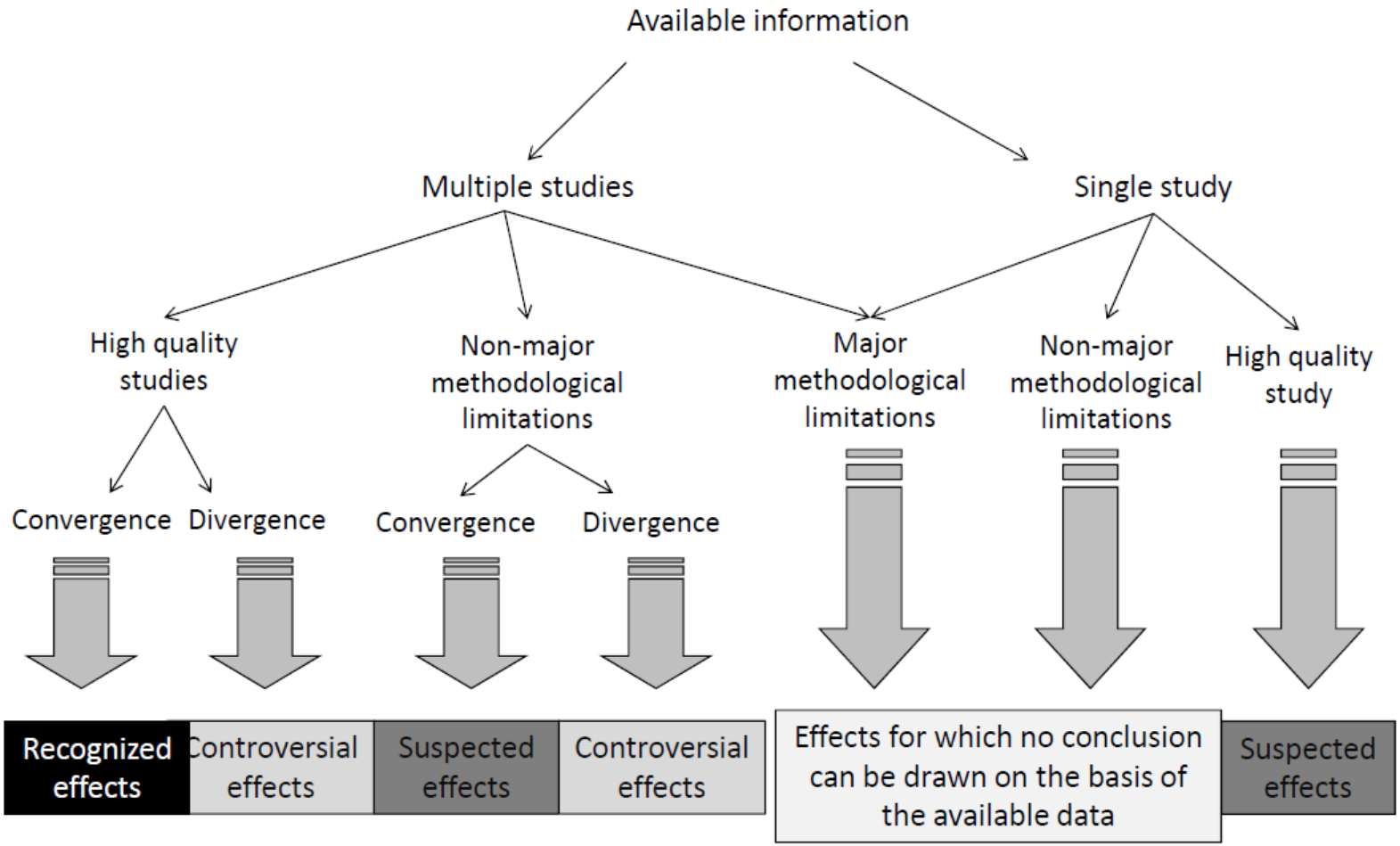


Figure 3: Decision tree

All the available information regarding a health effect was assessed using the decision tree, which can be interpreted as follows:

- When the available information was obtained from one or more studies, each study was analysed and considered either to be of 'good-quality', having 'non-major methodological limitations' or having 'major methodological limitations'.
  A 'good-quality' study was defined as containing an appropriate methodology (coherence of the exposure model, confounding factors taken into account, etc.) and a sufficient number of observations.
  A study was considered to have 'non-major methodological limitations' when one of the above aspects was not considered to be fully satisfactory. Nevertheless, the study could be taken into account in light of its contribution to the expert appraisal. Moreover, co-exposure had to be controlled (composition of feed for laboratory animals, type of cage, type of drinking container, etc.) or the way in which it was managed at least had to be mentioned. When a study had unacceptable shortcomings, it was considered as having 'major methodological limitations'.

- When the results of multiple 'good-quality' studies undertaken by different scientific teams:
  - converged: the effect was considered to be 'recognised',
  - diverged: the effect was considered to be 'controversial'.
- When studies having 'non-major methodological limitations':
  - converged: the effect was considered to be 'suspected',
  - diverged: the effect was considered to be 'controversial'.
- Studies having 'major methodological limitations' were excluded as they could not be used to draw conclusions.
- Lastly, when information was reported in only one study, the methodology was assessed:
  - when it was 'good-quality', the effect was considered to be 'suspected',
  - when it had 'major or non-major methodological limitations', the study was considered to be excluded and could not be used to draw conclusions regarding the effect under consideration.

The classification of effects according to this decision tree was supported by an expert judgment.

Lastly, once the various types of effects had been characterised according to their level of evidence, the WG was invited to interpret the results in terms of transposition to humans in order to undertake a Health Risk Assessment (HRA). The significance of the observed biological effects was thus discussed in order to estimate their relevance for the HRA in humans. This raised several questions: from what level of change in a biological parameter, whether human or animal, is an effect considered to be a significant effect? From what level of change in a biological parameter, whether human or animal, is an effect considered to be an adverse effect? How should one go about interpreting the results of '-omics' studies that show changes in gene or protein expression

without there being any perceptible change in the phenotype? Although some of these questions have already been raised in this document, they will be examined more closely in the final HRA report on BPA.

Further to the hazard analysis, critical effects and key studies for the various exposure routes will be determined in order to define human toxicity values so as to undertake a quantitative assessment of the health risks.

5. Toxicokinetics

EFSA concludes, in its opinion in 2010, on the basis of works by Doerge *et al.*, that the BPA elimination rate is higher in humans than in rodents (Doerge *et al.*, 2010a, 2010b). Consequently, for the same exposure, the internal dose in humans (including in premature babies) will be lower than that in rodents. The standard uncertainty factor of 10 for taking into account inter-species variability when producing the TDI is therefore conservative (EFSA, 2010).

In addition, after oral administration of the same dose in adult rats and monkeys (100 µg/kg bw), the proportion of free BPA is very similar in the two species (< 1%). The only notable difference is a longer elimination half-life in rats. On the other hand, in newborns, not only is the elimination half-life longer in rats, but, in addition, the proportion of free BPA in serum is ten times higher than in monkeys. These data confirm the difference in metabolism in monkeys and in rats, due to the fact that rats are more immature at birth (in relation to the UGT (or Uridine Diphosphate Glucuronosyl Transferase) activity).

Fifteen recent articles were identified in the toxicokinetics field, among which three relate directly to humans. Among all these articles, some confirm previously established data, while broadening them. Others make it possible to advance knowledge significantly. It is these articles that will be examined as a priority.

5.1. Absorption

5.1.1. By oral route

Studies in various species (rats, mice, monkeys) have shown that after oral administration, BPA is rapidly and widely absorbed (INRS, 2010). Analysis of the areas under the plasma concentration time curve (AUC) shows that gastrointestinal absorption is greater than 85% in rats and monkeys. Experiments carried out in adult humans at relatively low doses (0.025 to 5 mg in total) show that BPA is completely and rapidly absorbed by the digestive tract (Tsukioka *et al.*, 2004; Volkel *et al.*, 2002; Volkel *et al.*, 2005). After a single dose, the plasma peak is reached approximately 80 minutes after ingestion.

5.1.2. By cutaneous route

Calculations for estimating the skin absorption of BPA mention a value of 10% of the dose applied, which the work by Kaddar *et al.* using a pig skin model appears to confirm (Kaddar *et al.*, 2008). However, recent work suggests that this level could be greatly underestimated.

In a recent study (Zalko *et al.*, 2011), the diffusion and biotransformation of BPA were studied on cultures of pig ear skin and on explants of human skin. The objective of this group was to develop a model for diffusion and study of metabolism on pig ear skin maintained in a state of survival. Four compounds were used for these studies: 7-ethoxycoumarin, testosterone, benzo(a)pyrene and bisphenol A. The publication by Zalko *et al.*, (2011) relates to the results obtained with bisphenol A (Zalko *et al.*, 2011). The authors report BPA penetration and metabolism results in two *"ex vivo"* models of skin maintained in a state of survival: pig ear skin (dermatomed at a thickness of 500 µm) and human skin (dermatomed at a thickness of 500 µm). The diffusion model is of the "static" type on cells 28 mm in diameter. Radiolabelled BPA was deposited in solution in 60 µl of a mixture of ethanol/0.1 M phosphate buffer, pH 7.4 (1:2, v/v), at various concentrations corresponding to 2.75, 5.5, 11, 22 and 44 $\mu g.cm^{-2}$. The culture medium was removed and the radioactivity was measured at 24, 48 and 72 h. The radioactivity in each compartment (surface, skin, culture medium, wells, inserts) was then determined at 72 h. Only the data at 72 hours are reported in the article. A significant part of the work relates to the analysis of the metabolism of BPA (identification of metabolites in the culture medium by radio-HPLC and comparison with reference products) and the comparison between the "pig ear skin" model and the "human skin" model. It appears that BPA, under the conditions of the experiment and after 72 h of incubation, diffuses significantly through the two skin models: absorption of about 65% for the pig ear skin and 46% for the human skin explants. This result is not surprising if one refers to the physico-chemical characteristics of the compound (MW = 228 and logKow = 3.2). On the other hand, the information provided by the publication does not make it possible to estimate, under saturating conditions, the penetration of BPA through human skin as a function of time. Moreover, it is not a penetration study as recommended by OECD guideline 428 (OECD, 2004). Furthermore, the incubation time of 72 h is well over the 24 h recommended for preserving the integrity of the explant. The exposure modes (for example: solid BPA present on a surface) do not represent presumed human exposure. This study, on the other hand, shows that, at the low concentrations applied to human skin, approximately 40% of the dose that diffuses into the recipient liquid is in the form of glucuronide and sulphate.

Another recent study determined the percutaneous absorption of BPA *in vivo* and *ex vivo* in rats and *ex vivo* in humans, after an exposure of 24 hours (Marquet 2011). The permeability was found to be 12 times greater in rats than in humans. However, inter-individual variability was found in humans. The authors reported skin penetration flux values of 120 $ng.cm^{-2}.h^{-1}$ using samples of human skin explants exposed to 200 µg of $BPA.cm^{-2}$. Finally, contrary to the study by Zalko *et al.*, 2011, the authors find most of the BPA in unchanged form in the recipient fluid, which can be

explained by the much higher dose of BPA applied to the skin samples in the study by Marquet and colleagues (Marquet 2011).

5.1.3. By inhalation

There are no data on BPA toxicokinetics after respiratory exposure. However, in view of the toxicity induced (variations in absolute weight of organs demonstrated in a study of repeat toxicity by inhalation in rats exposed for 13 weeks) and of a favourable octanol/water partition coefficient (3.2), it would be prudent to consider that respiratory absorption can occur. In the absence of data, the bioavailability via the respiratory route cannot be quantified (EC, 2010b). For the risk characterisation part conducted in the European report of 2008, absorption orally and by inhalation was set at 100% and skin absorption at 10% (EC, 2010b).

5.2. Distribution

Once absorbed, BPA is rapidly distributed in all the tissues. BPA has no real affinity for one particular organ. However, in rodents, a few hours after oral administration of radiolabelled BPA, the highest concentrations are found in the liver and the kidneys.

The toxicokinetic data obtained in rats and humans show a considerable first-pass effect and indicate that the plasma residues are mainly (92-99%) in glucuronide form. Several reports indicate a difference in toxicokinetics between the two species, related to the existence of an enterohepatic cycle in rats after hydrolysis of the glucuronide in the intestine, which results in a relatively slow elimination compared with humans (EC, 2010b; INERIS, 2010b; INFOSAN, 2009). This difference has frequently been emphasised in order to underline the limits of the rodent model in assessing BPA risks for humans (Mielke and Gundert-Remy, 2009; Ginsberg and Rice, 2009). While recent studies combining the use of BPA labelled with tritium or with deuterium and specific, sensitive detection techniques (LC-MS/MS) confirm the existence of an enterohepatic cycle in rodents, unlike primates, they indicate that this cycle has very limited consequences on BPA clearance (Doerge *et al.*, 2010a; Doerge *et al.*, 2010b; Taylor *et al.*, 2011) and plead in favour of the relevance of the rodent model for humans with regard to oral exposure to BPA. The studies carried out in rodents indicate that gestation has little effect on the tissue distribution of BPA and of its metabolites. However, they show that BPA crosses the placental barrier and that the concentrations found in foetal tissues are of the same order of magnitude as those measured in the mother. Analysis of the foetal compartment shows that the BPA is free BPA (in the minority) and its glucuronide conjugate (in the majority).

There are no data on the distribution of BPA after cutaneous and/or subcutaneous administration (EC, 2010b).

5.3. Metabolism

In all the species studied, the major metabolic pathway is the conjugation of BPA with glucuronic acid to form BPA-glucuronide (BPA-GA) (Figure 4). This conjugation takes place mainly in the liver and, to a lesser extent, in the intestine. It is catalysed by UGT2B1 in rats, whereas in humans, it is the UGT2B15 and UGT2B7 isoforms which are responsible for this glucuronidation (Mazur *et al.*, 2010). Genetic polymorphism of UGT2B15 could lead to inter-individual differences in the ability to detoxify BPA (Hanioka *et al.*, 2011; INSERM, 2011).

In studies of **pharmacokinetics in humans**, the urinary metabolite profile is made up exclusively or almost exclusively of BPA-glucuronide. Monitoring studies carried out using urine samples collected from adults (Ye *et al.*, 2005) indicate different proportions (9.5% of free BPA, 69.5% of BPA-glucuronide and 21% of BPA-sulphate). Kim *et al.* analysed the proportion of BPA and its metabolites obtained from 15 women and 15 men (Kim *et al.*, 2003). The average urinary composition observed in men is 29.1% of free BPA, 66.2% of BPA-glucuronide and 4.78% of BPA-sulphate, whereas in women, the proportions are 33.4% of free BPA, 33.1% of BPA-glucuronide and 33.5% of BPA-sulphate. The authors conclude that women have a better sulphation capacity than men (NTP-CERHR, 2008).

It has also been possible to identify other metabolites using urine or bile samples **in rodents** or during incubations with hepatocytes in primary culture. These are mainly BPA-sulphate and hydroxylated BPA (Figure 4). In total, these metabolites only very rarely exceed 5 to 10% of the total metabolites in the urine in rodents. Other minor metabolites, such as double conjugates or methoxylated derivatives, have also been identified in rodents.

It has been possible to identify, ***in vitro***, several other metabolites, formed by oxidation, using subcellular fractions (4-methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, isopropylhydroxyphenol, glutathionylphenol, glutathionyl 4-isopropylphenol, and bisphenol A dimers); however, to date, they have not been described *in vivo* (INRS, 2010).

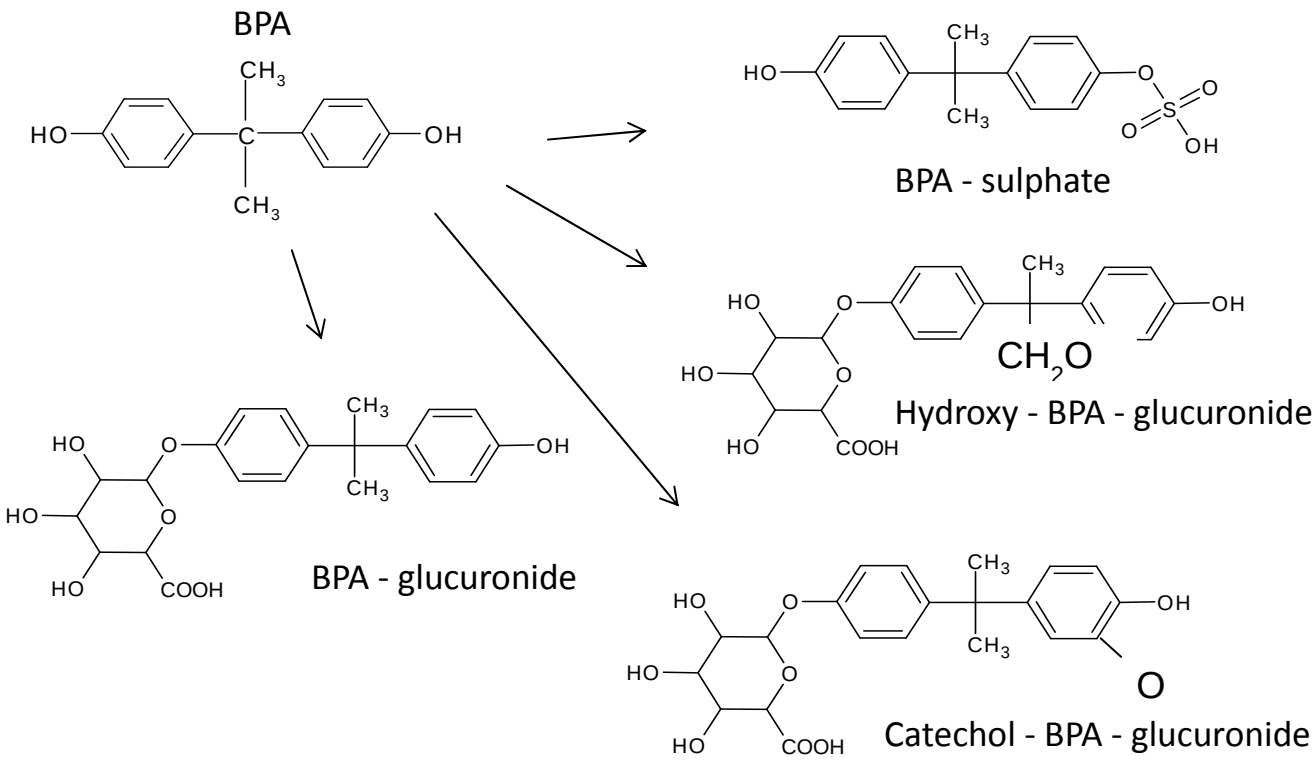


Figure 4: Major BPA metabolic pathways in mammals (INSERM, 2011)

The BPA-glucuronide and BPA-sulphate forms represent BPA detoxification pathways insofar as they are not active on oestrogen receptors. Ginsberg *et al.* have suggested that deconjugation at specific tissue sites by β-glucuronidase and arylsulphatase C enzymes could convert conjugated and sulphated metabolites into "free" BPA which is active on oestrogen receptors (Ginsberg and Rice, 2009). β-Glucuronidase is present in the intestines but also in the placenta and the foetal liver, which could lead to exposure of the foetus to "free" BPA (Aschberger *et al.*, 2010).

The route of administration has an effect on the forms and the circulating levels of BPA, the proportion of free BPA relative to the conjugates (Doerge *et al.*, 2010b; Pottenger *et al.*, 2000; Taylor *et al.*, 2008). The data collected in rodents relate to oral, subcutaneous and intraperitoneal routes and reveal free BPA/total BPA proportions that are much higher in the case of subcutaneous and intraperitoneal administrations. The differences are much smaller in the urine compartment.

5.4. Elimination

There are considerable differences between humans and rodents in the process for elimination of BPA and of the corresponding glucuronide (Aschberger *et al.*, 2010).

In humans, BPA is predominantly metabolised to BPA-glucuronide in the liver, and is then eliminated in this form via the urine. The circulating "free" BPA concentration peak is considered to be very low after oral exposure, given the first-pass effect and the high speed of metabolisation. The plasma half-life time of total BPA is approximately 4-6 h. Several studies have shown a limited excretion of BPA in maternal milk (Vandenberg *et al.*, 2007a).

In cynomolgus monkeys, exposed orally to low doses of BPA (<100 µg/kg bw), most of the dose administered (85 to 100%) is excreted in the urine, as in humans. The plasma elimination half-life of total BPA in rhesus monkeys is between 3.5 and 9 h, i.e. comparable to that of humans (Doerge *et al.*, 2010a; Taylor *et al.*, 2011).

In rodents, after glucuronidation of BPA in the liver, BPA-glucuronide is excreted in the bile and then hydrolysed by a glucuronidase and reabsorbed in the intestine in the form of "free" BPA. Thus, a large proportion of the administered dose (45%-66%) is rapidly excreted in the bile in the form of BPA-glucuronide, with a biliary excretion rate that tends to be higher in males than in females. This enterohepatic cycle results in a slower elimination in rodents than in humans.

The excretion of BPA and of its metabolites in the urine accounts for 10-40% of the dose administered, and the main route of excretion in rats is via the faeces (50-83% of the dose administered), partly in the form of free BPA. Seven days after oral administration of BPA, approximately 80% and 70% of the dose is eliminated via the faeces in males and females, respectively. The major part of the dose is excreted 72 hours after administration.

In the urine, BPA is mainly eliminated in the form of BPA-glucuronide (82% of the radioactive labelling found in the urine), and then in the form of "free" BPA (14%) and BPA-sulphate (4%) (Aschberger *et al.*, 2010; EC, 2010b).

Most of the BPA excreted in the faeces is in the form of "free" BPA which probably mostly comes from the hydrolysis, in the gastrointestinal tract, of the BPA-glucuronide excreted in the bile, rather than from non-absorbed BPA.

In a recent study, it was determined that BPA passes into milk, on the basis of experiments carried out in rats exposed orally (Doerge *et al.*, 2010b). Suckling female rats (n=5) were subjected to daily gavage for one week with deuterated BPA (100 µg/kg bw), from the day on which the newborns were born. A control group (n=3) was treated with the vehicle only (ethanol/water, 1:9 v/v). The milk samples were taken after injection of oxytocin exactly one hour after the administration of BPA. The milk and serum analyses were carried out by LC/MS-MS. They were carried out at PND7

for the milk and at PND10 for the serum (for the mothers and their young). The serum analyses confirmed the low percentage of aglycone (0.5%). The assays carried out on the milk indicated average concentrations of free BPA and total BPA corresponding, respectively, to 0.87 and 7.6 nM, i.e. a milk/serum ratio of 1.3 for the free BPA and of 0.062 for the total BPA.

This article clearly shows that exposure of newborns to free BPA, following exposure of the mother, is very low. The serum concentrations of total BPA were 300 times lower in the young than in the mothers, the free BPA being undetectable in the young. The results obtained, compared with data previously obtained by the same authors in young rats at PND10, indicate that the serum concentrations are in this case 500 times lower than those obtained with administration via gavage of a dose of 100 µg/kg bw, i.e. the dose administered in this study with the mothers.

5.5. Toxicokinetics of BPA during gestation and in the foetus

The toxicokinetics and metabolism of BPA appear to be different in gravid females, foetuses and newborns in comparison with non-gravid adults (Health Canada, 2008).

In pregnant women, several studies have measured BPA (free + conjugated) concentration levels in the serum, in umbilical cord blood and in the plasma of foetuses (Vandenberg *et al.*, 2007a). The results of these studies show that BPA crosses the placental barrier. In one study (Ikezuki *et al.*, 2002), the concentrations found in the maternal serum varied from 1.4 to 2.4 ng/mL, whereas the concentrations found in the amniotic fluid (15-18 weeks of gestation) were higher (approximately 8.3 ng/mL).

In F344 **gravid female rats**, after oral administration of a single dose of BPA of 1 g/kg of feed, BPA absorption and distribution were rapid in the organs. After 20 minutes, the substance had reached the foetus via the placenta, and after 40 minutes, the foetal concentration exceeded the mother's blood concentration (Health Canada, 2008).

In a recent study, exposure of the foetus to free BPA was investigated by studying not only the crossing of the placental barrier, but also the possibility of *in situ* release of free BPA from the deconjugation of BPA-glucuronide (Nishikawa *et al.*, 2010). This study was carried out in pregnant female rats, whose uteruses were perfused with BPA-glucuronide at a concentration of 10 µM. The BPA and BPA-GA contents are determined from LC/TOF-MS analyses. No contamination of BPA-GA with BPA was observed. This study aimed to assess the transfer of BPA to the foetus and the amniotic fluid.

The results show that BPA-GA is found in the foetus (110 pmol), as to a lesser extent is free BPA, with a 95/5 ratio, showing for the first time that the foetus is capable of deconjugating BPA-GA. The

amniotic fluid analyses indicate the presence of BPA (31.4 pmol), whereas no trace of BPA-GA was detected (LOQ = 20 pmol/mL). This result is explained by the fact that the action of beta-glucuronidases, which are deconjugating enzymes, is expressed in the young before the conjugating enzymes (UDPGT); this shift in equilibrium is in favour of BPA deconjugation. UDPGTs appear late or even after birth in primates. The UGTB1 measurements confirm that they are very weakly expressed in the foetus. The authors also demonstrated in the placenta a high level of expression of the Oatp4a1 and Mrp1 transporters, which might be involved in the management of BPA-GA in the placenta. This publication shows that the foetus may be exposed to free BPA despite the fact that circulating levels in the mother are mainly in the form of glucuronide conjugate. Contamination of the samples with free BPA is not very likely since many samples prove to be negative with regard to the presence of BPA.

5.6. Toxicokinetics of BPA in newborns

In human newborns, several metabolic pathways, such as, for example, glucuronidation (two to five times lower in premature newborns), and several excretory functions, such as, for example, glomerular filtration (1.7 times lower), have a lower efficiency compared with that of adults; these functions reach their full capacity one month and seven months after birth, respectively (EFSA, 2008). In 2008, a request was made to EFSA to re-examine the toxicokinetics of BPA according to age and the implication of these toxicokinetics in risk assessment and therefore in establishing the TDI. EFSA concluded that this immaturity in the glucuronidation capacity of newborns could be compensated for by the presence of sulphotransferases (SULTs), which would result in efficient detoxification of BPA (Aschberger *et al.*, 2010; EFSA, 2008). Indeed, unlike UGTs, sulphotransferases, for which the substrates of UGTs have a strong affinity, are active in developing foetuses and are functional at birth. These enzymes efficiently catalyse the formation of BPA-sulphate *in vitro* in humans. Finally, EFSA concluded that BPA's capacity for biotransformation into inactive metabolites was sufficient in human newborns.

Studies in rats have shown that, in newborns, the glucuronidation pathway is more saturable than in adults, which could induce a higher concentration of "free" BPA in the target tissues. The glucuronide conjugation capacity through UGT activity is also low after birth and remains low after weaning (Aschberger *et al.*, 2010).

The studies by Doerge *et al.* in rat and rhesus monkey newborns confirm that most of the toxicokinetic parameters are significantly different from those determined in adults, particularly with regard to total BPA (Doerge *et al.*, 2010a; Doerge *et al.*, 2010b). Nevertheless, regarding the maximum serum concentrations (C_{max}) of free BPA, while they are significantly higher in newborn rats (at PND3 or PND10) than in adults, the same does not appear to be true in monkeys.

These same authors also showed, in adult and newborn rats, that the subcutaneous administration of BPA considerably modified the toxicokinetic parameters, as well as the free BPA/conjugated BPA ratio in serum in newborn rats (Doerge *et al.*, 2010b).

In conclusion, there are toxicokinetic differences between rodents and humans in terms of BPA, but these differences are minor and do not call into question the value of the rat or mouse model for understanding the toxic effects of this compound. In particular, the influence of the enterohepatic cycle present in rodents, but not in monkeys or humans, is limited. The main BPA detoxification pathway is conjugation with glucuronic acid. The conjugated forms are not active on oestrogen receptors, but deconjugations releasing BPA may occur in certain tissues. They have been demonstrated in the rat foetus.

There are still several uncertainties regarding the toxicokinetics of BPA. Pregnant women, foetuses and newborns can be considered to be susceptible subpopulations. The current pharmacokinetic models do not make it possible to fully characterise the absorption, distribution, metabolism and excretion of BPA in these subpopulations. They also all reveal an inconsistency between the exposure values calculated on the basis of food contamination and the values measured in the blood or urine of the general population.

6. Health effects

6.1. Information from epidemiological studies

Epidemiological studies have been evaluated by organisations such as EFSA, NTP-CERHR, OEHHA, FAO/WHO, AFSSA, INSERM, etc. According to INSERM (2011): “In conclusion, overall, the epidemiological studies are too few to determine the probability in humans of the effects observed in animal experiments. At present, studies conducted in women concerning the risk of breast cancer or endometriosis are all based on a retrospective approach (particularly limited for a non-persistent compound like bisphenol A), and convenient clinical populations, without a specific sampling plan. In men, a study in China suggests an association between occupational exposure to BPA and impaired sexual function; in addition, two studies, one from the same population and another from a population of men seen in an infertility clinic in Massachusetts, reported a decrease in sperm concentration associated with exposure to BPA in adulthood at doses corresponding to the level found in the general population. The consistency of these two studies and their cross-sectional character suggest seriously considering the hypothesis of a BPA effect on sperm concentration in adulthood. Confirmation or refutation of the probability of finding in humans some of the effects demonstrated in animal experiments following exposures during the developmental phase would involve studies with regular monitoring of exposure in pregnant women, and health monitoring of their progeny; long and difficult methodologically, requiring large populations” (INSERM, 2011).

Epidemiological studies have been conducted over the last decade, including an analysis of the possible effects of BPA. These studies aim to explore the impact on reproductive function in men and women, the risk of miscarriage or prematurity, endometriosis, obesity, the effects on child behaviour, the age of onset of puberty, breast development, and especially an association with breast cancer.

The results of these studies have been discussed in various sections of this expert report, with a particular focus on recently conducted studies.

A summary table of the epidemiological studies analysed in this report is available in the Annex (Annexe 7). This table includes a reference to the quality of these studies, as well as to the section(s) concerned.

6.1.1. Studies of high quality, or not presenting major methodological limitations

The cross-sectional study of Itoh *et al,* did not find any association between urinary BPA and **endometriosis** in 140 Japanese patients from an infertility clinic (Itoh *et al.*, 2007). Women who had a pregnancy with childbirth were excluded. For unknown reasons, the authors recruited nine women with no fertility problems "to increase the statistical power." BPA was analysed in urine collected just before laparoscopy. The diagnosis was made according to the laparoscopic criteria of the American Fertility Society Association, stage 0 to IV. The median level of the group 0-I was 0.80 µg/g creatinine versus 0.93 µg/g creatinine in the group II-IV.

The study by Wolff *et al,* (**growth parameters at birth**, 367 subjects) demonstrated no correlation with the BPA analysed once during the third trimester of pregnancy (Wolff *et al.*, 2008b). The authors analysed five phenols including BPA and ten phthalate metabolites. In a recruited population of 479 women, 75 (16%) were excluded from the analysis, mainly because of complications of pregnancy or loss to follow-up (n=19). Concentrations of BPA ranged from the limit of detection to 35.2 µg/L (median 1.3 µg/L).

The study by Wolff *et al,* (**puberty in girls, breast development**, 192 subjects) demonstrated no relationship between BPA and the stage of puberty (Wolff *et al.*, 2008a). In addition to BPA, phytoestrogens, lead, DDE and PCBs were analysed. This result was confirmed in a cohort of 1151 girls (Wolff *et al.*, 2010). The latest study included the analyses of phytoestrogens, phthalates, triclosan, and phenols other than BPA. In both cases, the authors do not provide the range of concentrations across the population; they are presented by study groups. The geometric means ranged from 1.6 to 2.4 µg/L.

Two studies (Lang 2008, Melzer 2010) **analysing cardiovascular disease, diabetes and biochemical blood parameters** in adults deserve special attention, because they concern an NHANES population representative of the general US population (Lang *et al.*, 2008) (Melzer *et al.*, 2010). In a sample of 1455 persons in 2003-2004, a positive association was observed between urinary BPA, certain liver enzymes, the risk of diabetes, and cardiovascular disease. In 2005-2006, the levels of BPA were significantly lower. Only an association with the risk of cardiovascular disease was observed. No animal studies have been done to support these observations. The authors have reservations about causality in these associations, and indicate that further studies are needed.

Another 2003-2006 NHANES study sought an association between BPA and **allergies** (n=2133) and **anti-CMV antibodies** (n=787). A positive association with cytomegalovirus antibodies was observed, but the extent and causality of this relationship remains uncertain. In addition, the authors worked only with a population with detectable levels of urinary BPA, which implies that

individuals with levels below the detection limit were excluded, which is questionable (Clayton *et al.*, 2011).

Two studies have examined the relationship between BPA and **psychomotor development**. That of Braun *et al.* has been quite criticised (Braun *et al.*, 2009), especially by AFSSA, which in 2010 did not consider it to be of acceptable quality. It is highly probable that the positive association found for the externalising behaviour found only in girls is related to chance. However, it should be noted that FAO/WHO experts consider it a priority to replicate this study in a large cohort, combined with several urinary measurements, particularly in early pregnancy (FAO/WHO, 2010). A second study by Miodovnik *et al.* sought to correlate the level of urinary BPA and phthalates analysed during pregnancy with the sociability of multi-ethnic urban children aged 7 and 9 years in 137 children. No significant association was found between urinary and social problems for BPA. BPA was positively correlated with the severity of social problems ('Social Responsiveness Scale'), but this relationship was not statistically significant (Miodovnik *et al.*, 2011).

The study by Hong *et al.* (**markers of oxidative stress and insulin resistance**, 960 subjects) demonstrated no correlation with markers of oxidative stress (Hong *et al.*, 2009). However, subjects with high fasting insulin levels had more urinary BPA. The HOMA index (Homeostatic Model Assessment) was not linked to BPA. BPA was analysed in 516 samples. In 24% of subjects, BPA was not detectable, and the median was 0.63 ng/mL.

Three studies of acceptable quality address the issue of **male sex hormones** (Table III).

Table III: Human epidemiological studies of high quality and with no major methodological limitations relative to the study of male sex hormones

	Number	Population	Results	Urinary BPA
(Galloway *et al.*, 2010)	715	General	men: ↗ total testosterone (n=316)	1.3 - 11.5 ng/mL (24h)
(Meeker *et al.*, 2010a)	167	Infertile couples	↗ FSH, FSH/inhibin B and oestradiol/testosterone, ↘ inhibin B	<0.4 - 36.4 ng/mL
(Mendiola *et al.*, 2010)	375	Fertile men	↘ free testosterone index (total testosterone/ SHBG) ↗ SHBG	<0.4 - 6.5 ng/mL

In the studies of Meeker *et al.* and Mendiola *et al,* two populations were studied: men in infertile couples and fertile men (Meeker *et al.*, 2010a; Mendiola *et al.*, 2010). In both populations, the concentrations of various proteins and hormones (FSH, LH, testosterone, oestradiol, inhibin B,

etc.) and urinary concentrations of BPA were measured. Hormonal and protein changes were observed in the two studies, as shown in the table above. Note that the observed changes were different according to the two studies. Concentrations of thyroid hormones and TSH were also measured in the work of Meeker *et al,* but no relationship was observed with concentrations of urinary BPA (Meeker *et al.*, 2010a).

The study by Galloway *et al.* is not inconsistent with these studies, but the choice of hormones investigated was restricted to gonadal hormones: free and total testosterone, oestradiol as well as SHBG (sex hormone-binding globulin) (Galloway *et al.*, 2010).

Four studies address the question of the **quality of gametes**, including three in men (Table IV).

Table IV: Human epidemiological studies of high quality and with no major methodological limitations, related to the study of male gamete quality

	Number	Population	Results	Urinary BPA
(Li *et al.*, 2011)	218	Workers	↘ concentration, mobility, vitality of sperm	Median=39 (6-354) µg/g creatinine
(Meeker *et al.*, 2010b)*	190	Infertile	↘ sperm concentration and percentage of typical forms; ↗ fragmentation of DNA in sperm nucleus	ND – 36.4 ng/mL
(Mendiola *et al.*, 2010)	375	fertile men	No relationship	ND – 6.5 ng/mL

* of 114 subjects having a parameter below the WHO standard of 99 (i.e., a sperm count of 40 x 10^6, a sperm concentration of 20 x 10^6/mL and mobility of 50%), an interquartile increase in BPA is associated with a decrease of 27% in concentration and 16% in morphology

The study by Mendiola *et al.* failed to show any correlation between urinary concentrations of BPA and sperm characteristics in urinary levels lower than 6.5 ng/mL (Mendiola *et al.*, 2010).

However, Meeker *et al.* have shown a relationship between a change in the characteristics of semen, increased fragmentation of sperm nuclear DNA, and the urinary concentration of BPA in a population of infertile men with urinary BPA concentrations not exceeding 36.4 ng/mL (Meeker *et al.*, 2010b). It is important to note that some parameters of semen analysis were not performed in accordance with the WHO recommendations (WHO, 1999).

Li *et al,* have shown a link between sperm parameters and urinary concentrations of BPA in a population of workers exposed to BPA (Li *et al.*, 2011). In this study, of 888 men approached, only 58% participated, and their motivations are unknown (fertility problem, age, etc.), which can constitute a selection bias. Measurements relating to semen quality were conducted in 218 subjects.

The study of Mok-Lin *et al,* conducted in women treated by *in vitro* fertilisation (IVF), demonstrated a negative association between BPA, oestradiol, the number and stage of oocyte maturation (Mok-Lin *et al.*, 2010). Urinary concentrations of BPA ranged from <0.4 to 25.5 µg/L. The geometric mean was 2.5 µg/L. The study was conducted on 112 cycles (total of 84 women), and 203 urine samples (2 samples for 91 cycles and one sample for 21 cycles).

The study by Fujimoto *et al.* examined the relationship between serum BPA and maturity of oocytes and fertilisation rate in 58 women treated with IVF (Fujimoto *et al.*, 2011). Urinary BPA was analysed in women and in 26 male partners. The median concentration of BPA was 2.53 ng/mL, with maximal concentrations of 67.4 ng/mL in women and 0.34 ng/mL in men (with maximal concentrations of 22.7 ng/mL). Of 59 cycles, 13 oocytes on average were collected per cycle. The authors report a significant association between serum BPA in women and decreased fertilisation rates. However, patients who used two procedures for *in vitro* fertilisation (with and without sperm microinjection) were considered as one group, despite the fact that the quality of male gametes was different in these two groups.

The analysis of all of these studies:

- does not allow a conclusion to be drawn about changes in hormonal and protein profiles, and the quality of sperm,
- allows some doubt to remain about the impact on the quality of gametes in sterile females followed for medically-assisted procreation (MAP).

Two studies of acceptable quality address the **problems of sexual function**. The cross-sectional study of Li *et al.* recruited male workers exposed to bisphenol A and estimated by questionnaire the frequency of certain disorders of sexual function declared in an interview (Li *et al.*, 2011). The authors concluded that there was a dose-response association between high levels of cumulative exposure to BPA and an increased risk of impaired sexual function. In a second article by Li *et al,* which completes the first study, a determination of BPA levels in the urine was performed in 427 workers exposed to BPA (Li *et al.*, 2010b). The results demonstrated an association between exposure to BPA and the existence of sexual dysfunction (high levels of BPA in the urine were significantly correlated with an increase in sexual dysfunction). However, these studies involved the use of questionnaires during individual interviews, probably not blinded to the worker's status

vis-à-vis the exposure. In addition, other questions remain about the choice of threshold values for sexual-life events.

The epidemiological studies available at present have failed to demonstrate an association between urinary BPA concentrations measured postnatally and the development of puberty in girls.

In addition, as noted in the introduction to this section, all these studies will be discussed in the different sections of this expert report.

6.1.2. Studies not selected due to major methodological limitations

When analysing the quality of epidemiological studies, it appeared that some of these studies had major methodological limitations, such as low population numbers investigated, not taking into account relevant confounders, determination of BPA using an unsuitable technique, an unsuitable method of sample storage, etc. The studies below have therefore not been taken into account when assessing the health effects of BPA:

- (Cantonwine *et al.*, 2010),
- (Cha *et al.*, 2008),
- (Cobellis *et al.*, 2009),
- (Hanaoka *et al.*, 2002),
- (Hiroi *et al.*, 2004),
- (Kandaraki *et al.*, 2011),
- (Meeker *et al.*, 2010a): investigation of the effects on thyroid hormones,
- (Padmanabhan *et al.*, 2008),
- (Sugiura-Ogasawara *et al.*, 2005),
- (Takeuchi *et al.*, 2004),
- (Yang *et al.*, 2009).

In addition, as noted in the introduction to this section, all epidemiological studies analysed are reported in a summary table (Annexe 7).

6.2. Effects on the male reproductive system

In men, three studies have examined the link between urinary BPA and semen quality (Mendiola *et al.*, 2010; Meeker *et al.*, 2010; Li *et al.*, 2011). The conclusions of the panel of experts convened under the auspices of the FAO/WHO indicate that these three epidemiological studies reported associations between the highest urinary concentrations of BPA and one or more parameters of impaired sperm quality (FAO/WHO, 2010). However, for two of these studies, the association was not statistically significant, and for one of the three studies, co-occupational exposures were partially studied (Li *et al.*, 2011). Given the weaknesses in these studies, the panel recommended further studies to investigate the effects of BPA on sperm quality, including several samples of semen and urine in men participating in the epidemiological studies.

In animals, nearly 100 studies *in vitro* and *in vivo*, most in the rat or mouse, have studied the effects of BPA on the male reproductive system. Their results are contradictory. These studies differ in many parameters, such as strain, age, or stage of development of the animals, nature of exposure, the effects investigated and mode of investigation (type of clinical and histological examinations, etc.) the number of animals per group, and so on. Prostate and testicular effects reported in some studies with low or intermediate doses of BPA administered at different stages of development were not consistently observed (OEHHA, 2009). Thus, the current data are not sufficient to conclude that exposure to BPA during pregnancy or adulthood can lead to a reduction in the quantity and quality of sperm (NTP-CERHR, 2008).

However, AFSSA, in its expert appraisal in 2010, questions the possibility of transgenerational effects related to fertility in males, and the expression of steroid receptors observed after perinatal exposure to low doses of BPA by Salian (2009a, b, c) because of too many methodological flaws. AFSSA considers the work of this team a warning signal without demonstrated significance for human health. The results do not establish a dose-response relationship (AFSSA, 2010a).

The expert panel convened under the auspices of the FAO/WHO in 2010 took into account studies published since 2008.

The experts on this panel believe controversial the evidence for:

- change in the age of puberty in F1 males,
- change in weight and histology of male reproductive organs in exposed adults,
- change in hormone levels in exposed adults and F1 males,
- change in weight, histology and sperm quality in F1 males.

The experts believe there to be considerable uncertainty about the conventional reprotoxic effects of BPA by oral or dermal route in rodents at doses < 1 mg/kg bw/d.

The experts estimate as significant the uncertainties about:

- the effects of oral or dermal exposure to BPA in rodents at doses <1 mg/kg bw/day on conventional targets related to reprotoxicity or development,
- potential effects in men at current exposure levels.

According to INSERM (2011), based on human data, the hypothesis of an effect of BPA on adult sperm production is to be seriously considered (INSERM, 2011). INSERM also indicates that in several recent studies in animals, performed in a single gestation/lactation period and with exposure to low doses, effects found in the subsequent unexposed generations suggest an action on the male germinal line, leading to fertility deficiencies in F1 and their male descendants, even those not exposed. However, the relevance of this work for predicting a risk for humans in whom the exposure is continuous remains relative (INSERM, 2011).

6.2.1. Human data

The cross-sectional study of Li *et al.* recruited male workers exposed to bisphenol A and estimated by questionnaire the frequency of certain sexual dysfunctions, declared during an interview (Li *et al.*, 2011). The frequency of these disorders was compared with that observed using a similar approach in a control population composed of men not exposed to BPA. The participation rate was 62% for those exposed vs 55% for those not exposed. The authors concluded that there was a dose-response association between high levels of cumulative exposure to BPA and an increased risk of impaired sexual function. However, some questions remain: were the interviews, during which it was necessary to answer a questionnaire, blinded? How were threshold values chosen regarding sexual activity? In a second article by Li *et al,* which completed the first study, the level of BPA in urine was obtained in 427 workers exposed to BPA (Li *et al.*, 2010b). The results demonstrated an association between BPA exposure and the existence of sexual dysfunction (high levels of BPA in the urine were significantly correlated with increased sexual dysfunction).

In 2011, Li *et al.* demonstrated a link between sperm quality and urinary BPA concentration in workers (high BPA was associated with decreased sperm count, mobility and vitality). The participation rate was 58% (n= 514 of 888 eligible) but the analysis was done on only 218 men for whom the authors had a semen analysis and urinary BPA concentration) (Li *et al.*, 2010a).

In a population of fertile men (partners of pregnant women), no correlation was found between sperm parameters and urinary concentrations of BPA (Mendiola *et al.*, 2010). Only an inverse relationship was observed between urinary BPA concentrations and the free testosterone index (FTI) and the FTI/LH ratio.

A similar study was carried out by Meeker *et al.* in a population of infertile men (n=190). The urinary concentration of bisphenol A was associated with increased plasma levels of FSH (in contrast to the study of Hanaoka *et al,* but the study population was not the same (Hanaoka *et al.*,

2002)), a decrease in the level of inhibin B and the oestradiol/testosterone ratio (considered by the author as a marker of aromatase activity), and an increase in the ratio of FSH/inhibin B (Meeker *et al.*, 2010a). These studies also suggest a link between BPA exposure and impaired sperm quality (only for urine samples obtained the same day as the semen collection) but these data remain to be confirmed (Meeker *et al.*, 2010b).

In a recent cross-sectional study in the general population, Galloway *et al.* demonstrated in men (n=307) a relationship between BPA in the urine and the concentration of total testosterone in the serum; i.e., the higher the testosterone level in the serum, the higher the urinary BPA concentration (Galloway *et al.*, 2010).

6.2.2. Animal data

6.2.2.1. Exposure during gestation/lactation

Several teams have studied the effects of BPA after exposure during gestation/lactation. These studies were conducted in rodents, either in or mice or rats, with great diversity in terms of exposure (dose, duration, exposure route, etc.). Some studies found effects (Kabuto *et al.*, 2004; Okada et Kai, 2008; Salian *et al.*, 2009c; Watanabe *et al.*, 2003), while others did not (Howdeshell *et al.*, 2008; Kobayashi *et al.*, 2002; Tinwell *et al.*, 2002).

In 2002, the work of Tinwell with rats (Wistar and Sprague Dawley) failed to show significant effects following exposure to BPA at doses of 20 mg/kg, 100 mg/kg on the male reproductive function (litter size, sex-ratio, weight and organ weights, anogenital distance, sperm reserves) (Tinwell *et al.*, 2002). Similarly, in the Long-Evans male rat, no modification of various reproductive parameters (anogenital distance, organ weights, sperm production, puberty, and hormone levels) was observed after exposure to ethinyl oestradiol (EE2) at relatively low oral doses of 0.05 to 1.5 µg/kg bw/day or BPA at doses of 2, 20, 200 µg/kg bw/day (Howdeshell *et al.*, 2008). It should be noted that in this study, the rats' feed contained phyto-oestrogens.

Watanabe *et al.* exposed pregnant rats and their pups (Sprague Dawley) by gavage on the sixth day of gestation to the twentieth day of life (pups, F1) to different doses: 0 (control), 4, 40, and 400 mg/kg bw/day (Watanabe *et al.*, 2003). The testes and blood of the animals were recovered at 9 and 36 weeks of life. The testicular weights were unchanged. On the other hand, serum testosterone levels in rats (9 weeks) were significantly increased, starting with the dose of 4 mg/kg bw/day, and this without changing the concentrations of LH, FSH, and oestradiol. These results suggest an effect of BPA on the homeostasis of testosterone in males, with notably a possible change in aromatase activity, or increased enzyme activity of cytochrome P450scc, or a decrease in 5-α-reductase. The Kabuto team worked with mice (Kabuto *et al.*, 2004). The aim of this work was to investigate changes in endogenous antioxidant capacity and oxidative damage in the brain,

liver, kidneys, and testes after exposure to BPA. The mice were exposed during gestation and lactation through drinking water at doses of 5 or 10 µg BPA/mL. Male mice were sacrificed at 4 weeks. It was shown that BPA could induce oxidative stress and peroxidation of tissues, leading notably to hypotrophy of the testes. Okada *et al.* also exposed mice via a subcutaneous implant at doses of 100 µg or 5000 µg during gestation/lactation(Okada and Kai, 2008). Male mice were sacrificed at 4 weeks. Different parameters (weight of reproductive organs, testosterone, histological analysis) were analysed. Only the percentage of seminiferous tubules with elongated spermatids was significantly lower in animals exposed to 5000 micrograms of BPA.

More recently, in the Salian study, rats (Holtzman) were exposed by gavage from the twelfth day of gestation to day 21 of life to two doses of BPA (1.2 and 2.4 µg/kg bw/day) (Salian *et al.*, 2009c). The diet was devoid of phytoestrogens. In parallel, a positive control group was treated with diethylstilbestrol, DES, at a dose of 10 µg/kg bw/day. At D75, male rats were bred with unexposed females. Males (not exposed) of subsequent generations (F2 and F3) were also studied. In the first generation (F1), a significant increase in post-implantation loss, a decrease in litter size and in the number and motility of sperm were observed, and a significant decrease in hormonal levels in adults (FSH, LH, testosterone, oestradiol). In the three generations, the testicular expression of receptors to oestrogen β and receptors to androgen was decreased. On the other hand, the expression profile of α receptors to oestrogen was only increased in the first generation. The effect maintained in subsequent generations (F2, F3) could suggest the involvement of genetic/epigenetic mechanisms.

6.2.2.2. Neonatal exposure

The only two works studying this period of exposure do not yield the same conclusions.

In the work of the Kato team, rats (Sprague Dawley) were exposed from birth to the ninth day of life to BPA (2 and 9700 µg/kg bw/day) by subcutaneous injection (Kato *et al.*, 2006). 17 β-oestradiol was used as positive control. The rats were observed on the tenth, thirty-fifth and one hundred and fiftieth days. The results showed no abnormalities of reproductive parameters, hormonal disturbances or changes in gene expression in the testis, regardless of the dose of BPA.

Salian's team exposed rats (Holtzman), via subcutaneous injections, to different doses of BPA (100, 200, 400, 800, 1600 µg/kg bw/day) from the first day of life until PND5 (Salian *et al.*, 2009b). The rats were then observed at various times after the end of exposure (PND15 to PND90). DES was used as positive control. Abnormalities in spermatogenesis were identified, with a decrease in epididymal reserves and also sperm motility, and increased post-implantation loss and decreased litter size. There was no dose effect. Immunohistochemical analysis revealed a significant

decrease in the expression of connexin 43, and an increased expression of N-cadherin and zona occludin-1, proteins constituting the adherens junctions of Sertoli cells. It is interesting to note that a recent study in an *in vitro* model (Li *et al.*, 2009) demonstrated that BPA can alter the haemato-testicular barrier, with a reversible reduction in the expression of connexin 43. These alterations could be one of the mechanisms leading to alterations in spermatogenesis.

6.2.2.3. Pubertal exposure

Only the study of Della Setta *et al.* examined the effects of BPA on male reproductive function at doses consistent with human exposure after pubertal exposure (Della Seta *et al.*, 2006). Rats were orally exposed during puberty (PND23 to PND30) to 40 µg/kg bw/day of BPA, and in parallel, another group of rats was exposed to 0.4 µg/kg bw/day ethinyl oestradiol (EE2). Observations on animal hormones and behaviour were made at PND37 and PND105.

A significant decrease in testosterone levels was observed with both BPA and EE2 in juvenile animals at PND37. This decrease persisted in the animals into adulthood (at PND105), and was statistically significant in animals treated with BPA alone. No significant change in the concentration of oestradiol was observed, either on PND37 or PND105. In addition, exposure to BPA altered sexual activity (sequence and duration of the coupling phases) of the males, reducing performance in adults, but less marked than with EE2.

6.2.2.4. Adult exposure

The study by Chitra, exposing adult rats (Wistar) orally to BPA for 5 weeks at doses of 0.2, 2, 20 µg/kg bw/day showed a significant decrease in the relative weight of the testes and epididymis, a significant increase in the relative weight of the ventral prostate, as well as a significant decrease in the mobility and number of epididymal sperm, and was dose-dependent (Chitra *et al.*, 2003). In addition, the antioxidant effect on sperm was also studied. The activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase were decreased, while the levels of H2O2 and lipid peroxidation were increased.

Herath *et al.* also studied the effects of BPA in rats (Wistar) during the same period of exposure, exposure of the young adult (D52) to BPA for 5 weeks, but by another route of administration (subcutaneous), and using a dose of 3000 µg/kg bw/day (Herath *et al.*, 2004). They noted a significant decrease in sperm production without change in motility, significant hormonal variations with a decrease in serum concentration of testosterone, an increase of progesterone, as well as an increase in the relative weight of the ventral prostate.

Therefore, even if the fertility of rats *in fine* was not evaluated, these studies found effects that point in the same direction on sperm production and the weight of the ventral prostate for the same period of exposure. A proven effect of BPA on sperm production during adult exposure can be established.

Sakau *et al.* did not find a significant change in sperm production; but this after exposure of only 6 days (unlike the two previous studies which exposed rats for 5 weeks) and one observation after 5 weeks; this was contrary to the changes in sperm production reported in an earlier study (Sakau *et al.*, 2001).

6.2.2.5. Multigeneration exposure from conception to adulthood

The two studies of Tyl *et al.* were performed according to guideline 416 of the OECD in rodents (rats (Tyl *et al.*, 2002) and mice (Tyl *et al.*, 2008), respectively) exposed to BPA by gavage (0.003; 0.03; 0.3; 5; 50 and 600 mg/kg bw/day in mice, and 0.001; 0.02; 0.3; 5; 50 and 500 mg/kg bw/day in rats). A positive control group was exposed at the same time to 17-β-oestradiol. Rodents were exposed 10 weeks before mating, then from conception to adulthood (chronic exposure). Males and females of the same group were mated as adults. Three generations of males and females were studied. Numerous fertility parameters were studied (organ weights, sperm reserves, sperm mobility, etc.).

For doses consistent with human exposure, no effects on male reproduction, whether in rats or mice, were observed.

In these studies, animal feed was not devoid of phytoestrogens.

Table V: Animal studies investigating the effects of bisphenol A on the male reproductive tract: summary table

Reference	Species/ strain	Route	Doses Exposure period	Effects NOAEL/LOAEL
(Howdeshell *et al.*, 2008)	Long Evans male rats	Oral	2 - 20 - 200 µg/kg bw/day GD7 - PND18	Observations made in adulthood: no significant effect observed on reproductive organ weights and epididymal sperm reserve
(Salian *et al.*, 2009a)	Holtzman rats	Oral	1.2 to 2.4 µg/kg bw/day GD12 - PND21	**F1, F2 and F3** 1.2 or 2.4 µg/kg bw/day: ↗ SRC-1 labelling in tubules at stage III. ↗ GRIP-1 labelling in stage IX tubules and elongated spermatids. ↗ labelling p/CIP in the early stages (only at 1.2 µg/kg bw/day F2 and F3). ↓ intensity of N-CoR labelling of Leydig cell (nucleus and cytoplasm).
(Salian *et al.*, 2009c)	Holtzman rats	Oral	1.2 to 2.4 µg/kg bw/day GD12 - PND21	**F1, F2, F3** 1.2 and 2.4 µg/kg bw/day: ↘ litter size ↗ significant post-implantation loss ↗ body weight (except F1 at 2.4 µg/kg bw/day) ↘ sperm count and motility ↗ coupling time ↘ ER β testicular expression profile ↘ AR testicular expression profile (except F2 and F3 at 2.4 µg/kg bw/day)
(Watanabe *et al.*, 2003)	Rats	Oral	4 - 40 and 400 mg/kg bw/day GD6 - PND20	Observations made in adult PND63 and PND252: Significant increase in testosterone at nine weeks only without change in the concentrations of LH and FSH
(Kabuto *et al.*, 2004)	ICR mice	Oral	5 or 10 µg BPA/mL drinking water one week before mating - PND28	Observations made at 4 weeks of life: Changes in endogenous antioxidant capacity and oxidative damage in different organs, including testes in mice, regardless of the dose. Decrease in testicular weight (not related) after exposure to 10 micrograms BPA/mL drinking water.
(Tinwell *et al.*,	Sprague	Oral	20 µg/kg,	Observations made at adult age: Significant

2002)	Dawley and Alderley Park rats (derived from Wistar)		100 µg/kg bw, 50 mg/kg with EE: 200 µg/kg GD6 - GD21	reduction in sperm reserves but only at a high dose 50 mg/kg in AP rats
(Okada and Kai, 2008)	ICR mice	Sub-cutaneous	40 µg/kg bw/day and 2 mg/kg bw/day 3 days prior to cross breeding until copulation	Observations at day 28: Decrease in the percentage of seminiferous tubules having mature spermatids in mice exposed to 5000 µg (60 µg/d) BPA. No change for the other observed parameters (reproductive organ weights, testosterone, histological analysis).
(Salian *et al.*, 2009b)	Holtzman rats	Sub-cutaneous	100 - 200 - 400 - 800 - 1600 µg/kg bw/day PND1-5	Observations made at adult age: Impairment of fertility parameters with a significant increase in copulation time and a significant increase in post-implantation loss, beginning with exposure to BPA at 200 µg/kg bw/day. Decrease in litter size beginning at the same dose. Significant decrease in sperm production and mobility. Testicular modification with a significant decrease in the expression of Cx-43 (PND 45 and 90) and an increase in the expression of N-cadherin (PND 45 and 90) and ZO-1 (PND 90).
(Kato *et al.*, 2006)	Sprague Dawley rats	Sub-cutaneous	2 - 97000 µg/kg bw/day PND0- PND9	No abnormalities of the reproductive parameters or hormone levels. No changes in gene expression in the testis, regardless of the dose of BPA
(Della Seta *et al.*, 2006)	Sprague Dawley rats	Oral	40 µg/kg bw/day PND23 - PND30	Observations made during puberty and adulthood: Decrease in sexual performance in adults, decreased testosterone in juvenile animals persisting in adults.
(Herath *et al.*, 2004)	Wistar rats	Sub-cutaneous	3000 µg/kg/day PND52 - PND87	Observations made at adult age: Significant decrease in serum testosterone levels and the number of epididymal sperm (no change in sperm motility). Significant increase in weight of the ventral prostate with high IGF-I
(Chitra *et al.*, 2003)	Wistar rats	Oral	0.2 - 2 - 20 µg/kg bw/day PND45 – PND90	Observations made at adult age: Significant decrease in relative weights of testis and epididymis, significant increase in the relative weight of ventral prostate. Significant decrease (dose dependent) in mobility and epididymal sperm count. Changes in enzymes involved in oxidative stress
(Sakaue *et al.*, 2001)	Sprague-Dawley rats	Oral	0.02 to 0.2 - 2 - 20; 200 mg/kg bw/day 6 days of exposure	No significant change in sperm production

			in adulthood (11 weeks)	
(Tyl *et al.*, 2008)	Mice	Oral	0.003 to 0.03 - 0.3 - 5 to 50 and 600 mg/kg bw/day Exposure 10 weeks before copulation until adulthood	In the wide range of doses studied, particularly at doses consistent with human exposure, no effect on reproduction. Presence of effects at higher doses (not relevant to human exposure)
(Tyl *et al.*, 2002)	Rats	Oral	0.001 to 0.02 - 0.3 - 5 to 50 and 500 mg/kg bw/day Exposure 10 weeks before mating until adulthood	In the wide range of doses studied, particularly at doses compatible with human exposure, no effect on reproduction. Presence of effects at higher doses (not relevant to human exposure).

6.2.3. Conclusion

The experts underline the difficulty of drawing a conclusion on the basis of epidemiological studies because these are not totally convergent. It is important to note that the populations studied are not always the same (fertile and infertile men). In men**, the effects of BPA on the male reproductive system are controversial**.

In animals, effects on sperm production (low sperm count) due to 5 weeks exposure to BPA as an adult are established. Indeed, the results of studies by Chitra *et al.* and Herath *et al.* via the subcutaneous route are convergent for the same period of exposure (exposure limited to adulthood) (Chitra *et al.*, 2003; Herath *et al.*, 2004).

In animals, effects on the male reproductive system due to exposure during prenatal, neonatal and postnatal (lactation) periods are controversial.

In animals, effects on the male reproductive system (decreased plasma concentrations of testosterone, changes in sexual activity) due to exposure during puberty, are suspected.

- **Impaired sperm production is an effect which should be taken into account for the health risk assessment.**

6.3. Effects on the female reproductive system

Data in women:

According to reports from Health Canada (2008) and the OEHHA (2009), epidemiological studies report a link between BPA exposure and endometrial hyperplasia, recurrent miscarriage, polycystic ovary syndrome and elevated levels of androgens. However, these studies have significant methodological flaws that prevent consideration of these effects as recognised (OEHHA, 2009; Health Canada, 2008).

According to the FAO/WHO (2010), in women, only one study with a small number (n=84) examined the link between BPA and oocyte production and peak serum oestradiol (Mok-Lin *et al.*, 2010). According to an expert panel, conclusions cannot be drawn from this sole study. Two studies have examined the link between BPA and the advancement of the age of puberty, but are of limited quality and not convergent (Wolff *et al.*, 2008a; Wolff *et al.*, 2010). The expert panel recommended a prospective study to investigate the association between BPA and the effects of BPA on the age of puberty. In addition, experts on the panel underlined the lack of any study undertaken in boys (FAO/WHO, 2010).

Animal data:

According to the NTP-CERHR, the results obtained in different animal models are conflicting, and some studies have methodological flaws that make it difficult to interpret them (NTP-CERHR, 2008). The results concerning early puberty are considered controversial.

However, according to the FDA, studies on delayed vaginal opening at high doses in animals are convincing, even if this parameter is not a direct measure of puberty, but an indicator of sexual maturation (FDA, 2008).

According to the FAO/WHO, many studies have been conducted in rodents and in other pets. Developmental or reproductive toxic effects were observed only in rodents, and their extrapolation to humans is still subject to discussion, and they believe it is important to consider species differences in terms of critical periods of development and sexual differentiation (FAO/WHO, 2010).

The experts on this panel believe that the evidence is controversial for the following effects:

- alteration of the age of puberty in F1 females,
- change in weight and histology of female reproductive organs of exposed adults,
- changes in hormone levels in exposed adults and F1 females.

According to INSERM, low doses of BPA during critical periods of development have an impact on the advance in the age of puberty, lead to changes in the oestrus cycle, sexual and maternal behaviour and benign, pre-malignant, and neoplastic effects on the female genital tract (histological alteration of the uterus and vagina, endometrial cystic hyperplasia, ovarian cysts) (INSERM, 2011).

6.3.1. Human data

6.3.1.1. Uterine effects

- **Endometriosis**

In an Italian study, BPA was more commonly detected in the plasma of women with endometriosis (n=58) than in women without endometriosis (n=11). BPA was not found in the control group. In 51.7% of endometriosis cases, BPA was above the detection limit. Only 25.9% of cases had levels of BPA greater than the limit of quantitation (LOQ) (Cobellis *et al.*, 2009). The methodology is questionable in terms of the constitution of the groups (inclusion criteria, study dates, very small number of subjects in the control group, and diseases existing in the control group). The analytical technique used is adapted (HPLC-fluorescence and MS detection). However the impact of deconjugation during the extraction was not evaluated. It should be noted that free BPA was never detected in the plasma of the control population.

A second study evaluated the association between endometriosis and urinary levels of BPA in 140 Japanese women seen for primary infertility between January 2000 and December 2001, divided into two groups: endometriosis stage 0-I, n=81; and stage II-IV, n=59 (Itoh *et al.*, 2007). A cross-sectional analysis was performed between the urinary level of conjugated BPA (unadjusted and adjusted for creatinine) and stage of endometriosis. The authors found no significant association. The urinary levels of conjugated BPA found appear consistent with rates found in Japan in several studies of the general population. Two main limitations weigh in the interpretation of this study. First, the determination of urinary BPA reflects not long-term, but recent exposure. Second, there is no true control group devoid of pathology.

- **Endometrial hyperplasia**

An *a priori* prospective study (inclusion criteria and dates not specified) suggests that circulating levels of BPA would be lower in women with complex uterine hyperplasia (n=9) and/or uterine adenocarcinoma (n=7) than in women with normal endometrial histology (n=11) or moderate endometrial hyperplasia (n=10) (Hiroi *et al.*, 2004). The analytical method (ELISA) is questionable and BPA was measured in a single plasma sample in uncontrolled conditions (it is present in all

subjects). In addition, the number of patients in each subgroup is very limited. This study was excluded.

6.3.1.2. Pregnancy

A case-control study evaluated the association between BPA exposure and the incidence of spontaneous miscarriages (Sugiura-Ogasawara *et al.*, 2005). The authors report a higher serum level of BPA in women with a history of three miscarriages. However, this study remains very controversial, especially in terms of the protocol for measuring BPA (ELISA method), the comparability of groups, because of other confounding miscarriage factors, in terms of analysing the results (median serum levels identical in both groups), and statistical tools chosen (Berkowitz 2006).

Cantonwine *et al,* studied the relationship between the rate of premature births and total urinary BPA on a single sample taken between 30 and 37 weeks of pregnancy in a Mexican population (Cantonwine *et al.*, 2010). The most conclusive result for the authors was a higher concentration among women delivering before 37 weeks, and that an increase of 1 log in BPA concentration was associated with an advance of the delivery date by 4.5 days ("odds ratio" method). Analysis of these data is problematic: only 12 of 60 women gave birth before 37 weeks. In addition, the difference compared to women who delivered at term is no longer significant if one normalises the concentrations of BPA in relation to urine specific gravity and/or creatinine concentration. Finally, the absence of certain information further limits the scope of the study (time of urine collection relative to the stage of pregnancy and in relation to food intake, etc.).

6.3.1.3. Ovarian effects

One prospective study that included women (n=84) following an ovarian stimulation protocol as part of an *in vitro* fertilisation indicated that there is a negative correlation between urinary levels of BPA (n=203 urine samples in 112 cycles of IVF) and ovarian response (number of oocytes collected and amplitude of the preovulatory oestradiol peak). A mean decrease of 12% in the number of oocytes recovered per cycle and of 213 pg/mL from the oestradiol peak for each log unit increase of urinary SG-BPA (BPA specific gravity, i.e., the BPA concentration corrected by the urine specific gravity) was observed (Mok-Lin *et al.*, 2010). The study compared urinary BPA concentrations to those observed in the general population in the NHANES 2003-2008 cohort. The concentration of urinary BPA found reflects exposure at the time of collection, and not during the period of follicular maturation several months earlier. In addition, it is difficult to extrapolate the results observed in a sample of infertile women seen for *in vitro* fertilisation to the general population. These results are nonetheless consistent with those of a recent study showing that exposure to BPA is associated with a decreased likelihood of success of IVF (fertilisation rate), attributed to impaired oocyte quality (Fujimoto *et al.*, 2011). Although this is a fairly limited group of patients, the authors indicate that the units of study were oocytes whose quantity was on average 13 per cycle and per woman.

A cross-sectional study was conducted in Japan in women with polycystic ovary syndrome (PCOS) (Takeuchi *et al.*, 2004). The women with PCOS were obese (n=6) or not (n=13), and the women without PCOS were divided into several categories: no disruption of the menstrual cycle and normal body weight (n=19), no cycle disorders and obesity (n=7), cycle disorders associated with hyperprolactinaemia (n=7), and cycle disorders associated with hypothalamic amenorrhea (n=21). BPA was measured in fasting plasma by a non-validated immunoassay method. BPA was present in all subjects. The statistical analysis was poorly detailed, the numbers were low; the final comparison was made with respect to non-obese women without cycle disorders (considered as controls). For the entire group, the study demonstrated a correlation between plasma concentrations of testosterone (free and total) and BPA on the one hand, and the concentration of BPA and body mass index on the other hand: the levels were significantly increased in women with PCOS (obese or not) and in the obese without ovulation disorders. The results remain difficult to interpret as they are, because of the imprecision of the sampling, the lack of information on inclusion criteria, and the lack of accounting in the results of disorders in the controls.

However, this study joins with that of Kandaraki *et al.* who found serum concentrations of BPA significantly higher in women with PCOS (n=71) (obese or not) compared to normal control women (n=100) (Kandaraki *et al.*, 2011). In addition, BPA concentrations were significantly correlated with testosterone concentrations and insulin resistance. Women with PCOS were divided into obese (n=33) or non-obese (n=38) and were compared to women with normal ovarian cyclicity (obese: n=49 and non-obese: n=51). The main limitation of this study is the analytical method (ELISA) which does not discriminate between different forms of BPA. However, the concentrations obtained can be considered as a global indicator of exposure to BPA.

6.3.2. Animal data

6.3.2.1. Prenatal, perinatal and pre-pubertal exposure

6.3.2.2. Effects on the reproductive tract and ovaries

Oral exposure to 1.2 mg/kg bw/day of BPA in rats during pregnancy and lactation is suspected of inducing an increase in the thickness of the epithelium and stroma of the uterus in the offspring, a decrease in apoptosis of the uterine epithelium, disorders of the oestrus cycle, and a decrease in ERα receptor expression in the epithelial cells of the uterus during the oestrus phase (Mendoza-Rodríguez *et al.*, 2011). These results compare with those obtained by Markey in 2005. In that study, female CD-1 mice aged 3 months from mothers treated with very low doses (25 and 250 ng/kg bw by subcutaneous pump from GD9 to PND4) had decreased vaginal weight, impaired DNA synthesis in the uterine epithelium (250 ng) and a significant increase in the expression of ERα and PR (progesterone receptor) at the lowest dose (Markey *et al.*, 2005).

In Balb-C mice, *in utero* exposure to BPA and before weaning (mothers treated at 100 and 1000 µg/kg bw/day by subcutaneous injection) was associated with the development of structures suggestive of endometriosis in the peri-uterine fat, an increased incidence of cystic ovaries, and endometrial hyperplasia (Signorile *et al.*, 2010).

According to the studies reviewed, BPA is suspected to be linked to the development of ovarian pathologies, in particular polycystic ovaries, in CD-1 mice from mothers treated with BPA from PND1 to PND5 (10, 100, 1000 µg/kg bw/day by subcutaneous injection) (Newbold *et al.*, 2007). In all groups treated with BPA and regardless of the dose, the animals developed ovarian and/or uterine pathologies (benign, pre-malignant, and neoplastic proliferative lesions of the uterus), with little or no representation in the control group. However, only the increase in the frequency of appearance of polycystic ovaries and cystic endometrial hyperplasia in the group treated with 100 µg/kg bw/day was statistically significant. The same team found similar results for exposure later in gestation (GD9 to GD16), from 10 µg/kg bw/day (Newbold *et al.*, 2009). The increase in the frequency of occurrence of ovarian cysts was significant at 1 µg/kg bw/day. Similar lesions were found by Signorile *et al.* in offspring of Balb-c mice exposed to higher doses of BPA (100 and 1000 µg/kg bw/day) during gestation and lactation (Signorile *et al.*, 2010).

Similarly, in rats, treatment with BPA subcutaneously in the neonatal period (0.25 to 25 mg/kg bw/day) was associated with the development of phenotypes similar to polycystic ovary syndrome. Although the effects were significant starting from the lowest dose, the doses used were high (Fernandez *et al.*, 2010). Adewale *et al.* reported a reduction in age of puberty, an increase in the proportion of acyclic animals, and ovarian dysfunction among the descendants of females treated

from PND0 to NDP3 with 50 µg/kg bw/day or 50 mg/kg bw/day of BPA (Adewale *et al.*, 2009). The positive control used was oestradiol benzoate (25 µg – the unit was not specified by the author). However, in the study of Nikaido *et al,* prepubertal neonatal exposure (15 to 19 days) to BPA (10 µg/kg bw/day subcutaneously) led to no change of the uterus or vagina or of mammary development, although over 80% of treated animals exhibited an anovulatory state (absence of corpora lutea) at 4 weeks (Nikaido *et al.*, 2005). Moreover, in this study, exposure to BPA did not affect the age at puberty or ovarian cyclicity.

Similarly, Long Evans rats in gestation were treated with BPA (2, 20, and 200 µg/kg bw/day) or ethinyl oestradiol (EE2, 50 µg/kg bw/d) from GD7 to PND18 orally (Ryan *et al.*, 2010a). Unlike the positive control (EE2), the female offspring of mothers treated with BPA demonstrated no change in body weight, age at puberty, anogenital distance, fertility, or sexual behaviour. Finally, F1 offspring from Sprague Dawley rats treated with BPA in drinking water during gestation and lactation (estimated ingested dose from 0.1 to 1.2 mg/kg bw/day) show no difference in age at puberty or anogenital distance at birth (Rubin *et al.*, 2001). In contrast, the females after puberty had irregular ovarian cycles and decreased LH secretion after castration. This study provides excellent confirmation, and suggests that developmental exposure to BPA could induce, in rodents, impaired ontogenesis of gonadotropin function.

Moreover, the descendants of CD1 mice treated with subcutaneous osmotic pumps with very low doses of BPA from the eighth day of gestation until day 16 of lactation, studied over several successive pregnancies, presented with reduced fertility and fecundity (number of pregnancies over 32 weeks and number of offspring per birth and total number of pups born over the 32 weeks of the study) at 25 ng/kg bw/day and 25 µg/kg bw/day, but not at 250 ng/kg bw/day. These effects are only apparent after 5-6 pregnancies (Cabaton *et al.*, 2010). These results could be explained by a non-monotonic U-shaped dose-response curve. However, further studies are needed, including a greater number of doses to better characterise this type of dose-response relationship. According to the authors, BPA accentuated the “physiological” decline in the number of pups per litter as a function of age, similar to the DES control. This study is interesting, first because it has excellent safeguards in terms of control of experimental conditions, but also because it could explain the lack of effect in other studies where similar observations were limited to the first pregnancy in F1 offspring from exposed mothers, such as the Ryan study (Ryan *et al.*, 2010a) and the Zoeller study (Zoeller *et al.*, 2005) in rats. In the latter, the BPA administered to pregnant rats from gestation day 7 until the end of gestation at oral doses of 1-50 mg/kg bw/day did not seem to affect the *in utero* development of pups (Zoeller *et al.*, 2005).

6.3.2.3. Effects on the hypothalamic-pituitary-gonadal axis

In rodents, the neonatal period (PND1 to 10) is a critical period for development of the hypothalamic-pituitary-gonadal (HPG) system. Exposure to BPA during this period causes changes

in the secretion of hypothalamic-pituitary hormones. These include the level and frequency of hormonal secretions and were responsible for disruption of reproduction in the long term.

Treatment of sheep during gestation over a period covering ontogenesis and sexual differentiation of the GnRH system (5 mg/kg bw/day intramuscularly for GD30-GD90) is associated with malfunctions of the HPG axis in female offspring: hypersecretion of LH in the prepubertal period, changes in the preovulatory LH surge (positive feedback of oestradiol) (Savabieasfahani *et al.*, 2006). This same treatment induces a decrease in GnRH gene expression, an increase in expression of the ESR1 (ERα) oestrogen receptor, and decreased ESR2 (ERβ) receptor expression in the preoptic area (Mahoney and Padmanabhan, 2010). The authors measured unconjugated BPA plasma concentrations during treatment, and argue that these concentrations are close to the maximum plasma levels described in pregnant women (~ 10 ng/mL). The sampling period compared to the administrations was not specified, but it is likely that these concentrations correspond to residual levels and are not representative of the exposure of the animal over 24 hours. Similarly, treatment of prepubertal sheep intramuscularly 2 times/week for 5 weeks with diethylstilbestrol (DES; 0.175 mg/kg) or BPA (3.5 mg/kg) led to a decrease in the frequency and the amplitude of LH pulses after ovariectomy in these animals compared to controls (Evans *et al.*, 2004). The treatment of prepubertal sheep with BPA for short periods (4 days) at different doses (intravenous infusion at 0.5 - 1 - 2.5 - 5 - 10 - 20 - 40 and 80 mg/kg bw/day), allowed detection of effects of BPA on the LH pulse generator system that are qualitatively similar to the effects of 17-β-oestradiol (positive control) and seem to obey two types of mechanisms: immediate inhibitory effects at high doses and delayed effects expressed at lower doses, resulting in plasma concentrations of about 38 ng/mL (double the highest values described in humans) (Collet *et al.*, 2010). BPA is significantly less potent than oestradiol as an inhibitor of pulsatile secretion of LH. The lowest plasma concentration of oestradiol associated with an inhibition of pulsatile secretion of LH is 2 pg/mL, compared to 38 ng/ml for BPA.

In light of these studies in sheep, BPA is suspected to alter the ontogenesis of the GnRH/LH system controlling the pulsatile secretion of LH. In addition, short term effects are found on the neuroendocrine system controlling the pulsatile secretion of LH, with an EC_{50} close to the highest plasma levels described in humans. However, the relevance of the model remains questionable, as the prepubescent sheep is indeed particularly more sensitive than humans to oestradiol negative feedback.

Fernandez *et al.* reported increased secretion of GnRH, of progesterone (significant effect at the lowest dose) and increased secretion of oestradiol and testosterone in rats treated with BPA by subcutaneous injection (0.25 to 25 mg/kg bw/day) in the neonatal period (PND1 to 10) (Fernandez *et al.*, 2010). However the effects are obtained for the two highest doses. Rubin *et al.* report an irregularity of the ovarian cycles and a decrease in LH secretion after castration of F1 offspring

from SD rats treated with BPA in drinking water (estimated intakes of 0.1 to 1.2 mg/kg bw/day) (Rubin *et al.*, 2001). However, Adewale *et al.* suggest that BPA disrupts ovarian development but not the sensitivity of GnRH neurons in the positive feedback of oestradiol at the origin of the genesis of the preovulatory LH surge (Adewale *et al.*, 2009). The rats received 50 µg/kg bw/day or 50 mg/kg bw/day of BPA. The induction of the expression of the proto-oncogene C-Fos in GnRH neurons following a preovulatory dose of oestradiol (positive feedback) was not altered in animals treated with BPA, while it was reduced in positive control treated animals (oestradiol benzoate).

The KiSS neuropeptide is involved in the central control of reproductive function, especially in puberty. It is expressed in two structures, among others; the anteroventral periventricular (AVPV) and arcuate nuclei (ARC). Exposure to BPA during the postnatal period in young Wistar rats (100 to 500 µg/rat from day 1 to day 5) decreases the amount of mRNA of the KiSS peptide measured by RT-PCR in the whole of the hypothalamus in prepubescent males and females at 30 days. This effect persists in males at 75 days (Navarro *et al.*, 2009). Fifty µg/kg bw/day and 50 mg/kg bw/day of BPA were administered subcutaneously from the first to the fifth day of life for young Long Evans rats (Patisaul *et al.*, 2009). Two positive controls were included: oestradiol benzoate (EB 25 µg/rat) and an ERα agonist (PPT 1 mg/kg). KiSS immunoreactivity was measured in intact pubescent males and ovariectomised females after puberty and subjected to replacement steroid treatment (10 µg of oestradiol benzoate followed at 48 hours by 500 µg of progesterone). In the AVPV, the EB and PPT induced a decrease in KiSS immunoreactivity over untreated controls; BPA had no significant effects. In the ARC, only EB decreased KiSS immunoreactivity. In males, KiSS immunoreactivity was not affected by any treatment, regardless of the structure.

6.3.2.4. Effects on age at puberty

In animals, exposures limited to pregnancy (the second half in mice) demonstrate a fairly consistent advance of sexual maturation, assessed by age at vaginal opening and/or age at first oestrus (Honma *et al.*, 2002; Howdeshell *et al.*, 1999; Nikaido *et al.*, 2004). It should be noted that the age at vaginal opening is an indicator of sexual maturation and provides only indirect assessment of the degree of advancement of puberty. In the study by Howdeshell, for example, no significant effect of BPA on age at vaginal opening was observed, while a sizeable decrease in the time between the opening and the onset of first oestrus was recorded. In addition, this study clearly demonstrates that the effect of BPA on pubertal maturation can be largely modulated by the intrauterine environment. Thus, the effect of BPA is only slightly or not at all expressed in female foetuses having been surrounded by two male foetuses during pregnancy. This study clearly highlights a major limitation of the rodent model, and consequently other studies having estimated the age at puberty without incorporating the concept of the intrauterine environment. One single study has been conducted in another animal model without this limitation (sheep) with exposure to high doses (5 mg/kg bw/day) subcutaneously (2/5th of gestation) and showed no impact on age at first oestrous cycle (Savabieasfahani *et al.*, 2006). However, it should be noted that the occurrence

of the first cycle in sheep may be influenced by the photoperiodic environment, which in this study could have attenuated the effects of BPA.

Studies in rodents on early postnatal exposure also indicate a fairly consistent advance of the age at vaginal opening for a range of doses large enough for subcutaneous exposures (50 µg/kg bw/day to 6 mg/kg bw/day) (Adewale *et al.*, 2009; Fernandez *et al.*, 2009).

Surprisingly enough, studies concerned with broader exposure, which include the second half of pregnancy and postnatal exposure until puberty in rats, reveal no effect of BPA on the age at vaginal opening and/or the first oestrus (Kwon *et al.*, 2000; Ryan *et al.*, 2010a; Takagi *et al.*, 2004; Yoshida *et al.*, 2004). Similarly, a study of peripubertal exposure showed no BPA effect.

In summary, an acceleration of puberty in mice following exposure *in utero* and/or in the early postnatal period can be considered as an established fact. This effect is not expressed during extended developmental exposure comprising part of gestation and a postnatal and peripubertal period. However, most studies evaluated for exposures *in utero* have a major drawback: not taking into account the intrauterine environment, which could notably explain such a lack of effect. Such a bias is generally not expected in humans, where pregnancy with twins of the opposite sex is rather rare.

6.3.2.5. Adult exposure

Overall, data resulting from exclusive exposure of animals in adulthood are piecemeal, and rely on high doses for short periods, with the exception of studies on implantation and gestation.

Exposure during implantation in CD1 mice subcutaneously at high doses (minimum 100 mg/kg bw/day, 20 times the NOAEL) leads to a decrease in the number of implantation sites (200 mg/kg bw/day), histological alterations of the uterine wall (cell height) and a decreased expression of ERα and PR receptors only at the highest dose (300 mg/kg bw/day) (Berger *et al.*, 2010). Oral doses of about 2 g/kg bw/day are necessary in order to observe an effect on gestation (Berger *et al.*, 2007). Similarly, exposure to BPA at 10 mg/kg bw/day from GD0 to GD7 subcutaneously in ICR mice induced a significant decrease in the number of embryos at D10 and D12, associated with decreased weight of the uterus and marked alterations in placental structure (Tachibana *et al.*, 2007). However, in C57BL6 mice, BPA at low doses in the diet (approximately 0.1 to 10 mg/kg bw/day) throughout gestation did not induce any modification of gestational parameters (duration, litter size, survival of young, etc.) (Kobayashi *et al.*, 2010). It is therefore likely that these low doses, pertinent in terms of human exposure, do not induce significant enough changes in the uterine wall to have a functional impact on gestation. In addition, BPA administered to pregnant rats from the seventh day to the end of gestation, at oral doses of 1 to 50 mg/kg bw/day, did not induce changes in litter size or pup weight at birth (Zoeller *et al.*, 2005).

Ovariectomy in rats induced changes in uterine morphological parameters (uterotrophic OECD TG 440) and an increased expression of oestradiol receptors in the uterus. The administration of BPA

at doses of 0.5 to 50 mg/kg bw/day for 5 days by subcutaneous injection in ovariectomised Wistar rats did not restore these uterine parameters to a level similar to that of non-castrated rats. Similarly, BPA does not, unlike oestradiol, suppress the increased expression of ERα and β receptors induced by ovariectomy. On the other hand, 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP), a potential liver metabolite of BPA, is used to cancel the effects of castration on the uterotrophic test and the expression of oestrogen receptors like oestradiol (positive control) (Okuda *et al.*, 2010). The oestrogen-mimicking potential of BPA in this model appears quite moderate, compared to its potential metabolite MBP and oestradiol.

Table VI: Animal studies examining the effects of bisphenol A on the female reproductive tract: summary table

Reference	Species	Routes	Dose Exposure period	Effects NOAEL/LOAEL
(Mendoza-Rodríguez *et al.*, 2011)	Wistar rats	Oral	10 mg/L in drinking water, estimated intake of 1.2 mg/kg bw/day GD6 - PND21	**F1** ↗ thickness of the epithelium and uterine stroma ↘ apoptosis in the uterine epithelium disorders of the oestrus cycle ↘ of ER-α receptor expression in the epithelial cells of the uterus during the oestrus phase
(Ryan *et al.*, 2010a)	Long-Evans rats	Oral	2 - 20 or 200 µg/kg bw/day GD7 - PND18	No effect (F0 and F1 weight, primary sexual characteristics, fertility, fecundity, sexually dimorphic behaviour) following a pre-and neonatal exposure to low doses of BPA Confirms the results of multigenerational studies (Tyl *et al.*, 2002) (etc.)
(Adewale *et al.*, 2009)	Long-Evans rats	Sub-cutaneous	50 and 50,000 µg/kg bw/day PND0-PND3	**F1 as adults** ↘ age at puberty (advancing age of vaginal opening, stronger effect at lower doses) modification of ovarian morphology (cysts, ↘ number of corpora lutea, degenerate follicles) ↗ proportion of acyclic animals No change in sexual behaviour No change in the expression of FOS in the GnRH neurons for the two BPA groups
(Fernandez *et al.*, 2010)	Sprague-Dawley rats	Sub-cutaneous	5 (0.25 to 0.62 mg/kg), 50 (2.5 to 6.2 mg/kg), 500 µg/50µL (25 - 62.5 mg/kg) PND1 - 10	↗ serum testosterone and oestradiol level and ↘ progesterone in adulthood and altered secretion of GnRH *in vitro* 50 µg/50 µL: reduced fertility 500 µg/50 µL: abnormal morphology of the ovaries with many cysts (morphology similar to that observed in the case of polycystic ovaries in women); all sterile females **LOAEL=2.5 mg/kg**
(Markey *et al.*, 2005)	CD-1 mice	Sub-cutaneous	0.025 and 0.25 µg/kg bw/day GD9 - GD23	**F1** 0.025 and 0.25µg/kg bw/day: Changes in ovarian morphology at 3 and 6 months and alteration of the uterus and vagina: ↘ dry weight of the vagina, ↘ endometrial volume, ↗ expression of ERα and PR in uterine epithelium, impairment of DNA synthesis in the uterine epithelium **LOAEL=25 ng/kg bw/day**
(Rubin *et al.*, 2001)	Sprague-Dawley rats	Oral	estimate of 0.1 mg/kg bw/day to 1.2 mg/kg bw/day (drinking water)	**F0:** Oestrus cycle disorders (longer than normal) ↘ secretion of LH in response to ovariectomy -> suggesting a neuroendocrine effect

			GD6 - pups were weaned	**F1** ↗ bodyweight (after birth and up to adult age) Alteration oestrogenic cyclicity and ↘ in LH at adult age after castration No difference in age at puberty nor difference in anogenital distance at birth
(Newbold *et al.*, 2007)	CD-1 mice	Sub-cutaneous	10-100-1000 µg/kg bw/d PND1-PND5	- No difference between **body weight** of the treated and control animals, irrespective of the dose. **Ovaries** - Appearance of ovarian cysts, significant only at the dose of 100 µg/kg bw/d of BPA - Decrease in the observation of corpora lutea when the dose increases (NS) - Appearance of para-ovarian cysts of mesonephric origin (absence in the control group) NS - Appearance in the BPA groups of progressive proliferative lesions (PPL), absent in the control group (NS) **Uterus** - Increase in the incidence of endometrial hyperplastic cysts, but only the BPA100 dose causes a significant effect - Tendency, at the highest doses, towards an increase in atypical hyperplasia of the endometrium, a precursor for adenocarcinoma - Appearance (NS) of leiomyomas (absence in control group) - Upper stromal polyps in the BPA100 group - Increased incidence of enlarged Wolffian ducts in the treated mice **NOAEL: 10 µg/kg** **LOAEL: 100 µg/kg**
(Newbold *et al.*, 2009)	CD-1 mice	Sub-cutaneous	0.1-1-10-100 and 1000 µg BPA/kg bw/d GD9-GD16	**Ovaries:** - No difference for the number of mice not having corpora lutea - Significantly increased incidence of ovarian cysts for BPA-1 only - Presence of prominent para-ovarian cysts (no associated statistical test) at BPA-10 - Neoplastic lesion in the ovary including cystadenoma present at BPA-10, 100 and 1000 (NS) - Progressive proliferative lesion observed in all the treated groups but not the controls (NS) **Uterus** - Cystic endometrial hyperplasia (CEH) incidence increased for all the groups except

				BPA-0.1 (even the control) at NS - Adenomyosis: In controls, BPA-0.1 and BPA10 - Adenomatous hyperplasia with CEH in BPA-1, BPA-100 but not in controls (NS) - Atypical hyperplasia of the uterus, considered to be a precursor for uterine adenocarcinomas, found in BPA0.1, BPA1 and BPA1000, not in controls (NS) - Wolffian remnants in the uterus comparable to those seen in the ovary and in the fallopian tubes in all groups except BPA-100 - Uterine polyps seen in BPA0.1, BPA1 and 10 (NS). Lesions of this type have been reported as being associated with the development of stromal cell sarcomas in rodents **Vagina** - One BPA-1000 mouse had a vaginal adenoma characterised by glandular structures at atypical locations **Premature death or euthanasia** - One BPA-1 mouse had a sarcoma which invaded the reproductive organs, but it was definitely a cancer of hematopoietic origin in view of the overall incidence of the lesions - There were significantly more lesions in the genital tract for BPA-0.1 than in the controls. - There were significantly more lesions (independently of location) for BPA-0.1 and BPA-1 than for the other doses. **$LOAEL_{est}$ = 0.1 µg/kg bw/d**
(Nikaido *et al.*, 2005)	CD-1 mice	Sub-cutaneous	10 mg/kg bw/d PND15-PND19	No acceleration of the beginning of age at puberty No modification of the uterus nor of the vagina nor of mammary development Anovulatory state for 80% of the animals treated with BPA *versus* control group No modification of ovarian cyclicity
(Signorile *et al.*, 2010)	Balb-C mice	Sub-cutaneous	100 and 1 000 µg/kg bw/d GD1 - PND7	Lesions of cystic hyperplasia type and atypical lesions of the endometrium <u>10 or 1000 µg/kg bw/d in F1 ♀ after 3 months:</u> ↗ frequency of appearance of structures (glands and stroma expressing ER and Hoxa10) of endometriosis type in the adipose tissue surrounding the genital tract. ↗ frequency endometrial hyperplasia, cystic ovaries and endometrial hyperplasia significantly more frequent in F1 ♀ BPA (free) in the liver of all treated F0 ♀ and F1 (no dose-dependant relationship) with no correlation with the occurrence of a pathological condition similar to endometriosis

				LOAEL = 100 µg/kg bw/d
(Cabaton *et al.*, 2010)	CD-1 mice	Oral	25 ng, 250 ng or 25 µg/kg bw/d GD8 - PND16	↘ in fertility and fecundity (↘ in the number of gestations over a period of 32 weeks, in the number of young per birth and in the total number of young born over the 32 weeks of study) **LOAEL = 25 ng/kg bw/d**
(Evans *et al.*, 2004)	Ewes	Intra-muscular	3.5 mg/kg twice a week 4-week-old ewes treated for 5 weeks	↘ in the frequency and amplitude of LH pulsatility after ovariectomy No modification of ovary weight
(Navarro *et al.*, 2009)	Wistar rats	Sub-cutaneous	100-500 µg/animal PND1-5	Suppression of KiSS-1 messenger RNA levels in the hypothalamus that may lead to a modification of the hypothalamic-pituitary axis and of gonadotropic hormone secretion
(Savabieasfahani *et al.*, 2006)	Ewes	Intra-muscular	5 mg/kg bw/d GD30-GD90	Hypersecretion of LH in the prepubescent period Modification of the preovulatory peak of LH
(Mahoney and Padmanabhan, 2010)	Sheep	Sub-cutaneous	5 mg/kg bw/d G30-G90	↗ in the expression of ESR1 and ↘ in the expression of ESR2 ↗ in the expression of gonadoliberin
(Collet *et al.*, 2010)	Ewes	Intra-venous	5-10-20-40 and 80 mg/kg bw/d Adult ewes treated for 4 days	Effects on the LH pulse-generating system qualitatively similar to the effects of 17β-oestradiol (positive control).
(Berger *et al.*, 2010)	CF-1 mice	Sub-cutaneous	100 - 200 - 300 mg/kg bw/d GD1 - GD4	↘ implantation sites Histological modifications of the wall of the uterine cavity Decrease in ERα and PR receptor expression
(Berger *et al.*, 2007)	CF-1 mice	Oral	Administration of BPA by addition to peanut butter in an amount of 0.11-9% or by addition to the feed in an amount of 3 and 6%. GD1-GD5	No modification of litter size or of parturition rate The dose of 68.84 mg of BPA/d/animal (corresponding to a BPA supplementation at 6%) causes the abortion of all gestations
		Sub-cutaneous	0.0005-0.0015-0.0046-0.0143-0.0416-0.125-0.375-1.125-3.375, and 10.125 mg/animal/day GD1- GD4	↘ in litter size at 3.375 mg/d ↘ in the proportion of females to be parturient at 10.125 mg/d ↘ in the number of implantation sites at the dose of 10.125 mg/d
(Tachibana *et al.*, 2007)	ICR mice	Sub-cutaneous	10mg/kg bw/d GD0 - GD7	↘ in the embryo number ↘ in the weight of the uterus and marked modifications of placental structure

(Kobayashi *et al.*, 2010)	C57BL/ 6J mice	Oral	0.05-0.5 or 5 mg/kg bw/d GD6-PND22	No modification of body weight, of gain in body weight, feed consumption, duration of gestation, litter size, or survival of the young in the F0 animals No difference between the sex ratio and the viability in the F1 animals No modification of body weight, feed consumption, developmental parameters, anogenital distance, or organ weight (liver, kidney, heart, spleen, thymus, testis, ovaries and uterus) in F1 and F2 adults. No modification of sperm number or motility in F1 and F2 animals
(Munoz del Toro *et al.*, 2005)	CD-1 mice	Sub-cutaneous	25 - 250 ng/kg bw/d GD9 - PND4	- No modification of TEB number, size and area - Increase in mammary gland sensitivity to oestrogen - Decrease in number of cells in apoptosis in the TEBs starting from 25 ng/kg bw/d - No proliferative effect - No increase in ERα receptors, but increase in progesterone receptors - Significant increase in side-branching of mammary glands at 25 ng/kg
(Nikaido *et al.*, 2004)	CD-1 mice	Sub-cutaneous	0.5 and 10 mg/kg bw/d GD15-GD18	Acceleration of weight gain in F1 females Precocity of vaginal opening. Increase in oestrogen cycle duration Genital tract abnormalities (acyclicity, hyperplasia) Acceleration of mammary gland differentiation
(Patisaul *et al.*, 2009)	Long Evans rats	Sub-cutaneous	50 µg/kg bw/d and 50 mg/kg bw/d PND1-PND5	No modification in immunoreactivity to KISS in the anteroventral periventricular nucleus, decrease in the ARC nucleus in females No modification in males

Table VII: Animal studies investigating the effects of bisphenol A on vaginal opening and on age at first oestrus: summary table

Exposure period	References	Species	Routes	Exposure period	Exposure dose	Effect evaluated on vaginal opening and age at first oestrus
Gestation	(Howdeshell *et al.*, 1999)	CF-1 mice	Oral gavage	GD11-GD17	BPA: 2.4 µg/kg bw/d	Vaginal opening: no effect Interval between vaginal opening and age at first oestrus: decrease by 2-4d
	(Nikaido *et al.*, 2004)	CD-1 mice	Sub-cutaneous	GD15-GD19	BPA: 0.5 or 10 mg/kg bw/d DES: 0.5 or 10 µg/kg bw/d	Vaginal opening: BPA 0.5 mg/kg bw/d: no effect BPA 10 mg/kg bw/d : advance of 1.2d DES: advance of 1.5 and 1.9d at doses of 0.5 and 10 µg/kg bw/d respectively
	(Honma *et al.*, 2002)	ICR Jcl mice	Sub-cutaneous	GD11-GD17	BPA: 2 or 20 µg/kg DES: 0.02-0.2 or 2 µg/kg	Vaginal opening and age at first oestrus: BPA 20 µg/kg: advance (~1d) DES: advance 1.5d minimum
	(Savabieasfahani *et al.*, 2006)	Sheep	Sub-cutaneous	GD30-GD90 (2/5th of gestation)	BPA: 5 mg/kg	No effect: on age at first oestrus cycle determined by the progesterone level
Second half of gestation and postnatal	(Yoshida *et al.*, 2004)	Donryu rats	Oral gavage	GD2-PND21	BPA: 6 µg/kg bw/d 6 mg/kg bw/d	Vaginal opening No effect of BPA
	(Takagi *et al.*, 2004)	Sprague-Dawley rats	Oral feed	GD15-PND10	BPA feed: 60-600-3000 ppm, i.e. ~7-300 mg/kg bw/d Ethynyl E2 0.5 ppm	Vaginal opening No effect of BPA
	(Kwon *et al.*, 2000)	Sprague-Dawley rats	Oral gavage	GD11-PND20	BPA: 3.2-32-320 mg/kg bw/d DES 15 µg/kg bw/d	Vaginal opening and age at first oestrus No effect of BPA nor of DES

	(Ryan *et al.*, 2010a)	Rats	Oral gavage	GD7-PND18	EE2: 0.05-0.5-1.5-5-15-50 µg/kg bw/d BPA: 2-20-200 µg/kg bw/d	Vaginal opening EE2 at the dose of 5 µg/kg caused a vaginal opening advance of 4d. BPA did not cause any effect.
Early postnatal	(Adewale *et al.*, 2009)	Rats	Sub-cutaneous	PND0-PND3	EB*: 25 µg BPA: 50 µg/kg BPA: 50 mg/kg PPT: 1 mg/kg	Vaginal opening: EB: Advance of 4d BPA: 50 µg/kg: advance of 2d BPA: 50 mg/kg: NS PPT 1 mg/kg: advance of 1d
	(Fernandez *et al.*, 2009)	Sprague-Dawley rats	Sub-cutaneous Castor oil	PND1-PND10	1st BPA dose tested: 2.5-6.2 mg/kg bw 2nd BPA dose tested: 25 to 62.5 mg/kg bw	Vaginal opening: 2.5d advance 4.8d advance
Postnatal	(Nikaido *et al.*, 2005)	CD-1 mice	Sub-cutaneous	PND15-19 prepubertal	BPA: 10 mg/kg bw/d DES: 10 µg/kg bw/d	Vaginal opening No effect with BPA 10 mg/kg bw/d DES 10 µg/kg bw/d: advance

6.3.2.6. Transposition to humans: interpretation issues

Many grey areas persist in the precise physiopathology and etiology of polycystic ovary syndrome (PCOS) in women. Hormonal imbalances associated with this pathological condition are quite well described and cross over in many respects with the hormonal modifications described in rats following developmental exposure to BPA associated with an increased incidence of polycystic ovaries (Fernandez *et al.*, 2010). These hormonal modifications are consistent with an endocrine clinical picture evoking POCS, which would tend to suggest that, from this point of view, rodents could prove to be relevant for assessing the risk of developing POCS in women.

Endometrial hyperplasia is clearly identified, both in women and in animals, as being the result of hyperoestrogenism. However, this physiopathological scheme is based essentially on a contemporary effect of the hormonal imbalance and is reversible. Studies concerning the effects of DES *in utero* demonstrate that developmental over-impregnation is also responsible for promoting hyperplasia and/or uterine cancers in rodents in adulthood, thus supporting the epidemiological observations in humans. This would tend to suggest that, at least for uterine pathological conditions with an oestrogen “component”, rodents could be used, to a certain extent, to predict the potential harmful effects of developmental exposure to endocrine disruptors on the uterus.

In women, it is essentially atypical hyperplasia of the endometrium which is considered to be a precancerous lesion. Indeed, less than 2% of glandular hyperplasias without an atypical aspect progress to an adenocarcinoma, whereas approximately 30% of atypical hyperplasias progress to invasive cancer. Not all adenocarcinomas are preceded by atypical glandular hyperplasia. Obesity (which is associated with hyperoestrogenism via the increased aromatisation of androstenedione to oestrone, in the adipose tissue) is a notable risk factor.

In summary, for the two pathological conditions of the female genital tract that are most commonly described and “recognised” in rodents in the context of developmental exposure to BPA (polycystic ovaries and endometrial hyperplasia), rodents appear to be relevant models in terms of analysing the risk to human health.

With regard to endometriosis, two main hypotheses prevail at the current time for the physio-pathological scheme of the development of this pathological condition: differentiation of totipotent peritoneal mesothelial cells to give endometrial cells in conjunction with a particular hormonal environment and/or an autograft of endometrial cells out of the uterus from a retrograde menstrual flow (Robboy and Bean, 2010). In the first situation, it is possible to envisage that such mechanisms may be expressed in rodents, and the Signorile study would in this sense be acceptable in the context of the analysis of the risk to human health. On the other hand, in the second situation, the absence of menstruation in rodents, as in most animal species, invalidates this model in terms of its relevance to humans. The experimental models most suitable for studying endometriosis in women appear rather to be primate models (baboons, Rhesus monkeys, etc.) since they spontaneously experience endometriosis, but the data are limited and sometimes not very probative.

6.3.3. Tissue/cell models of human origin

Various cell models derived from human cells have been developed and used in order to evaluate direct effects of BPA on endometrial and/or ovarian cells. This approach may be advantageous from two points of view:

- the endometrium and the ovary are targets for oestrogens and the type of responses induced in these tissues by oestrogens is well documented;
- cell cultures lend themselves particularly well to dose-response type approaches for establishing the relative potency and efficacy of BPA with respect to endogenous steroids.

The work carried out on various cell lines proposes mechanisms of action other than those of the oestrogen type.

However, the relevance of these results can only be very limited from the point of view of risk analysis since the doses to which the cells are generally exposed are very much greater than the concentrations measured *in vivo* (10-20 ng/mL for the highest values, i.e. 0.04 to 0.08 µM). These results should therefore be interpreted as tests for “prescreening” the oestrogen-mimicking potential of BPA on this type of tissue. The limits inherent in this type of approach should be emphasised: firstly, the metabolism of the toxic compound by the cells does not necessarily result in the same exposure scheme as *in vivo*; next, this most commonly involves cancer lines, which therefore probably have modified regulating schemes compared with normal endometrial cells.

On Ishikawa cells, which are adenocarcinoma-derived endometrial cells, BPA induces progesterone receptor expression, but is approximately 10,000 times less powerful than oestradiol (Schaefer *et al.*, 2010). Genomic screening on these cells indicates that there is a cluster of genes that are modified in a qualitatively similar manner by oestradiol (1 µM) and BPA (100 µM ~ 20 µg/mL). A subgroup of this cluster is also modified *in vivo* in the uterus of prepubescent female rats exposed to low doses of BPA (10 µg/kg) (Naciff *et al.*, 2010).

Bredhult *et al.* exposed a primary culture of endometrial endothelial cells, originating from donors with no endometrial pathological condition and/or disruption of the menstrual cycle, to a high dose of BPA (50 µM; 11 µg/mL) (Bredhult *et al.*, 2009). This study demonstrates the regulation of cell cycle signalling pathways probably associated with increased cell death.

BPA (0.1 to 1 µM) inhibits the expression of genes of key enzymes in the production of steroids by granulosa cells in primary culture (P450aromatase and P450scc) without, however, inducing any decrease in the steroid concentrations in the culture media (Jiang *et al.*, 2007). At 40 µM, it inhibits the response of granulosa cells (KGN lines and luteinising cells in primary culture, expression of IgF1 and aromatase CYP19 genes) to FSH, together with a decrease in FSH-induced oestradiol production (Kwintkiewicz *et al.*, 2010).

Placental explants (first trimester) subjected to low BPA concentrations (1 nM) respond through an increase in β-HCG secretion and pro-apoptotic pathway activation (Morck *et al.*, 2010). These results suggest that BPA could modify the differentiation sequence of the placental tissue and in particular of the syncytiotrophoblast, which could impair correct functioning of the placenta. The effect at the molecular level is convincing, but the functional meaning of these modifications is not discussed.

A study carried out on human cytotrophoblasts in culture shows, under the experimental conditions (24 h of exposure on a non-controlled device), effects of BPA at very low doses (as low as 0.2 ng/mL in nominal concentrations in the culture medium) on cytotoxicity and apoptosis (Benachour and Aris, 2009). However, these results need to be analysed with great care: there is no characterisation of the exposure of the donor placentas at the time they were collected; it is not specified whether the culture device contains BPA and especially whether or not BPA interferes with the analytical methods.

Although these studies show that BPA has a certain potential for modifying the steroidogenesis functions of ovarian cells and/or the physiology of endometrial cells, the doses used are too high for these data to support the epidemiological data in women. The data on placental cells are consistent and demonstrate effects expressed at low nominal doses of the same order of magnitude as the plasma concentrations reported in pregnant women. However, these studies remain too imprecise from a methodological point of view, with in particular a lack of controls regarding the doses and the possible biases related to the experimental conditions, to be conclusive.

6.3.4. Conclusion

There are relatively few epidemiological studies examining a link between exposure to BPA and effects on reproduction in women. These studies have methodological limits (size of the population studied, selection of participants, statistical analysis, etc.) which make them difficult to interpret. Correlations in populations (with many possible confounding factors) can only be convincing on the basis of a very large number of individuals, regardless of the statistical approach used to analyse these data. The human data are therefore to be considered with a great deal of circumspection and are in no way conclusive of an effect of BPA on the parameters studied. The experts thus express reservations regarding all the epidemiological studies and consider that, as the current knowledge stands, **the human data relating to the effects of BPA on the endometrium (endometrium, hyperplasia), polycystic ovaries and the outcome of pregnancy (miscarriages and premature birth) do not allow any conclusions in women to be drawn**.

The effect of BPA on oocyte maturation in women, in a medically-assisted procreation context, is suspected on the basis of a study of high quality (Mok-Lin *et al.*, 2010) and of another which has non-major methodological limitations (Fujimoto *et al.*, 2011).

In animals, on the basis of the convergence of results from various studies carried out under various conditions and on various models, **the following effects can be considered to be** "**recognised in animals**" in protocols of exposure during development (pre- and postnatal exposure):

- **Increase in the occurrence of ovarian cysts,**
- **Hyperplastic modifications of the endometrium,**
- **Advancement of the age at puberty when there has been early pre- and postnatal exposure.**

The effects on the hypothalamic-pituitary-gonadal axis due to exposure *in utero* or to early postnatal exposure lead to variations in sex hormone levels, and modification of sex hormone receptor expression has been found in several studies. These effects are recognised in animals.

In animals, the potential effects of exposure in adults are observed for doses well above the NOAEL selected by EFSA (for example, number of implantation sites, histological modification of the uterine wall, morphology of the genital tract, etc.).

- **The effects that are "recognised in animals" are effects that should be taken into account for the health risk assessment.**

6.4. Effects on the brain and behaviour

According to the FAO/WHO, a prospective cohort study in humans (Braun *et al.,* 2009) showed changes in behaviour (aggressiveness, hyperactivity) in girls; this association was stronger when urinary concentrations of BPA at the start of pregnancy were higher in the mothers. This expert panel considers it a priority to undertake a prospective study in a large cohort using several urinary measurements, particularly at the start of pregnancy (FAO/WHO, 2010). A new study by Braun *et al.* confirming these results is under publication, according to this panel's conclusions. This study may also show a positive relationship between urinary concentrations of BPA measured in mothers during pregnancy and anxiety observed in children, which has also been reported in animals.

In animals, according to the expert panel that met in Chapel Hill in 2007 (Richter *et al.,* 2007), exposure to low doses of BPA in the critical period of development can have persistent effects on cerebral structure and function, and on behaviour in rats and mice, including:

- Increased ERα and β receptors in various brain structures in response to development exposure (Ramos *et al.*, 2003; Khurana *et al.*, 2000; Ceccarelli *et al.*, 2007; Kawai *et al.*, 2007),
- Alteration of the hypothalamic-pituitary-thyroid axis (Zoeller *et al.*, 2005),
- Effects on the cell signalling pathways,
- Effects on cerebral structure.

In adults, the onset of such effects appears to require exposure to higher doses of BPA and during a longer period.

According to the ECB (EC, 2010b), more than 30 studies, including three on subcutaneous exposure, have assessed neurotoxicity in animals (locomotive and exploratory activity, sexual, cognitive, emotional, social, maternal behaviour, expression of genes and receptors and immunotoxicity, etc.) but their protocols had limitations (small number of animals, inappropriate statistical analysis, results and methods reported in insufficient detail, one single dose, etc.). Therefore, confidence in the reliability of the results is limited and the observed effects lack coherence.

The NTP-CERHR has also expressed concern for humans ("some concern for adverse effects") as to the effects on cerebral development and behaviour related to BPA (NTP-CERHR, 2008). According to the (FDA, 2008), some studies suggest that exposure to BPA during development may, in rodents, alter brain development and have the following effects:

- possible effects on brain development and sexual differentiation,

- alteration of endocrine function in offspring: reduced testosterone in males, altered levels of thyroxine and genes responding to thyroxine, altered levels of retinoid receptors and thyroid hormone receptor coactivators,
- modulation of monoaminergic neural pathway development after exposure during development, suggested by significant changes in the behaviour of adult offspring.

According to the OEHHA, these effects, and particularly those involving changes in maternal behaviour, are consistent with BPA's oestrogenic potential; the statistical data analysis is appropriate and the doses seem to be consistent with those encountered in human exposure (around one µg/kg). The potential mechanisms of action behind developmental toxicity include regulation of gene expression in the embryo, action at membrane oestrogen receptor sites, and modulation of second messenger systems (OEHHA, 2009).

However, these effects at low doses remain controversial (NTP-CERHR, 2008; OEHHA, 2009) due to:

- the lack of study repeatability,
- doubts as to the relevance of the protocols used: a complete developmental neurotoxicity study of BPA has not been not undertaken despite routine protocols being available,
- the relevance of the animal model and its extrapolation to humans: the relationships between BPA exposure and neurological or neurodegenerative syndromes and behaviour in children have not been explored,
- a lack of consensus as to the harmful nature of the reported effects: for example, an observed effect in foetuses, newborns or prepubertal animals has generally not been investigated in adult animals to determine whether or not it is reversible and establish its level of severity.

AFSSA, in its Opinion of 29 January 2010 and the corresponding Annex (May 2010), analysed several studies on the neurotoxic effects of BPA (Palanza *et al.,* 2008; Nakagami *et al.,* 2009; Stump *et al.,* 2009; Braun *et al.,* 2009; Monje *et al.,* 2009; Ryan *et al.,* 2010) and considered that some of these publications, and particularly those by Nakagami *et al.* (2009) and Palanza *et al.* (2008), indicate alert signals after *in utero* and postnatal exposure to doses lower than the one used to establish the TDI (AFSSA, 2010b; AFSSA, 2010a). The reported effects included, firstly, feminisation in the behaviour of male offspring, and secondly, changes in exploratory behaviour and anxiety. However, other studies were not considered to be alarming (Braun *et al.,* 2009, Monje *et al.,* 2009). The studies by Stump and Ryan did not show effects at doses lower than 5 mg/kg bw/day.

EFSA considers that the data that are currently available do not provide sufficient proof that BPA affects behaviour at doses lower than 5 mg/kg bw/day (EFSA, 2010).

Lastly, the expert panel that met in 2010 under the leadership of the FAO/WHO considered that exposure to BPA during development does not appear to affect the sensory organs, spontaneous behaviour or female sexual behaviour in laboratory animals (FAO/WHO, 2010). The available experimental data are not in favour of cerebral neuropathological effects after oral exposure during gestation, at doses lower than 164 mg/kg bw/day (Stump *et al.,* 2010). Biochemical (monoaminergic, cholinergic, glutamatergic systems, etc.), morphometric and cellular changes, in the anatomical regions involved in sexual dimorphism and in certain neuroendocrine targets, have been reported after oral exposure during gestation to doses lower than 5 mg/kg bw/day. However, these studies had methodological limitations, and the observed effects had no functional equivalence, which means it is difficult to interpret them. On the basis of the available data, this panel considers that effects related to anxiety and sex differences in the brain, in both males and females, are potentially relevant critical effects in humans, but supplementary studies are required to reduce these uncertainties.

Data in rodents and sheep suggest effects on the organisation of the hypothalamic-pituitary-gonadal axis in females (>50 µg/kg bw/day for non-oral exposure) and its activity (>5 mg/kg bw/day for non-oral exposure).

This panel's experts recommend undertaking studies to examine specific effects related to stressful behaviour after exposure during pregnancy:

- by implementing various study protocols with several doses and in both sexes,
- by testing several ages,
- by examining the functional impact of changes in cerebral sexual differentiation,
- by undertaking dose-response analyses of anatomical changes linked to cerebral sexual differentiation.

6.4.1. Human data

The study by Braun *et al.* describes epidemiological monitoring of mothers exposed to BPA and their children at the age of 2 years (Braun *et al.,* 2009). Exposure to BPA was determined by analysing residues in the urine of mothers at around 16 and 26 weeks of pregnancy and at their children's birth. Prenatal exposure to BPA was linked to externalised behaviours, especially in girls (hyperactivity, multiple aggressions). These behaviours are usually dominant in boys and may also be interpreted as increased anxiety in girls, and perhaps also in boys, but in the latter case they could be confused with behaviours in boys linked to behavioural sexual dimorphism. Regarding this study, Longnecker expresses reservations about absolute differences in the scores observed for externalised behaviours associated with BPA, which cannot be determined using the sex-

standardised data presented in the study by Braun *et al.* (Braun *et al.,* 2009; Longnecker 2009). Thus, the size of the association with BPA in girls cannot be compared with the size of the male-female difference. As such, it is impossible to know whether the girls developed masculine behaviour or whether they still behaved like girls. It should also be mentioned that due to the methodological limitations noted by AFSSA in its 2010 expert appraisal, the conclusions of the study by Braun *et al.* were not taken into consideration (AFSSA, 2010a). Furthermore, AFSSA pointed out that the authors concluded that BPA impacts behaviour on the basis of scores that fell within the normal range of individual variation. For example, the highest mean score was 53.9 (standard deviation of 1.3), whereas the score was normalised for the American population to a value of 50 with a standard deviation of 10. However, it should be noted that the FAO/WHO experts consider that replicating this study using a large cohort with several urinary measurements, particularly at the start of pregnancy, is a high-priority research need (FAO/WHO, 2010).

Miodovnik *et al.* studied the correlation between urinary levels of BPA and phthalates analysed during pregnancy and the sociability of multiethnic urban children aged 7 to 9 years, in 137 children (Miodovnik *et al.,* 2011). Sociability was assessed using a Social Responsiveness Scale (SRS) that contained 65 items. Urinary concentrations of low molecular weight phthalate metabolites were associated with greater social deficits, with poorer social cognition, communication and awareness. However, no significant association was found between urinary levels of BPA and social impairment. BPA was positively correlated with the severity of social impairment (Social Responsiveness Scale), but this relationship was not statistically significant.

6.4.2. Animal data

6.4.2.1. Prenatal and perinatal exposure

6.4.2.2. Effects on behaviour

Effects on exploratory behaviour

Changes in maternal, exploratory and emotional behaviour have been reported after *in utero* exposure. The results obtained by Poimenova *et al.* show that BPA modifies the behaviour of F1 females born to mothers who were orally exposed to BPA at 40 µg/kg bw/day in their diet during gestation and lactation (Poimenova *et al.,* 2010). The F1 females had a sharp decrease in exploratory behaviour and a deterioration of spatial memory, but this study had methodological limitations (very small number of animals, one single dose, number of animals not always specified in each trial, etc.). (Table VIII). The developmental neurotoxicity study by Stump *et al.,* undertaken in accordance with OECD guideline 426 (tests, histopathologic evaluations, etc.) and with GLP (Good Laboratory Practice), established an NOAEL for developmental neurotoxicity effects at the highest tested dose of 2250 ppm (164 mg/kg bw/day for gestation and 410 mg/kg bw/day for

lactation) (Stump *et al.*, 2010). No effects on the exploratory behaviour of offspring were highlighted.

Effects on anxiety

Behavioural effects in mice were observed by Cox *et al.*, in the F1 offspring of mothers exposed during gestation (from E9 to the end of gestation) to doses of 50 mg/kg BPA administered in feed corresponding to 8 mg/kg bw/day (Cox *et al.*, 2010). In this study, the offspring were weaned, either with their biological mother, or with a foster dam. The results show a clear increase in anxiety in the offspring of mothers exposed to BPA. In general, the type of mother weaning the offspring (biological mother versus foster dam) modified the effects of BPA. However, in order to be able to properly interpret the studies by Cox *et al.*, two additional procedures would have needed to be undertaken: (i) newborns born to control mothers and fed by foster dams exposed to BPA and (ii) newborns born to mothers exposed to BPA and fed by foster dams exposed to BPA. That said, these results can be compared with those of Poimenova *et al.* which also show that BPA alters behavioural coping to stress in a sex-dependent manner in F1 rats born to mothers which were exposed to 40 µg/kg bw BPA daily during gestation and lactation (Poimenova *et al.*, 2010). For example, compared to males, F1 females exposed to BPA had increased anxiety and far lower exploratory behaviour.

In the study by Tian *et al.* using 100 and 500 µg/kg bw BPA daily in mice, prenatal and postnatal exposure (from GD7 to PND36) to BPA induces anxiolytic behaviour (at 100 µg/kg bw/day), unlike the anxiogenic effect reported by Cox *et al.* at the dose of 8 mg/kg bw/day (Cox *et al.*, 2010; Tian *et al.*, 2010). It remains to be known whether an 80-factor dose difference can explain the differential anxiogenic/anxiolytic effects of BPA. Moreover, the studies by Tian *et al.* should be considered with caution since the experimental groups of individuals contained only two mothers (Tian *et al.*, 2010).

Effects on behavioural sexual dimorphism

Exposure to BPA may result in a decrease or even loss of this dimorphism:

- in the locus ceruleus (Funabashi *et al.*, 2004; Kubo *et al.*, 2001; Kubo *et al.*, 2003);
- in the anteroventral periventricular nucleus, but with inconstant findings (Patisaul *et al.*, 2006; Patisaul *et al.*, 2007; Rubin *et al.*, 2006).

The lowest *in utero* or perinatal exposure doses that have shown such effects are 0.03 mg/kg bw/day after oral exposure (Kubo *et al.,* 2003), 0.000025 or 0.000250 mg/kg bw/day after subcutaneous perfusion (Rubin *et al.,* 2006) and 100 mg/kg bw/day after subcutaneous injection (Patisaul *et al.*, 2006).

In 2009, Nakagami *et al.* undertook a study examining the effects of prenatal exposure to BPA in monkeys by analysing infant-mother behaviour in F1 cynomolgus monkeys (*Macaca fascicularis*) (Nakagami *et al.,* 2009). The behaviour of male and female offspring was studied during the early lactation period. The behavioural analysis in the offspring examined clinging to the mother, environmental exploration, outward looking, proximity and social exploration. In general, for the behaviours under study, the male F1 individuals behaved like females. After subcutaneous administration of BPA (using an osmotic pump) at doses of 10 µg/kg bw/day to gestational day GD20, the authors examined five types of behaviour: clinging behaviour, environmental exploration, outward interest, proximity and social exploration in the offspring, and approach, locomotion, orientation, outward interest and social exploration in the mothers. Each behaviour type was studied in detail in the male and female offspring and the mothers. The scores obtained for each behaviour type were summarised by a score encompassing the 5 discriminant behaviours. In general, BPA decreased maternal behaviour in a way that was distinguishable between the male and female offspring, and feminised behaviours in the male offspring exposed to BPA, often with the same behaviours as the female offspring. This study was analysed during the AFSSA expert appraisal (2010) and the following methodological limitations were reported:

- regarding exposure to BPA: plasma levels of BPA measured in mothers only on the 50th day of gestation, and lower than the Limit of Detection (12.5 ng/mL); no measurements were available for the offspring. Differences in metabolism between routes of administration were not taken into account. No dose/effect relationship could be established (only 1 dose).
- in terms of the interpretation of results: only 1 to 3 variables out of 14 were modified in the short-term (10-minute) recordings of the monkeys' behaviour. Their significance remains to be determined especially since, as affirmed by the authors, the results cannot be explained in psychological terms.
- it is difficult to interpret this study given the route of administration (non-oral), the lack of data on the offspring's actual exposure, phyto-oestrogen levels in food and BPA levels in water, the fact that only one dose was tested and doubts regarding the significance of the observed effects.

In conclusion, the AFSSA expert appraisal considered this study's results to be an alert signal (AFSSA, 2010a).

No studies have reported changes in the nucleus of the preoptic area, also a sexually dimorphic area in humans, up to doses of 320 mg/kg bw/day in rats. It therefore remains difficult to interpret these effects in rodents and their consequences.

In the recent study by Ryan *et al.* that was mentioned above, no effects on behavioural sexual dimorphism were observed with BPA at doses of 2, 20 and 200 µg/kg bw/day whereas, for comparable doses, ethinyloestradiol EE2 had the following notable effects: reduced lordosis

behaviour, increased anogenital distance, reduced pup weight at PND2, early vaginal opening, reduced F1 fertility and reduced litter sizes (Ryan *et al.,* 2010a). This work does not necessarily indicate that BPA has no effects but rather that it may exert oestrogenic action at different exposure levels from those at which EE2 has effects.

Behavioural effects were highlighted in mice by Cox *et al.* when observing the F1 offspring of mothers exposed during gestation (from GD9 to the end of gestation) to doses of 50 mg/kg feed corresponding to 8 mg/kg bw/day (Cox *et al.,* 2010). The study by Cox *et al.* showed a loss of behavioural sexual dimorphism in the offspring of mothers exposed to BPA during gestation.

Adewale *et al.* examined the effects of neonatal subcutaneous exposure to BPA in rats. Four injections of BPA were administered to female newborns at PND0, PND1, PND2 and PND3 at doses of 50 µg/kg bw/day and 50 mg/kg bw/day (Adewale *et al.,* 2011). Two positive controls were used, one by injection of PPT (ERα agonist, 1 mg/kg bw) and the other by injection of oestradiol benzoate (EB 25 µg; the publication does not specify whether it was µg/per rat or per kg). BPA did not modify sexual behaviour at any dose, but increased body weight was observed at the age of 99 days, only at the dose of 50 mg/kg bw/day of BPA and also with EB. It should be noted that controls can be considered as positive only in relation to an expected effect, which here is oestrogenic action. In the absence of an expected action, the positive character of a control has no toxicological significance.

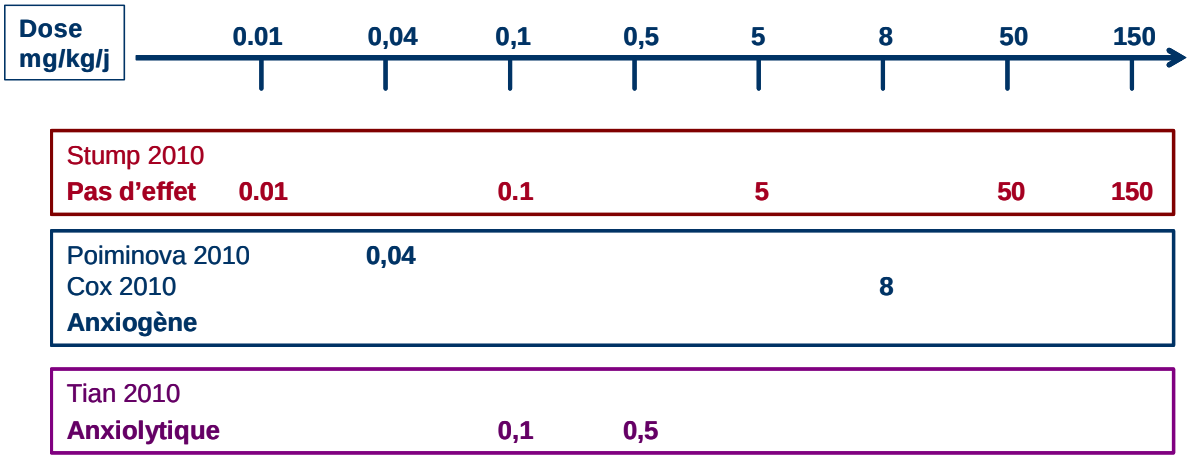


Figure 5: Effects of BPA on anxiety and exploratory behaviour according to exposure

6.4.2.3. Effects on cerebral development

Effects on neural development

The review by Hajszan and Leranth is particularly focused on how BPA affects synaptic remodelling (Hajszan and Leranth, 2010). It underlines that, in rats and non-human primates, BPA

negates the 70-100% increase in the number of hippocampal and prefrontal spine synapses induced by both oestrogens and androgens.

Kim *et al.* undertook a prenatal exposure study in ICR mice and *in vitro*. In prenatal exposure, the mothers were exposed between embryonic stages GD 14.5 and GD 18.5 by subcutaneous administration of 0, 5, 10 and 20 mg/kg bw/day (Kim *et al.*, 2009). Studies of hippocampal neurogenesis were undertaken by exposing offspring for 3 days, from postnatal week PNW8, at a rate of two daily injections of one 20 mg dose of BPA/kg in the presence of BrDU to examine neurogenesis. The *in vivo* studies showed that at PNW3, an increase could be observed in body weight at the dose of 5 mg/kg and a decrease at the dose of 20 mg/kg. These changes were not observed at PNW8, which led the authors to suggest effects mediated by the mother. Formation of the dentate gyrus was accelerated at PND1 at the dose of 20 mg/kg. The authors suggest that BPA blocks the proliferation of neural stem cells and promotes cellular differentiation in a relatively early stage. However, at PNW3, BPA did not have any observed effects on the cortical structure of the hippocampal neuronal cells or cell density. In adult mice, BPA had no observed effects on hippocampal neurogenesis. In the *in vitro* studies, mouse neural progenitor cells were exposed to BPA at concentrations of 1 nM to 500 µM. BPA reduced the proliferation of neural progenitor cells, in a concentration-dependent manner starting at 200 µM, and induced cytotoxicity at the highest concentration (500 µM). At low concentrations, BPA stimulated the differentiation of neural progenitors into neuronal phenotypes.

Effects on aminergic systems[14]

Tian *et al.* reported that perinatal oral exposure (GD7 to PND36) in mice to BPA at doses of 100 and 500 µg/kg bw/day induced an increase in dopamine D2 receptors and a decrease in dopamine transporters (DAT) in the putamen (Tian *et al.*, 2010).

BPA induces changes in cerebral development. Perinatal exposure in mice (embryonic day GD0-PND21) by subcutaneous injection at a dose of 20 µg/kg bw/day increases dopamine and its metabolites in the putamen and the dorsal raphe nucleus and increases serotonin and its metabolites in the putamen, dorsal raphe nucleus, thalamus and substantia nigra (Nakamura *et al.,* 2010). No differences in the synaptogenic effects of BPA have been observed between oral and subcutaneous exposure (Hajszan and Leranth, 2010).

[14] The **dopaminergic system** plays a role in cognitive function as lesions of dopaminergic neurons reduce performance associated with various learning and cognitive tasks.

In rats injected intracranially with BPA at PND2 with doses of 0 – 0.1 and 1 µg/kg, significant changes in certain monoamines could be observed 7 days and 28 days after the injection (PND9 and PND30) (Matsuda *et al.,* 2010). Significant increases in 5-HT (serotonin) in the hippocampus, 5-HIAA (5-hydroxyindoleacetic acid) and 5-HT in the brain stem, and DA (dopamine) and DOPAC (3,4-Dihydroxyphenylacetic acid) in the striatum were observed 28 days after the injection. Seven days after the injection, increases in 5-HT and norepinephrine (NE) and decreases in DOPAC and 5-HIAA were observed in the hippocampus. In this study, the authors analysed the degradation speed of BPA in the brain. BPA disappeared from brain tissues within 5 hours of the injection, even at the highest dose of 1000 µg/kg. The authors concluded that BPA can have effects on cerebral monoamines over 28 days after its disappearance. The authors do not describe the analytical method used to assay BPA or the Limits of Detection and Quantification. Thus, residual levels of BPA, lower than the Limit of Detection, could induce effects within the 28-day period after exposure. However, this does not change interpretation of the results. This study should be considered with caution since the doses in relation to the individuals' body weights were administered in the brain, and therefore the size of exposure cannot be assessed. Furthermore, the *in situ* injection of BPA significantly modifies the toxicokinetics and consequently the potential effects of BPA.

In the study by Adewale *et al.* that was mentioned above, the effects of neonatal subcutaneous exposure to BPA in rats were studied (Adewale *et al.,* 2011). BPA did not change serotonin fibre density in the ventrolateral subdivision of the ventromedial nucleus at any dose, whereas an increase was observed with EB and PPT which were used as positive controls.

Effects on the glutamatergic system

In the study by Tian *et al.,* which used 100 and 500 µg BPA/kg bw/day in mice, in perinatal exposure (GD7 to PND36), decreased NMDA receptors were observed in the frontal cortex, dentate gyrus (DG) and cornu ammonis 1 and 3 regions (CA1 and CA3) of the hippocampus (Tian *et al.,* 2010). Xu *et al.* studied the effects of perinatal oral (intra-gastric) exposure to BPA (GD7-PND21) at doses ranging from 0 – 0.05 – 0.5 – 5 and 50 mg/kg bw/day in mice (Xu *et al.*, 2010c) and from 0 – 0.05 – 0.5 – 5 – 50 and 200 mg/kg bw/day in rats (Xu *et al.*, 2010b). They showed that BPA negatively affects the expression of hippocampal NMDA receptors in male rats and mice. BPA at doses of 0.05 to 50 mg/kg bw/day reduced the expression of hippocampal NMDA receptors (subunits NR1, NR2A and NR2B) in F1 males. However, in rats, compared to the lower doses, the effects of BPA on the NMDA receptor subunits NR2A and NR2 at the highest dose of 200 mg/kg bw/day were less marked, which suggests that BPA has differential action at low and high doses. These changes in NMDA receptor expression were associated with reduced learning capacities.

These results were supported by studies of hippocampal neurons cultured *in vitro* exposed to BPA at concentrations from 10 to 1000 nM (Xu *et al.,* 2010a). Changes in the dendritic morphology of the hippocampal neurons (enhanced filopodial motility and density) and enhanced NMDA receptor

phosphorylation (subunit NR2B) via action exerted by BPA on the oestrogen receptors (effect suppressed by the oestrogen receptor agonist ICI 182780) were observed.

Developmental effects on NMDA receptors should be considered carefully knowing that these receptors are involved in memory and learning processes. They are also supported by the role 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).

Effects on systems involving sex hormones

Adewale *et al.* showed that, in female newborn rats subject to postnatal subcutaneous exposure with 4 injections at PND0, 1, 2 and 3, BPA increased the number of oxytocin neurons in the paraventricular nucleus, a sexually dimorphic hypothalamic region responsive to oestradiol, at BPA doses of 50 µg/kg bw/day and 50 mg/kg bw/day (Adewale *et al.,* 2011). This postnatal exposure did not affect sexual behaviour but was linked to increased body weight at the age of 99 days, only at 50 mg BPA/kg bw/day, which was also observed with oestradiol benzoate. No changes in ERα receptor density were observed in the ventrolateral subdivision of the ventromedial nucleus (VMNvl), the medial preoptic area (MPOA) or the arcuate nucleus (ARC).

In sheep, prenatal exposure to BPA (GD30-GD90) at 5 mg/kg bw/day has differential effects on the expression of hypothalamic oestrogen receptors ESR1 (ERα) and ESR2 (ERß), with increased expression for ESR1 and decreased expression for ESR2 (Mahoney and Padmanabhan, 2010). These changes were associated with increased gonadotropin-releasing hormone (GnRH) expression. In rats (Xu *et al.*, 2010b) and in mice (Xu *et al.*, 2010c), perinatal exposure to BPA (GD7-PND21) at doses of 0.05 to 50 mg/kg bw/day decreases oestrogen receptor ERβ expression and increases aromatase in the hippocampus. These studies confirm the work of Salian *et al.* which showed increased oestrogen receptor ERα expression and decreased ERβ receptors in the testes of rats whose mothers had been exposed during a period ranging from gestation (from GD12) to weaning (PND21) (Salian *et al.,* 2009c). These results were observed in the F1 offspring of exposed mothers as well as in the untreated F2 and F3 generations.

A study undertaken in SD rats, in a protocol of perinatal exposure to a low dose (sc injection of 2 µg/kg bw/day) from GD10 to PND7, clearly indicates that this exposure could modify sexual differentiation of the GnRH system in male offspring, particularly through increased kisspeptin expression in the anteroventral periventricular nucleus (AVPV) of the hypothalamus (Bai *et al.,* 2011). BPA increased the number of AVPV kisspeptin neurons at PND30, PND50 and PND90. BPA decreased the number of GnRH neurons by 40% at PND30, this was followed by a constant increase at PND50 and PND90. As a result, castrated adult males developed the ability to generate a pre-ovulatory surge-like LH release in response to a ‘pre-ovulatory’ dose of oestradiol. In rodents, this ability was considered to be a characteristic sign of feminisation in the nervous component of the gonadotropic axis. This ability was fully expressed only in males after the age of 90 days. Furthermore, in non-castrated animals, exposure to BPA increased LH concentrations, decreased testosterone concentrations in adult offspring (PND30 and 50) and increased oestradiol

concentrations at PND50 and 90. These endocrine effects are interpreted by the authors as indicative signs of long-term peripheral aromatase activity stimulation in animals exposed to BPA.

6.4.2.4. Postnatal exposure

Changes in maternal behaviour have been reported after oral exposure to 10 µg/kg bw/day of BPA from birth to adulthood (Palanza *et al.,* 2008): F1 generation mice exposed in the postnatal period showed a decrease in nursing time and an increase in time spent away from the litter. However, no effects on body weight were highlighted in the offspring (which would suggest an adequate level of care). As the significance of the effects observed in mice (nursing and nesting time) for human health has been demonstrated by only one team, they can be considered as suspected.

Table VIII: Studies examining the effects of bisphenol A on the brain and behaviour: summary table

Reference	Species/ strain	Route	Dose Exposure period	Effects NOAEL/LOAEL
(Poimenova *et al.*, 2010)	Wistar rats	Oral	40 µg/kg bw/day GD1 - weaning (42 days)	↗ levels of corticosterone and ↘ levels of GR in males in basal state and in the 2 sexes after stress No effects on the MR receptor in normal conditions, but ↘ MR level in females in the 2 groups of females ↘ spatial memory in the 2 sexes ↘ exploratory behaviour in females and appearance of anxious behaviour
(Stump *et al.*, 2010)	CD-SD rats	Oral	0.15 – 1.5 - 75 - 750 and 2250 ppm feed Gestation: 0.01 – 0.12 – 5.85 – 56.4 - 164 mg/kg bw/day Lactation: 0.03 – 0.25 – 13.1 – 123 - 410 mg/kg bw/day GD0 - PND21	No effects on exploratory behaviour <u>For systemic effects</u>: NOAEL = 5.85 mg/kg bw/day for gestation and 13.1 mg/kg bw/day for lactation <u>For neurotoxic effects</u>: NOAEL = 5.85 mg/kg bw/day for gestation and 13.1 mg/kg bw/day for lactation
(Nakagami *et al.*, 2009)	Cynomolgus monkeys	Subcutaneous	10 µg/kg bw/day (blood level equivalent to ingestion of 5 mg/kg bw/day in rats) PND31-60 and PND61 - 90	Univariate analysis: significant effects on 3 infant behaviours and 1 maternal behaviour: - <u>in ♂ F1 offspring</u>: 'embracing' and 'social exploration' behaviours ↘ at 2 months and 'outward looking' behaviour ↗ at 2 and 3 months. - <u>In mothers of ♂,</u> 'outward looking' behaviour ↗ at 2 and 3 months. Multivariate analysis: discriminant scores of F1 ♂ were closer to the F1 ♀ controls than the F1 ♂ controls. No effects in ♀. Regarding maternal behaviour, the mothers of F1 ♂: discriminant scores closer to those of the control mothers of F1 ♀ than those of the control mothers of F1 ♂.
(Kubo *et al.*, 2001)	Wistar rats	Oral	1.5 mg/kg bw/day GD0 - PND21	No sexual dimorphism compared to control No changes in reproductive organs or sex hormones

(Kubo *et al.*, 2003)	Wistar rats	Oral	0.03 - 0.3 mg/kg bw/day GD0 - PND21	Effects on sexual dimorphism: elimination and reversal of differences in openfield behaviour (locomotive activity, hyperactivity, exploratory behaviour and anxiety) **LOAEL = 0.03 mg/kg bw/day**
(Funabashi *et al.*, 2004)	Wistar rats	Oral	2.5 mg/kg bw/day GD0 - PND21	Difference in the number of CRH (corticotropin-releasing hormone-immunoreactive) neurons between females and males in the preoptic area but no difference in the BST (bed nucleus of the stria terminalis).No significant difference in the number of CRH neurons between exposed and non-exposed animals, all sexes combined
(Patisaul *et al.*, 2006)	CD-SD rats	Subcutaneous	500 µg/animal/day PND1 - PND2	Demasculinisation of tyrosine hydroxylase immunoreactivity in the anteroventral periventricular nucleus of the hypothalamus
(Patisaul *et al.*, 2007)	CD-SD rats	Subcutaneous	500 µg/animal/day PND1 - PND2	No change in SDN (sexually dimorphic nucleus) volume in the preoptic area Increased number of calbindin neurons in the SDN No demasculinisation of AVPV (anteroventral periventricular nucleus of the hypothalamus) volume but the neuron-dependent activation model was not affected
(Rubin *et al.*, 2006)	CD-1 mice	Subcutaneous	0 – 25 - 250 ng/kg bw/day GD8 - PND16	↘ sex differences in the number of tyrosine hydroxylase neurons due to a ↘ in the number of TH neurons in females Altered sexual dimorphism in the exposed animals **LOAEL = 25ng/kg bw/day**
(Ryan *et al.*, 2010a)	Long-Evans rats	Oral	2 - 20 or 200 µg/kg bw/day GD7 - PND18	No effects on behavioural sexual dimorphism
(Cox *et al.*, 2010)	Mice	Oral	8mg/kg bw/day (BPA administered in feed) GD9 - PND0	Suppression of behavioural sexual dimorphism in offspring exposed during embryogenesis No effects on dietary intake, caring behaviour or urinary marking in offspring irrespective of the mother's origin (treated or not). Increased anxiety (elevated plus maze) No effects of BPA exposure during gestation on

				the gonadal weight of male or female offspring No effects on corticosterone levels in male or female offspring **LOAEL 8 mg/kg bw/day (corresponding to 50 mg/kg feed)**
(Adewale *et al.*, 2011)	Long-Evans rats	Subcutaneous	50 µg/kg bw/day and 50 mg/kg bw/day PND0 - PND3 (4 injections)	No change in sexual behaviour ↗ body weight at the age of 99 days, only at the dose of 50 mg/kg bw/day No change in serotonin fibre density or in the density of ERα receptors in the ventrolateral subdivision of the ventromedial nucleus ↗ in the number of oxytocin neurons in the paraventricular nucleus at BPA 50 µg/kg bw/day and 50 mg/kg bw
(Kim *et al.*, 2009)	ICR mice	Sub-cutaneous	5-10-20 mg/kg bw/day GD14.5 - GD18.5 then injection of 20mg/kg twice a day for 3 days from PNW8	**F1** At PNW3, ↗ body weight at 5 mg/kg and ↘ at 20 mg/kg but not at PNW8 Accelerated formation of the dendate at PND1 at the dose of 20 mg/kg. →BPA may block the proliferation of neural stem cells and promote cell differentiation in a relatively early stage. BPA has no observed effects on the cortical structure of the neural cells, hippocampus or cell density. In adult mice, BPA has no observed effects on hippocampal neurogenesis.
(Tian *et al.*, 2010)	ICR mice	Oral	100 and 500 µg/kg bw/day GD7 - PND36	↗ dopamine D2 receptors and decreased dopamine transporters (DAT) in the putamen ↘ NMDA receptors in the frontal cortex, dentate gyrus (DG) and cornu ammonis 1 and 3 (CA1 and CA3) regions of the hippocampus
(Matsuda *et al.*, 2010)	Rats	Intracranial	0.1-1-10 µg/kg Single injection at PND2 (1st experiment) 1000 µg/kg single injection at PND2 (2nd experiment)	significant ↗ in serotonin in the hippocampus, 5-HIAA and 5-HT in the brain stem, dopamine and DOPAC in the striatum 28 days after the injection. Seven days after the injection, ↗ in 5-HT and norepinephrine (NE) and ↘ in DOPAC and 5-HIAA were observed in the hippocampus. BPA disappeared from brain tissues within 5 hours of the injection, even at the highest dose of 1000 µg/kg. → BPA may have effects on cerebral

				monoamine levels over 28 days after its disappearance
(Xu *et al.*, 2010b)	Mice and rats	Oral	0 – 0.05 – 0.5 – 5 – and 50 mg/kg bw/day in mice and up to 200 mg/kg bw/day in rats GD7 - PND21	BPA negatively affected the NMDA and ER receptor expression in the hippocampus in male rats and mice Doses 0,05 to 50 mg/kg bw/day ↘ expression of hippocampal NMDA receptors (subunits NR1, NR2A and NR2B) in F1 males. ↘ expression of ERβ oestrogen receptors and ↗ aromatase in the hippocampus
(Mahoney et Padmanabhan, 2010)	Sheep	Sub-cutaneous	5 mg/kg bw/day G30-G90	↗ expression of ESR1 and ↘ expression of ESR2 ↗ gonadotropin-releasing hormone expression
(Palanza *et al.*, 2008)	CD-1 mice	Oral	10 µg/kg bw/day 3 scenarios 1) GD14 -GD18 2) during gestation and continued after birth until adulthood 3) only after birth until adulthood	Changes in maternal behaviour in F1 offspring only after *in utero* or adult exposure (scenarios 1 and 3), but not in scenario 2 ↘ time spent by mothers caring for their offspring and ↗ time where they remained alone in the cage (isolated resting time). no effects on the weight of offspring at birth

6.4.3. Conclusion

In humans, the WG considers that the human data that are currently available are inadequate to draw a conclusion as to the effects of BPA on behaviour.

In animals, the effects on **cerebral development** linked to pre- or perinatal exposure to BPA have been confirmed by several studies that show, in particular, changes in neural differentiation, alterations of the NMDA aminergic and glutamatergic systems, changes in oestrogen receptor ERα and ERß expression, and changes in the number of neurons responsive to oxytocin and serotonin. These changes particularly occur in regions such as the hypothalamus (more precisely in regions involved in sexual dimorphism) and the hippocampus, a region involved in cognitive activities and anxiety, namely those associated with NMDA receptors. These neural effects could partly explain the behavioural effects of BPA and allow research to confirm or refute the effects of BPA on behavioural sexual dimorphism, anxiety and exploratory behaviour, and guide future research. **The WG considers that these histological changes in neurogenesis are recognised effects in animals**.

In animals, studies examining the effects of pre- or perinatal BPA exposure **on anxiety** have been conducted with exposure levels that cannot be directly compared. BPA has been shown to have no effects (Stump *et al.,* 2010), an anxiogenic effect (Cox *et al.,* 2010; Poimenova *et al.*, 2010) and an anxiolytic effect (Tian *et al.,* 2010). Thus, considering these results and those prior to 2010, **the effects of pre- or perinatal exposure to BPA in animals on anxiety, exploratory behaviour and behavioural sexual dimorphism are considered to be controversial.**

In animals, changes in maternal behaviour related to pre- or postnatal exposure to BPA are considered to be suspected effects.

- **These histological changes in neurogenesis are effects that should be considered for the health risk assessment.**

6.5. Effects on metabolism and the cardiovascular system

Metabolic syndrome, associated with a state of insulin resistance, is a combination of several criteria, including those that follow, in the same individual: central (abdominal) obesity, hypertriglyceridemia, low HDL-cholesterol, elevated blood pressure and fasting hyperglycaemia. It is a predisposing factor for cardiovascular risk and type 2 diabetes (see glossary).

The expert panel that met in Chapel Hill in 2007 considers that the *in vivo* results are contradictory. For example, certain studies show a decrease in body weight or no effect in response to developmental exposure to BPA. Other studies show an increase in postnatal growth after exposure during *in utero* development (Richter *et al.*, 2007).

The NPT-CERHR also indicates that the available data are not sufficiently conclusive to link prenatal BPA exposure with obesity (NTP-CERHR, 2008). It reports 2 animal studies that assessed disruption of the regulation of fat and carbohydrate metabolism. In male rats, sub-cutaneous doses of 0.01 and 0.10 mg/kg bw/day of BPA cause decreased glucose levels and increased insulin levels (Alonso-Magdalena *et al.*, 2006). Furthermore, increased insulin production by the pancreas and insulin resistance were described at 0.10 mg/kg bw/day (administered orally or by SC injection) after a 4-day period. The study by Miyawaki *et al.* reports effects on body weight, adipose tissue weight, serum leptin levels, triglyceridemia, non-esterified fatty acids and glucose (Miyawaki *et al.* 2007). However, the NTP considered that these studies were non-admissible due to methodological problems.

Some studies have assessed mechanisms likely to interact with fat and carbohydrate metabolism: BPA has been found to stimulate the oestrogen receptors α found in the pancreatic beta cells (Richter *et al.,* 2007; Ropero *et al.,* 2008; Nadal *et al.,* 2009; Alonso-Magdalena *et al.,* 2006, 2008), while oxidative stress may contribute to insulin resistance (Hong *et al.,* 2009). Likewise, the NPT-CERHR reports accelerated differentiation of fibroblast cells into adipocytes, and altered glucose transport in adipocytes (Masuno *et al.*, 2002 et 2005; Phrakonkham *et al.*, 2008; Sakurai *et al.*, 2004) (NTP-CERHR, 2008).

According to Aschberger *et al.* (2010), epidemiological studies and *in vivo* and *in vitro* studies suggest that exposure to BPA is related to 'metabolic syndrome' (Aschberger *et al.,* 2010). Liver enzyme abnormalities are also described (Takeuchi *et al.,* 2004; Lang *et al.,* 2008 ; Newbold *et al.,* 2009a; Elobeid *et al.,* 2008 reported by Aschberger *et al.*, 2010).

The FAO/WHO experts considered that the two studies in humans that reported a positive relationship between urinary concentrations of BPA and cardiovascular diseases or diabetes (Lang *et al.,* 2008; Melzer *et al.,* 2010) have weaknesses that limit their interpretation (FAO/WHO, 2010).

The experts consider that it is necessary to implement prospective studies linking BPA measurements during various windows of susceptibility and the onset of cardiovascular diseases or diabetes several years later. Two studies have examined birth defects and body weight index but the results are difficult to interpret (Padmanabhan *et al.,* 2008; Wolff *et al.,* 2008); the experts recommend undertaking studies assessing the link between BPA exposure during pregnancy (urinary BPA levels sampled on several occasions) and body weight index and adipose mass at birth.

In animals, according to this panel, the available data do not clearly show that BPA has cardiovascular effects, and in particular, studies undertaken in accordance with GLP using large samples have not shown toxicity to the cardiovascular system. Changes in VEGF expression, NO production and ion channels have been reported, but with no related adverse effects to date. These experts have been informed that studies examining the cardiotoxicity of BPA are in progress.

Regarding effects on metabolism, the available data are not sufficient to draw conclusions as to the effects of BPA. According to this panel, the 2008 conclusions of the NTP-CERHR indicating that BPA does not affect obesity at doses < 5000 µg/kg bw/day remain valid. However, examining newborn weight is not sufficient to draw a conclusion regarding obesity, unlike a direct measurement of body fat and its distribution. Yet the available data on glucose intolerance, hyperinsulinaemia, adipose hypertrophy, etc. suggest that supplementary studies need to be undertaken to examine the effects of BPA on the regulation of fat, carbohydrate and insulin metabolism and other effects related to diabetes and metabolic disorders. These effects should be investigated in adult animals exposed during pregnancy, including older animals (FAO/WHO, 2010).

6.5.1. Human data

Hong *et al.* studied levels of oxidative stress in an urban adult population in Korea exposed to various contaminants between April and December 2005 (Hong *et al.*, 2009). A total of 960 (85%) people out of 1131 identified subjects, of whom 46% were men and 54% were women took the questionnaire. A questionnaire on lifestyle habits was developed and environmental exposure studies were undertaken. Furthermore, urine and blood samples were taken. The aim was to assess the relationship between chemical exposure and oxidative stress, and the potential role of certain environmental chemicals in insulin resistance. The authors found a significant positive relationship between urinary concentrations of chemical contaminants, particularly phthalates and BPA, and oxidative stress markers in a simple regression analysis. Nevertheless, this relationship disappeared for BPA in a multiple regression model after controlling for age, sex, smoking and exercise. Oxidative stress marker levels were correlated with levels of insulin resistance in peripheral tissues. A positive association was found between urinary levels of BPA and fasting glycaemia. The authors concluded that exposure to chemical contaminants is associated with

oxidative stress in urban adult populations and suggested that exposure to certain environmental chemicals might contribute to insulin resistance.

In 2008, Lang *et al.* undertook a cross-sectional study in 1455 adults aged 18 through 74 years in the United States. They used data from the 2003-2004 National Health and Nutrition Examination Survey (NHAHES) (Lang *et al.*, 2008). Regression models were adjusted for age, sex, race/ethnicity, education, income, smoking, Body Mass Index (BMI) and waist circumference. Urinary concentrations of total (free and conjugated) BPA were measured using HPLC-MS and adjusted for creatinine. High BPA concentrations (5 and 13 ng/mL) were associated with a higher risk of cardiovascular disease, only after age and sex adjustment. An association with diabetes was found, but not with other types of diseases. A significant increase in alkaline phosphatase and γ-glutamyl transferase concentrations was associated with high BPA concentrations. The authors remain general in their conclusion and speak of a possible association between high BPA exposure and adult morbidity. The group of Melzer *et al.*, which was part of the Lang *et al.* team, used the data for the NHANES adult sub-population (Melzer *et al.,* 2010). This new analysis partly confirmed the results of the 2003-2004 campaign. It showed that high BPA exposure, reflected by high urinary concentrations of BPA, were associated with cardiovascular diseases (coronary diseases) in the 2005-2006 campaign and in the two pooled campaigns, and with diabetes in the two pooled campaigns but not in the 2005-2006 campaign.

The mechanisms by which BPA results in cardiac disease are not yet absolutely known. However, Asano *et al.* reported a possible route of action that might involve the Maxi-K potassium channels (Kca1.1), which are sensitive to both oestrogens and BPA (Asano *et al.*, 2010). One of the limitations of the study by Asano *et al.* is that activation of the Maxi-K channels is observed at a pharmacological concentration (10 µM) of BPA, which is not compatible with environmental exposure levels for BPA (Asano *et al.*, 2010).

A cross-sectional study was undertaken in Japan in order to examine the influence of BPA, age and BMI on hormonal changes in the blood (Takeuchi *et al.,* 2004). In total, 73 women were recruited, then divided up after medical consultation into 6 groups including: women diagnosed as normal (normal weight; no related disease), obese (no related disease), with hyperprolactinemia, with hypothalamic amenorrhea and with polycystic ovary syndrome (PCOS) including a subgroup of obese and non-obese women. The authors identified a strong relationship between serum levels of BPA and the effects on androgen metabolism. More precisely, Takeuchi *et al.* reported a positive correlation, in the group of women diagnosed as normal, between serum BPA concentrations and free testosterone, androstenedione and dehydroepiandrosterone sulphate (DHEAS) concentrations. They also showed a positive correlation taking into account all of the women from the 6 groups and calculating a correlation between BPA and concentrations of testosterone (free and total), androstenedione and DHEAS. The authors concluded that there is a strong relationship between serum BPA and androgen concentrations, which they attribute to the

effect of androgen on the metabolism of BPA. However, it remains difficult to interpret these results as is, due to the imprecision of the sampling plan and the lack of information about the inclusion criteria (particularly for the constitution of the control group). Moreover, the fact of taking into account all of the women in the 6 groups together introduces a selection bias on account of the various diagnosed diseases. The calculated correlations, even though they are significant, range from 0.391 and 0.684. These low correlation values could be due to the small population size, the variability of the measured parameters, biases linked to the summation of the diseases or the analytical technique that was used (ELISA).

6.5.2. Animal data

6.5.2.1. Prenatal and perinatal exposure

- **Effects on glucose metabolism**

Alonso-Magdalena *et al.* studied the effects of BPA on glucose metabolism in female mice, during gestation, and their male F1 offspring (Alonso-Magdalena *et al.,* 2010). BPA was administered sub-cutaneously to the mothers, from GD9 to GD16, at doses of 0, 10 and 100 µg/kg bw/day. In the F1 offspring, 6-month old males had reduced glucose tolerance, increased insulin resistance, and higher plasma levels of insulin, leptin, triglycerides and glycerol. Moreover, the islets of Langerhans presented altered calcium signalling. The authors note that BrdU incorporation into insulin-producing β cells was reduced, yet their surface was unchanged. However, the latter results, although very likely, should be considered with caution, since they were obtained with cultured cells from exposed individuals. Therefore, taking into account isolation and culturing methods, cultured cells have different phenotypes than *in situ* cells. Such an approach is relevant when undertaking an instant analysis of the cellular state after rapid fixation and treatment of the tissues. However, it is not appropriate when examining differences in cell functioning between controls and individuals exposed to a stress agent.

Ryan *et al.* tested the hypothesis that perinatal exposure to BPA, at a dose consistent with environmental exposure (0.25 µg BPA/kg bw/day), results in increased susceptibility to high-fat diet-induced obesity and glucose intolerance in CD-1 mice (Ryan *et al.,* 2010b). F1 individuals were exposed to BPA in the perinatal period (1 µg/kg via the mothers' feed, equivalent to around 0.25 µg/kg bw/day) from the embryonic stage GD0 to weaning (PND21). In the weaned F1 individuals, increased body weight was observed in males and females at 3 weeks and increased body length was observed in males at 4 weeks, these biometric differences disappearing in adulthood. No significant effects on glucose tolerance were observed. The authors concluded that the increased body length and weight were due to a faster rate of growth in the exposed mice rather than a state of obesity.

- **Effects on lipid metabolism**

Somm *et al.* studied the effects of BPA in F1 rats (Sprague-Dawley) subject to perinatal exposure (GD6 to PND21), by administering drinking water containing BPA at a concentration of 1 mg/L (corresponding to 70 µg/kg bw/day) to the mothers (Somm *et al.,* 2009). In general, BPA did not alter sex ratio or litter size. The male and female F1 individuals exposed to BPA had higher weights than the controls at PND1. At PND21, body weight was increased only in females, whose white adipose tissue weight increased threefold, this was combined with adipocyte hypertrophy and overexpression of lipogenic genes such as C/EBP-α (CCAAT enhancer binding protein α), PPAR-γ (peroxisome proliferator-activated receptor γ, SREBP-1C (sterol regulatory element binding protein-1C), LPL (lipoprotein lipase), FAS (fatty acid synthase) and SCD-1 (stearoyl-CoA desaturase). In addition, C/EBP-α, FAS and ACC (acetyl-CoA carboxylase) gene expression was also increased in the liver of exposed females at PND21, with no significant change in circulating glucose and lipid levels. After weaning, there was a sex- and diet-dependent predisposition to excess weight in F1 individuals exposed to BPA. Thus, no difference in body weight was observed between BPA-exposed individuals and control animals on a standard chow diet whereas exposed individuals fed a high fat diet were 7% overweight. This excess weight was not associated with increased food intake.

Miyawaki *et al.* studied the effects of BPA on hyperlipidemia, from gestation to PND10, and the development of obesity in mice (Miyawaki *et al.,* 2007). This group subjected mice to (1 µg/kg bw/day) (low dose = LD) or 10 µg (2.5 µg/kg bw/day) (high dose = HD) of BPA/mL in drinking water. They then measured anatomical and physiological changes at PND31. In females, they noted that the body weight of the mothers increased by 13% (LD) and 11% (HD) compared to the control group, adipose tissue weight increased by 132% in the LD group and cholesterol increased by 33% (LD) and 17% (HD). In males, body weight increased by 22% (LD) and 59% (HD) and the triacylglycerol level increased by 345% (LD) compared to the control group. In light of these results, they concluded that BPA, during pregnancy and in postnatal exposure during lactation, causes hyperlipidemia and the development of obesity.

6.5.2.2. Exposure in adults

Alonso-Magdalena *et al.* studied the effects of BPA on glucose metabolism in mice, considering mothers during gestation and their male F1 offspring (Alonso-Magdalena *et al.,* 2010). BPA was administered sub-cutaneously on gestation days GD9 to GD16 at doses of 0, 10 and 100 µg/kg bw/day. In mothers, BPA exposure increased insulin resistance associated with gestation at the dose of 10 µg/kg bw/day versus the control group and had a tendency to increase insulin sensitivity at the dose of 100 µg/kg bw/day (not significant at p=0.05), and reduced glucose tolerance at 10 µg/kg bw/day. IT caused a dose-dependent increase in plasma levels of insulin at

10 µg/kg bw/day, leptin at 100 µg/kg bw/day, triglycerides at 100 µg/kg and glycerol at 100 µg/kg bw/day. At 10 µg/kg, BPA reduced insulin-stimulated Akt[15] phosphorylation in the liver and blunted it in the gastroc-nemius muscle. Long-term effects were also observed in the mothers, 4 months post-partum, with increased body weight and higher concentrations of insulin, leptin, triglycerides and glycerol in BPA-treated individuals.

[15] Akt is a serine/threonine protein kinase that plays a role in glucose metabolism and is activated by the 3-phosphoinositide-dependent protein kinases PDK1 and PDK2. PI3K is involved in the signalling pathway associated with the synthesis and secretion of adiponectin.

6.5.3. In vitro studies

Effects on lipogenesis

In vitro, BPA at concentrations ranging from 100 pM to 1 µM promotes adipogenesis in mouse preadipocyte 3T3-L1 cells (Sargis *et al.,* 2010). The activation of this lipogenesis is mediated by glucocorticoid receptors. BPA increases lipogenesis in differentiating adipocytes and activates the expression of specific adipocytic proteins (adiponectin, transcription factor CCAAT enhancer binding protein α (C/EBP-α), a factor induced in the terminal phase of adipogenesis). However, the action of BPA on adiponectin induction shows a bell curve with a visible effect from 10 nM, peaking at 100 mN and disappearing at 1000 nM. An identical dose-response relationship was observed with dexamethasone. It should be noted that in this study, the other compounds under consideration, dicyclohexyl phthalate, tolyfluanid, troglitazone and triphenyltin had lesser effects at the highest concentration of 1 µM.

In the studies by Kidani *et al.*, 3T3-L1 cells were exposed to various forms of bisphenol (BP): BPA, BPB, BPE and BPF at concentrations of 0, 20, 40 and 80 µM. In a dose-dependent manner, BPA decreased the concentration of cellular adiponectin and was secreted in the extracellular medium (Kidani *et al.,* 2010). Forms of BPA can be classified as follows according to their ability to reduce adiponectin secretion: BPB > BPA > BPE > BPF. BPA negatively regulates the Phosphatidylinositol 3-Kinase (PI3K)-Akt signalling pathway by reducing Akt and p-Akt expression.

However, the inhibition of adiponectin expression by BPA should be compared with the results obtained by Sargis *et al.* showing a bell-shaped dose-response relationship between BPA and adiponectin (Sargis *et al.*, 2010). The negative effects on adiponectin expression observed by Kidani *et al.* are therefore not surprising in that they were produced at concentrations greater than 1 µM (Kidani *et al.,* 2010). Thus, BPA may induce adiponectin expression at low doses and suppress it at high doses (which are already very low).

Asahi *et al.* undertook studies in cultured non-parenchymal hepatocytes, NCTC Clone 1469 cells (Asahi *et al.*, 2010). The cells were exposed to BPA at concentrations of 0, 1, 10, 50, 100 and 200 µM for 48 hrs. or at a concentration of 100 µM for a period of 120 hours, with an analysis of BPA's effects at various times. After having examined the cytotoxicity of BPA at various concentrations, the studies continued, exposing the cells to BPA at the concentration of 100 µM. At this concentration, BPA induced apoptosis which was expressed by DNA fragmentation, phosphatidylserine externalisation on the outer plasma membrane leaflet, an increase in caspase-12, the GRP78/BiP protein (involved in endoplasmic reticulum homeostasis) and transcription factor CHOP (C/EBP homologous protein, a transcription factor involved in stress-induced apoptosis in the endoplasmic reticulum), and a slight decrease in the anti-apoptic protein Bcl-2. These results strongly suggest that the endoplasmic reticulum plays a role in the apoptosis induced by BPA. The effects of BPA are accompanied by oxidative stress, with an increase in

reactive oxygen species (ROS) counteracted by antioxidant N-acetylcysteine (N-AC). At the concentration of 100 µM, the effects of BPA do not appear to be mediated by oestrogen receptors; the oestrogen receptor inhibitors 4-OHT and ICI do not prevent the cytotoxicity of BPA and 4-OHT enhances it (Note: 4-OHT has a partial agonist effect on oestrogen receptors).

Table IX: Studies examining the effects of bisphenol A on metabolism and the cardiovascular system: summary table

Reference	Species/strain	Routes	Dose Exposure period	Effects NOAEL/LOAEL
(Alonso-Magdalena *et al.*, 2010)	Mice	Sub-cutaneous	0 - 10 and 100 µg/kg bw/day GD9 to GD16	In F1 offspring, 6-month males had ↘ glucose tolerance, ↗ insulin resistance, and ↗ plasma levels of insulin, leptin, triglycerides and glycerol, altered calcium signalling in islets of Langherans ↘ BrdU incorporation into insulin-producing β cells , whereas their surface was unchanged. In mothers, ↗ insulin resistance induced by gestation and ↘ glucose tolerance. dose-dependent ↗ in plasma levels of insulin, leptin, triglycerides and glycerol. ↘ insulin-stimulated Akt phosphorylation in gastrocnemius skeletal muscle and liver. 4 months post-partum: higher body weight, higher concentrations of insulin, leptin, triglycerides and glycerol
(Ryan *et al.*, 2010b)	CD-1 mice	Oral	0.25 µg/kg bw/day GD0 to PND21	In F1 offspring, ↗ body weight in males and females at 3 weeks ↗ body length in males at 4 weeks, these biometric differences disappearing in adulthood. No significant effects on glucose tolerance were observed.
(Somm *et al.*, 2009)	Sprague Dawley rats	Oral	70 µg/kg bw/day GD6 - PND21	**At birth**: BPA treatment during gestation did not affect sex-ratio or litter size. Newborns (♀ and ♂): ↗ weight **PND21** ↗body weight in females Increased parametrial fat associated with adipocyte hypertrophy and overexpression of lipogenic genes and lipogenic enzymes In the liver, increased RNA levels of C/EBP-α, SREBP-1C, ACC and FAS k. Circulating lipids and glucose were normal. **4 to 14 weeks**: no difference in body weight observed between BPA-treated males and control animals on standard chow diet. ↗ body weight in BPA-exposed males fed a

				high-fat diet. ↗ body weight in females for the 2 tested diets. In males fed a high-fat diet, normal glucose tolerance test results. <u>Conclusion</u>: Perinatal exposure to BPA. ↗Adipogenesis at weaning in ♀. In adult ♂, ↗ body weight observed if high-fat diet.

6.5.4. Conclusion

In a cross-sectional study **in humans** (Melzer *et al.,* 2010), a correlation was observed between the highest urinary levels of BPA and cardiovascular diseases (coronary diseases) and diabetes. The WG considered that these effects are suspected effects.

In animals, studies examining effects on enzyme activity, growth and metabolism suggest that rodents exposed in adulthood or during gestation undergo metabolic changes in various organs such as the liver, adipose tissue and pancreas. Moreover, a few authors have noted changes in the expression of protein-coding genes intervening in the cell signalling pathways involved in lipogenesis and carbohydrate metabolism. There is a trend showing *in vivo* effects on lipogenesis. *In vitro* mechanistic studies support these observations.

However, the effects on carbohydrate metabolism cannot be confirmed on account of insufficient repeatability.

Thus, in animals, BPA increases blood lipid levels, leads to excess body weight and enhances lipogenesis. The effects on lipogenesis (*in vivo* and *in vitro* data), after pre- or perinatal exposure or exposure in adulthood, are considered to be recognised. The effects on glucose metabolism after pre- or perinatal exposure to BPA are considered to be controversial.

- **Changes in lipid metabolism are effects that should be taken into account for the risk assessment.**

6.6. Effects on the thyroid

The panel of experts that met at Chapel Hill in 2007 mentions an anti-thyroid effect of BPA (see works of Zoeller *et al.*, 2005) (Richter *et al.*, 2007).

The NTP-CERHR also states that BPA may interact with the thyroid hormone receptors based on studies *in vitro* (NTP-CERHR, 2008). BPA may inhibit transcription mediated by the thyroid hormone receptors, the action of triiodothyronine (T3) or its binding to the thyroid hormone receptors. The NTP-CERHR also reports the results of Zoeller *et al.* (2005) suggesting an antagonist effect of BPA on the TRβ receptors (NTP-CERHR, 2008).

6.6.1. Human data

The study by Meeker *et al.* relating to a population of men consulting for fertility problems (n=167) shows a negative correlation between the urinary concentrations (0.4 to 36.4 ng/mL) of BPA (geometric mean from 1 to 3 samples taken) and the serum TSH concentrations at the first sampling. Reservations can be expressed concerning this study. Firstly, it relates to a particular population of men consulting for problems of infertility in a couple. Secondly, the thyroid aspects appear as "opportunistic", as the study was undertaken for a problem of fertility, and the protocol does not take into account features specific to investigation of thyroid function (existence of a nychthemeral rhythm, food intake, etc.). Finally, the significance of this correlation depends on the number of urine samples included in the calculation of the geometric mean (from 1 to 3 for each individual). Yet only 75 patients underwent repeated sampling. Although the analysis relates to the correlation between TSH and the only measurement of urinary BPA carried out on the day of sampling for all the patients (n=167), or between TSH at the first sampling and the geometric mean of the BPA concentrations for the 3 samples, limited to 75 patients from whom multiple samples have been collected, no significant correlation can be demonstrated. Taking these reservations into account, it seems difficult to accept the study for evaluating the disrupting effects of BPA on the thyroid in humans (Meeker *et al.*, 2010a).

6.6.2. Animal data

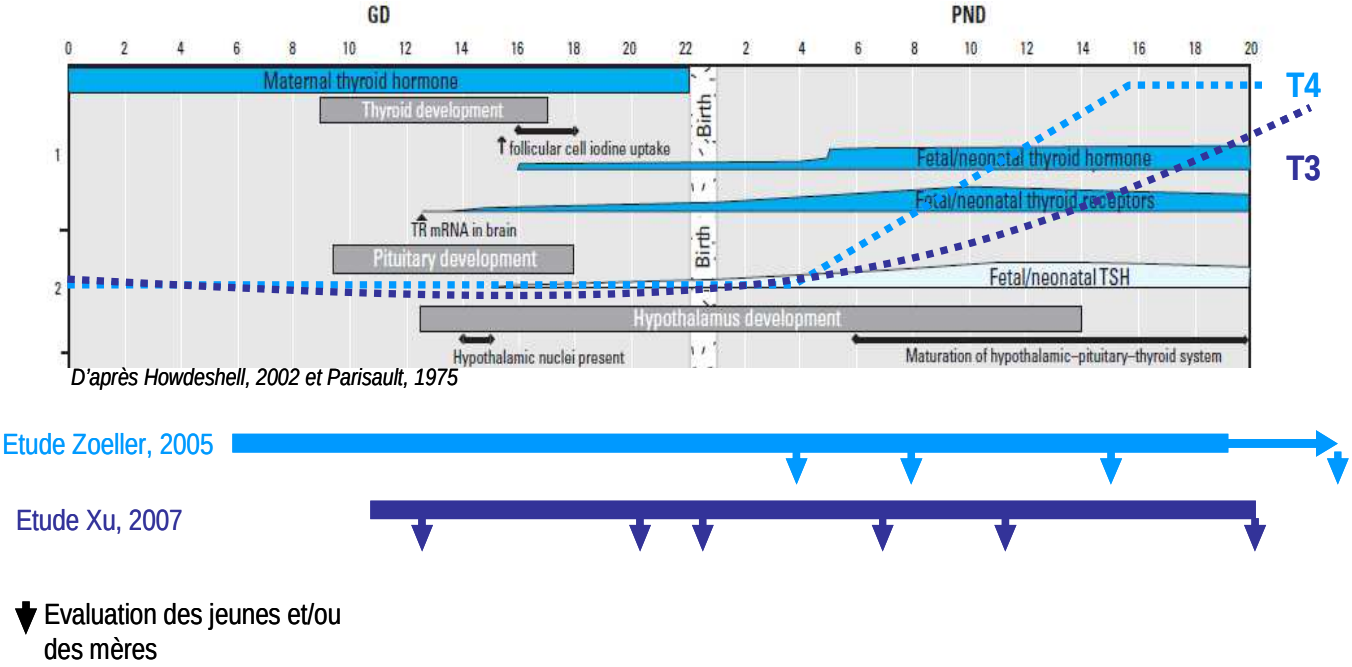
A first study was conducted in rats (Zoeller *et al.*, 2005). Exposure to BPA was initiated *in utero* (GD6) then continued throughout the neonatal period in female rats by the oral route in palatable biscuits made available in limited amounts to ensure consumption of the whole dose (negative control, 1; 10; 50 mg/kg bw/d). The young from these mothers were sacrificed at PND4, 8, 15 and 35. The results show, in the young, an interaction between the animals' age and the treatment on the total serum T4 concentrations. The total serum T4 concentrations increase physiologically between PND4 and PND16 (Dussault and Labrie, 1975). This increase is necessary for maturation of the central nervous system (Howdeshell 2002). In Zoeller's experiment, this increase in T4 is

greater in the animals (male and female) from mothers treated with BPA at PND15 (but not at PND8) compared with the animals from control mothers, starting from the lowest dose (1 mg/kg bw/d) (Zoeller *et al.*, 2005). In contrast, exposure to BPA in the mothers had no effect on serum concentrations of circulating TSH measured at PND15 in the males. Faced with an increase in concentrations of T4, the negative feedback mechanism is normally reflected in a decrease in TSH levels. The absence of a difference in serum TSH accompanying higher concentrations of T4 in the animals from mothers treated with BPA leads the author to express the hypothesis that there could be a fault in negative feedback connected with a state of resistance to thyroid hormones established in the animals exposed to BPA. Such a hypothesis would be consistent with the assumed mechanism of action of BPA on thyroid function, reflected in an antagonist effect of BPA on the TH receptors leading to a decrease in negative feedback of the THs on the secretions of TRH and/or TSH at the hypothalamo-pituitary level. However, owing to the set of specific transport systems of the THs, changes in total T4 are not necessarily reflected in changes in free bioactive T4. As Zoeller's study did not measure free T4, it is difficult to conclude that this study reveals inhibition of the action of the THs at the hypothalamo-pituitary level (Zoeller *et al.*, 2005). It would seem moreover that certain areas of the brain may be sensitive to this state of hyperthyroidism, as indicated by the increase in expression of neurogranin RC3 (protein of dendritic branching of neurons, expression of which is under the control of the THs) in the dentate gyrus in young males aged 15 days from mothers treated with BPA. The cortex, for its part, does not respond to this increase in T4. According to the studies *in vitro*, the mechanism of the TH antagonist action of BPA may result from increased recruitment of the co-repressors of the TH receptors. The author suggests that the antagonist effect of BPA might be "tissue-specific" and may depend notably on the level of expression of these corepressors in the target tissues.

This study offers excellent methodological guarantees although the animals were kept in plastic cages and is based on the use of a route of administration that is appropriate for analysis of the risk to human health (oral route of spontaneous ingestion and dose below the NOAEL).

Another study was also conducted in the pregnant rat (Xu *et al.*, 2007). These female animals were treated with BPA in the drinking water at doses of 0.1 and 50 mg/L from GD11 to PND21. Although the authors state that they measured the daily consumption of water, no information is given which allows the dose ingested by the animals to be estimated. Assuming an average consumption of 15 mL/100 g/d, a rough estimate shows that the doses ingested by the mothers were below 100 µg/kg bw/d (probably around 15 to 20 µg/kg bw/d) for the lowest dose and 50 mg/kg bw/d (8 to 10 mg/kg bw/d) for the highest. The mothers displayed significant transient hypothyroidism (lower serum concentrations of free T4) only for the lowest dose at PND0 and PND7 (no effect of BPA during gestation or after PND7). In the young, effects of BPA on the serum concentrations of free T4 were only significant in the males. Hyperthyroidism was noted at 7 days of age but was only significant for the lowest dose. Hypothyroidism was observed for the highest dose at 21 days.

Expression of the gene of TRα and of neurogranin RC3 in the hippocampus was unchanged in the animals treated including at PND15. It is difficult to understand whether the author dissociates the dentate gyrus from the horn of Ammon in his immunohistochemical analysis of the hippocampus, so it is difficult to make a direct comparison with the study of Zoeller *et al.* (Changes in expression of neurogranin specific to the dentate gyrus) (Zoeller *et al.*, 2005).



The blue bars represent the periods of exposure of the mothers in relation to the critical phases of ontogenesis of thyroid function in the rat shown in the upper part of the diagram.

Figure 6: Schematic representation of experimental protocols analysing the effect of developmental exposure to BPA on thyroid function of the young

The only two available studies *in vivo* in mammals are very difficult to compare, not from the standpoint of the doses and the methods of administration, but from that of the thyroid parameters investigated: total T4 and TSH for Zoeller (Zoeller *et al.*, 2005) and free T4 for Xu (Xu *et al.*, 2007). The balance between free (bioactive) and bound form of a hormone is complex and closely dependent on the specific binding proteins of these hormones. In the case of T4, this figure is even more complex as several binding proteins coexist and their expression profile is regulated during neonatal development in the rat, suggesting that the free T4/total T4 ratio might be extremely variable in relation to the stage of development. The two studies are in agreement in suggesting the possibility of a transient thyroid disturbance at the moment of maturation of this function in the newborn rat (PND4 to 16) following exposure *in utero* and neonatal exposure. This effect would involve amplification of the physiological increase of T4 in young rats from treated mothers. However, the dynamics of establishment of this effect as well as the interactions with the sex of the

young rats differ between the two studies. In both cases, an increase in circulating T4 is observed postnatally in the young rats from mothers exposed to BPA, but not before 8 days (difference observed at 15 days) in males and females for one (Zoeller) and starting from 7 days and only in males for the other (Xu). These two results are not necessarily contradictory since the two studies did not measure the same thing (total T4 constituting more than 70% of T4 bound to specific transport proteins for Zoeller *vs.* free T4 for Xu) (Xu *et al.*, 2007; Zoeller *et al.*, 2005). Thyroxin binding globulin (TBG), the most effective thyroid hormone binding protein, is still expressed at this stage in young rats. The expression and stability of said TBG are modulated in many species by the sex steroids with a positive effect of oestrogens and a negative effect of androgens. Although this is not documented in the newborn rat, the expression profiles of TBG could be expected to differ between the sexes. The females could have higher levels of TBG and the resultant increase in T4 binding capacity could contribute to the effects expressed on total T4 not affecting free T4.

The effects on thyroid function can be regarded as suspected.

However, the question of the relevance of the rodent model relative to humans and more particularly for the neonatal period is still one of the most critical. Unfortunately, no data are available in other animal models closer to humans and the question of a possible disrupting effect of BPA on the thyroid remains unanswered.

6.6.3. Data in vitro or in amphibians

6.6.3.1. Antagonistic effects of THs

- **Cellular models**

BPA may act as antagonist of the thyroid hormones and could thus block the effects of these hormones. The mechanism demonstrated to date is based on binding of BPA to the thyroid hormone receptor, which would potentiate interaction of the receptor with its corepressors thus leading to inhibition of the effects of the THs. A complete study was conducted, investigating the binding capacities of BPA to the nuclear receptors of the thyroid hormones and its mode of interaction with these receptors. BPA can displace T_3 from its binding sites on nuclear extract of rat hepatocytes with an inhibition constant of the order of 200 µM (low affinity). It reduces the affinity constant of T3 by a factor of 2 without changing the maximum binding capacities. These results suggest that BPA would be a very weak competitor of the THs for their receptors. This is confirmed by a study carried out on nuclear fractions of the MtT/E-2 rat pituitary cell line (Kitamura *et al.*, 2005) showing that BPA (10^{-8} to 10^{-4} M) does not permit significant displacement of T3 from its binding sites.

On embryonic kidney cells of human origin (TSA 201) transfected with either the gene of TRα, or the gene of TRβ associated with a reporter gene, BPA at concentration levels of the order of 1 µM is capable of reducing the transcriptional response of these cells induced by T3 for both receptors. Similar results are obtained with the human hepatocyte line HepG2 expressing the TRs naturally. This study indicates, moreover, that BPA may act by increasing the recruitment of the corepressor of the TH receptors, N-cor (Moriyama *et al.*, 2002) explaining the antagonist effect of BPA on the effects of the THs. A cellular model of monkey kidney cell line (CV1) transfected by the gene of the beta receptor of TH+luciferase, indicates that BPA only has an effect in the presence of TH and inhibits the response of these cells to T3. These effects are expressed for concentrations of BPA of the order of 10^{-4} to 10^{-5} M at nominal concentration in the culture media (Ghisari and Bonefeld-Jorgensen, 2005; Ghisari and Bonefeld-Jorgensen, 2009; Sun *et al.*, 2009). These results confirm that BPA might have a TH antagonist effect. However, it should be noted that the concentration levels associated with these effects are high (10^{-5} M or approximately 2.3 µg/mL, 100 times the highest plasma/serum concentrations found in humans).

BPA inhibits differentiation of precursors of murine oligodendrocytes induced by THs *in vitro* via a TRβ-dependent mechanism (Seiwa *et al.*, 2004). This study clearly poses the problem of the link that might exist between disturbance of thyroid function during development and ontogenesis of the central nervous system.

Overall, the aforementioned cellular models produce consistent results, suggesting a potential of BPA as antagonist of the effects of the thyroid hormones.

However, other studies notably conducted on cell lines of pituitary origin lead to a divergent result.

The model of pituitary cell lines GH3, growth of which is dependent on the thyroid hormones, shows a potentiating effect of BPA on the effect of T3 on cellular growth (Ghisari and Bonefeld-Jorgensen, 2005).

On balance, the antagonist effect of BPA relative to the effects of the THs (Thyroid Hormones) *in vitro* depends on the cellular model used and in any case is only expressed at relatively high nominal doses.

- **Amphibian models**

Results consistent with the hypothesis of an antagonist effect of BPA have been obtained *in vivo* from different amphibian models (oocyte, xenopus at the metamorphic stage, tadpole of transgenic xenopus expressing a reporter gene encoding a fluorescent protein under the control of a promoter bearing a thyroid responsive element).

Thus, the effects of BPA at doses of 0.1 to 10 µM (considered as relevant to human exposure), alone or in coadministration with T3, indicate that 60% of the genes whose expression is inhibited by BPA in the intestine of the xenopus tadpole in pre-metamorphosis are genes under positive control of T3 and that nearly 50% of those whose expression is stimulated by BPA are under negative control of T3 (Heimeier *et al.*, 2009; Heimeier and Shi, 2010). BPA greatly inhibits the process of metamorphosis induced by T3 at all levels (anatomy of the head, disappearance of the gills, growth of the limbs, etc.), starting from the lowest dose of 0.1 µM. Moreover, BPA (10^{-6} M) inhibits expression of GFP induced by T3 of tadpoles of transgenic xenopus (Fini *et al.*, 2007).

The results concerning the effects of BPA on metamorphosis of amphibians in response to T3 are all consistent, irrespective of which parameters are investigated (expression of fluorescent proteins on transgenic animals, degradation of DNA in the tail (Goto *et al.*, 2006) and occur for concentrations in the growing medium of the order of 1 µM. Taken together, these results speak in favour of a potential effect of BPA as thyroid hormone antagonist in lower vertebrates.

6.6.3.2. Effects on the TH transport proteins

The thyroid hormones in the bloodstream are managed by extremely efficient transport systems, which would thus play a protective role of the THs with respect to hepatic catabolism. These systems are therefore regarded as key elements in maintaining homeostasis of thyroid function to such a degree that interspecies differences in these transport systems are sometimes used for refuting the results obtained in rodent models regarding their relevance to humans. Two studies suggest that BPA would not compete with the THs for these proteins, whether considering transthyretin (Meerts *et al.*, 2000) and/or thyroxine binding globulin (Marchesini *et al.*, 2008).

Another study conducted in the Japanese quail suggests, however, that BPA might act as a competitor for binding of T3 to transthyretin proteins; it would nevertheless be half as effective as T3 (Ishihara *et al.*, 2003). A recent study *in vitro* examined determination of the quantitative parameters characterising the binding capacity of BPA to the three transport proteins of the THs in humans (serum albumin, transthyretin (TTR) and thyroxin binding globulin (TBG) (Cao *et al.*, 2011). This study clearly shows that BPA has the capacity to bind to the three types of proteins. This binding may take place with an affinity equivalent to that of the THs for serum albumin, which constitutes a non-specific transport system of low affinity (affinity constant of the order of 10^4 to 10^5 L/mol). Conversely, as regards the specific transport proteins, the affinity constants of BPA are respectively 300 and 3000 lower than those of T4 for TTR and TBG. The authors therefore conclude from this that at the levels of serum concentrations of BPA most often described in humans, the potential of BPA to interfere with the specific transport systems of the THs is insignificant.

6.6.3.3. Effects on secretion of TSH

A model of amphibian pituitary culture suggests that BPA might inhibit the secretion of TSH induced by CRH and/or TRH and that this effect would be additional to the negative feedback exerted by T3 and T4 (Kaneko *et al.*, 2008). This study is unacceptable for the following reasons: 1) what is the functional significance in mammals of the secretion of TSH induced by CRH, 2) the concentrations at which BPA acts are high (10^{-4} M) and inconsistent with the internal exposures obtained *in vivo.* It should be noted that Meeker relies on this study to explain the negative correlation that would exist between urinary BPA and TSH in men (Meeker *et al.*, 2010a). However, it is difficult to imagine that the urinary concentrations of total BPA in Meeker's study (0.4 to 36.4 ng/mL) could result from sufficiently high plasma concentrations (10^{-4} M or 23 µg/mL) to induce the pituitary effects described.

Table X: Animal studies examining the effects of bisphenol A on the thyroid: summary table

Reference	Species	Route	Dose Exposure period	Effects NOAEL/LOAEL
(Zoeller *et al.*, 2005)	Rats	Oral	1-10-50 mg/kg bw/d GD6-PND35	interaction between animals' age and treatment on total serum T4 concentrations ↗ in the concentration of T4 at PND15 No effect on serum concentration of TSH ↗ in expression of the neurogranin RC3 gene
(Xu *et al.*, 2007)	Rats	Oral	Estimated concentration: <100 µg/kg bw/d and 8-10 mg/kg bw/d GD11- PND21	In the mother, ↘ serum concentration of free T4 at PND0 and PND7 for the low dose In F1 males, ↗ free T4 at PND7 for the lowest dose and ↘ free T4 at PND21 for the highest dose No change in expression of the TRα gene and neurogranin RC3

6.6.4. Conclusion

The experimental models suggest that BPA might act as an antagonist of THs by increasing the recruitment of corepressors of the TH receptors. The scant availability of *in vivo* data on mammalian models does not contradict this hypothesis but can in no case confirm it. No solid data for humans can currently support the hypothesis of an effect of BPA on thyroid function. The studies on the interactions with the TH transport systems suggest that this mechanism of action is unlikely. Regarding possible interaction between BPA and thyroid function, the only finding that can be made at present is that there is a lack of data. Accordingly, there is nothing to rule out or assert that BPA could act as a thyroid disrupting factor at the doses to which human populations are exposed.

In humans, the data are considered to be limited and inconclusive because, on the one hand, the protocol seems poorly suited to evaluation of thyroid function and, on the other hand, the effects observed (negative correlation between BPA and TSH) depend on the model used for analysing the data (Meeker *et al.*, 2010a). The Working Group cannot draw a conclusion based on the human data.

In animals, the data on the metamorphosis of **amphibians** in response to T3 show a potential effect of BPA as antagonist of the thyroid hormones in these vertebrates. This effect is regarded as established in amphibians and could be explained by the mechanisms described on the basis of studies *in vitro*. Although the amphibian model is useful in terms of screening and investigation of the mechanisms of action, it is not, however, suitable for characterising the hazard in humans.

In **rodents,** the experimental data are based on relatively similar approaches (developmental exposure, oral routes taken spontaneously, etc.) and tend to show an effect of BPA on thyroid function in a period corresponding to final maturation of the hypothalamic-pituitary-thyroid axis. **The effects on the thyroid linked to neonatal exposure to BPA are suspected in rodents.**

6.7. Effects on the immune system

BPA is classified as skin sensitising, but clinically, prevalence remains low. The cases described are limited to industries and activities using epoxy resins. In a report from 2003, a group of experts from the Assessment of Chemicals department of the German Federal Institute for Risk Assessment (BfR) classified BPA in category B (solid-based indication for contact allergenic effects because of less frequency proven contact allergenic effect in humans taking into account existing positive animal data) (Schlede *et al.*, 2003).

In general, development of the immune response with predominance of Th2 cells predisposes individuals to pro-inflammatory, allergic reactions, atopic reactions and asthma. This immune response is the consequence of a programming that might persist.

According to the panel of experts that met at Chapel Hill in 2007, "BPA may modulate the production of cytokines associated with the proliferation of Th1 and Th2 lymphocytes" and "may alter the production of antibodies" (Richter *et al.*, 2007).

EFSA states that investigation of modulation of the immune system associated with BPA is a recent area for research. Several studies have reported changes in levels of cytokines, populations of T lymphocytes and other immunomodulating effects. However, all of these studies suffer from methodological bias which makes their interpretation difficult (EFSA, 2010). EFSA considers that in the current state of knowledge, these data cannot be used for establishing a TDI.

The FAO/WHO panel of experts also states that several studies *in vivo*, *ex vivo* or *in vitro* according to various protocols have recently investigated the effects of BPA on the immune system (FAO/WHO, 2010). The results observed in rodents exposed *in utero* or in adulthood suggest an immunomodulating effect of BPA (activity of cytokines, synthesis of nitric acid by macrophages, secretion of TNF-α, change in the immune response to predominance of Th2 cells, etc.). Among these studies, several have also investigated effects on weight or histological changes of the thymus or of the spleen. Thus, changes in the immune response have been observed in the absence of lesions in these organs. These results did not enable the panel of experts to confirm the effect of BPA on the immune system. Complementary studies according to standardised protocols would be useful but the panel does not judge this area of research to be urgent.

The literature is limited on this subject and all of the available publications were therefore examined for this work.

6.7.1. Human data

A NHANES 2003-2006 study looked for associations between BPA and **allergies** (n=2133) and **anti-cytomegalovirus antibodies** (Clayton *et al.*, 2011). A positive link with anti-cytomegalovirus antibodies was observed in adults but not in children. However, the extent and causality of this relation remain uncertain. No link between BPA and allergy has been reported. Moreover, the authors worked solely on a population with detectable levels of urinary BPA. This method of selection is controversial.

6.7.2. Animal data

6.7.2.1. Prenatal and perinatal exposure

In 2004, Yoshino *et al.* exposed BDA/1 J mice, by gavage every day for 18 days since mating, to 4 doses of BPA: 3, 30, 300 and 3000 µg/kg bw/d (Yoshino *et al.*, 2004). Only male mice pups were used (5 animals per group). The article does not state how the effect of gestation was taken into account, and does not give information on the cages and feeding. This study did not include a positive control.

The results of this study show an increase in the antigen-specific response connected with stimulation of Th1 and Th2 at a dose of 300 µg/kg for most of the responses and at 30 µg/kg for anti-HEL IgG2 (Hen Egg Lysozyme). Stimulation of the Th1s is greater than that of the Th2s. The number of CD3+, CD4+ and CD8+ cells is increased in the spleens of the mice exposed *in utero* to BPA.

In 2007, Ohshima *et al.* crossed BALB/c mice with DO11.10 OVA-TCR-Tg transgenic males [16], and exposed them, during pregnancy (without further details on the period of treatment) and lactation, to BPA via the feed, at two doses: 0.1 and 1 ppm of BPA (Ohshima *et al.*, 2007). Male mice pups which were heterozygotic for OVA-TCR-Tg were used (1 male per litter, selected at random, 6-8 animals per exposure group). They received, by stomach tube at age 14, 16 and 18 days, 20 mg or 0.2 mg of ovalbumin. One group was not given ovalbumin. They were then sacrificed at 21 days. Blood was collected and the spleen was removed. The proliferation of the antigen-specific T cells, the production of cytokines and anti-ovalbumin antibodies were measured. The experiments *in vitro* on the isolated cells were repeated 4-6 times, the data from the experiments were combined for each individual. The authors do not give any information on the cages and the feed. BPA was

[16] D011.10 transgenic mice whose T cells bear the TCR receptor that recognises the 323-339 peptide fragment of ovalbumin

analysed by ELISA in the mothers and the F1s on postnatal day 21 in the 1 ppm group only (1.41 ± 0.66 ng/mL (0.70-2.33 ng/mL) and 3.971 ± 2.40 ng/mL (0-6.74 ng/mL), respectively). In most of the experiments, only the 1 ppm group was used. There was no positive control.

The results of this study show that cellular proliferation in response to oral exposure to ovalbumin is not changed in mice pups exposed to BPA during pregnancy relative to unexposed mice pups. In contrast, an increase in the production of IL-4 and IL-13 and IFN-γ by the splenocytes in response to ovalbumin is observed in mice pups exposed to 1 ppm of BPA relative to mice pups not exposed to BPA. This suggests that exposure to BPA *in utero* promotes acquisition of a profile of response to Th2 predominance. After oral exposure to ovalbumin, the splenocytes of the mice pups previously exposed to BPA produce more IFN-γ and IL-13 than those of mice pups not exposed to BPA. The serum levels of IgG1 and IgG2a are higher in the mice pups previously exposed to BPA, following stimulation *in vivo* by OVA (oral antigen).

Finally, this study shows that *in utero* exposure to BPA can act on the mechanisms of induction of tolerance to oral antigens.

In Yan's study, BALB/c mice and C57BL/6 mice (strain resistant to *Leishmania major*) were treated 2 weeks before mating, and then 1 week during pregnancy with drinking water containing BPA at three doses: 1, 10 and 100 nM; the doses received were estimated at 0.07, 0.7 and 7.0 nmol, the dose of 100.0 nM corresponding to a supply of 3 µg/kg bw/d (Yan *et al.*, 2008). Only male mice pups exposed *in utero* were exposed at the age of 10 weeks to *Leishmania major* (between 3 and 4 animals per group). The article does not state how the effect of gestation was taken into account. The cages were made of TPX polymethylpentene, the bottles were of glass, the feed was standard (FR-2 from Funabashi Farm, Japan) and there was no positive control.

The results of this study show an increased, dose-dependent reaction to infection by *Leishmania major* and an increase in production of cytokines (INF-γ[17], IL-4[18]) in adulthood in male mice exposed *in utero,* following induction of Th1 and Th2, in the 10 and 100 nM groups. In contrast, the percentage of CD4+ and CD25+ was reduced. Changes to the immune system linked to exposure to BPA in adult mice were also observed in this study; they are described in the next section. According to the authors, exposure to BPA *in utero* could in theory lead to a risk of allergy and of asthma (Yan *et al.*, 2008).

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[17] Indicator of the Th1-mediated response

[18] Indicator of the Th2-mediated response

Finally, Holladay *et al.* injected BPA intraperitoneally at a dose of 1 mg/kg bw/d in pregnant mice from GD9.5 to the end of lactation. The production of cytokines was evaluated in the male offspring at 20 weeks (Holladay *et al.*, 2010). An increase in production of cytokines is reported by the authors. This study has, however, been rejected because the number of litters per group is too limited (n=2 for the control group and n=3 for the BPA group).

To summarise, each study has faults, but overall there is consistency among the results observed.

The study by Miao *et al.* examined the effect of BPA from day GD0 to day PND30 by the oral route (gavage) at doses of 4, 40 and 400 mg/kg bw/d on the expression of cytokine RNA in the spleen (Miao *et al.*, 2008). The authors demonstrate a dose-dependent decrease in expression of cytokines IL-2, IL-12, INF-γ and TNF-α in the young and in the parents. The number of animals per group was 10. Significant differences were observed in all the groups including the lowest concentration (4 mg/kg bw/d).

Midoro-Horiuti *et al.* examined, in BALB/c mice, the effect of exposure to BPA via drinking water (5 or 10 µg/mL, which corresponds to concentrations from 10^{-8} to 10^{-7} M in the tissues of neonates) throughout gestation and lactation on the response to the OVA antigen at "suboptimal" dose and bronchial inflammation/hyperreactivity (Midoro-Horiuti *et al.*, 2010). In mice pups exposed to BPA, the response to OVA was reflected in a significant increase in IgEs, eosinophilic inflammation of the respiratory tract and hyperreactivity of the respiratory tract. This study demonstrates the possible involvement of BPA in the development of allergies and of asthma. It is a study of excellent quality.

6.7.2.2. Exposure in adults

In Yan's study cited above, BALB/c mice and C57BL/6 mice (strain resistant to *Leishmania major)* were treated subcutaneously with BPA at doses of 5.7; 11.4; 22.8 and 45.6 mg/kg bw/d for 1 week and were then infected with *Leishmania major* (Yan *et al.*, 2008). The results of this study show an increased, dose-dependent reaction to infection by *Leishmania major* and an increase in production of cytokines (IL-4, IL-10 and IL-13) but not INF-γ. In contrast, the percentage of CD4+ and CD25+ decreased. The changes in the immune system linked to exposure to BPA in mice pups exposed *in utero* were greater than those observed in the adult mice.

The study by Goto *et al.* uses transgenic mice expressing the receptor specific to the protein OVA (Goto *et al.*, 2007). Exposure to BPA was by the oral route via water *ad libitum* for 2 weeks. The concentration of BPA was 10 mg/L, which corresponds to about 1.5-1.8 mg/kg. The T cells were cultured and the production of cytokines and proliferation were studied in response to the antigen. According to the authors, BPA reduces the secretion of IL-2 and IL-4 and of INF-γ and increases

the secretion of IgA and IgG2a. In the conditions of administration of the antigen, BPA increased the production of IFN-γ by the T cells and modified the antigen presenting cells that act on the T cells, suppressing the production of cytokines. One of the limitations of the study is the small number of subjects (n=3) in each experiment.

6.7.3. In vitro data

Yang *et al.* studied, by techniques of proteomics, the effect of BPA administered in drinking water at doses of 15 and 300 mg/L corresponding to average exposures of 8.9 mg/kg bw/d and 171 mg/kg bw/d in mice from the 7th day of gestation (GD7) and up to the end of lactation (PND21), a total exposure time of 34 to 36 days (Yang *et al.*, 2008). Analysis of thymus and spleen proteins from mice pups showed that 7 of them were over- or under-expressed in a dose-dependent manner (only 2 doses). In particular, this study offers the advantage of identifying biomarkers (apo-A1, DPPIII and VAT1) that could be used for quantifying the exposure of the immune system to BPA. For the time being, any relation that might exist between the nature of the proteins whose expression is modulated and the response of the immune system is still very uncertain.

The study by Guo *et al.* confirms BPA's potential for modulating the interaction between the dendritic cells (involved in the immune response acquired by presenting the antigen to the T cells) and the T cells, and for orienting the immune response towards a profile of the Th2 type (Guo *et al.*, 2010). The authors demonstrated, on a model of dendritic cells derived from monocytes (Mo-DCs), that the presence of BPA (0.1 µM) does not affect maturation of the dendritic cells but induces an increase in secretion of IL-10 and of IL-12p70. The capacity for activation of naive T lymphocytes by these Mo-DCs was examined and it was possible to demonstrate a dose-dependent production (0.001-0.1 µM) of the cytokines associated with a Th2 profile in the presence of BPA and TNF-α.

Table XI: Studies examining the effects of bisphenol A on the immune system: summary table

Reference	Species/ strain	Route	Dose Exposure period	Effects NOAEL/LOAEL
(Yan *et al.*, 2008)	BALB/c and C57BL/6 mice	Oral	1 - 10 and 100 nM, i.e. 0.07 - 0.7 and 7.0 nmol, the 100 nM dose being equivalent to an intake of 3 µg/kg bw/d 2 weeks before mating and then 1 week during gestation	Increased reaction to *Leishmania major* infection ↗ cytokine production (INF-γ, IL-4) in adulthood in male mice exposed *in utero* following induction of Th1 and Th2 in the 10 and 100 nM groups.
		Sub-cutaneous	5.7 - 11.4 - 22.8 and 45.6 mg/kg bw/d 1 week in adults	↗ in the dose-dependent reaction to *Leishmania major* infection ↗ cytokine production (IL-4, IL-10 and IL-13) but not INF-γ. ↘ in percentage of CD4+ and CD25+
(Yoshino *et al.*, 2004)	BDA/1 J mice	Oral	3 - 30 - 300 and 3000 µg/kg bw/d GD0 - GD18	↗ in the antigen-specific response to Th1 and Th2 stimulation at 300 µg/kg for most responses and 30 µg/kg for anti-HEL IgG2.
(Ohshima *et al.*, 2007)	Female BALB/c mice crossed with transgenic male DO11.10 OVA-TCR-Tg mice	Oral	0.1 and 1 ppm Prenatal exposure and exposure during lactation period	No cell proliferation in response to ovalbumin exposure, ↗ IL-4 and IL-13 and IFN-γ production by splenocytes in response to an ovalbumin dose of 1 ppm ↗ IFN-γ and IL-13 production by splenocytes after oral ovalbumin exposure ↗ *in vitro* T cell response, T cell clonal change (does not induce CD4+CD25+ accumulation) ↗ IgG1 and IgG2a, following *in vivo* OVA stimulation (oral antigen)
(Miao *et al.*, 2008)	F344 rats	Oral	4 - 40 - 400 mg/kg bw/d GD0 - PND30	↘ dose-dependent expression of cytokines IL-2, IL-12, INF-γ and TNFα in offspring and in the parents **LOAEL = 4mg/kg bw/d**
(Midoro-Horiuti *et al.*, 2010)	BALB/c mice	Oral	5 - 10 µg/mL 1 week before gestation - weaning	significant ↗ in IgEs Respiratory tract eosinophilic inflammation and hyperreactivity of respiratory tract after sensitisation
(Goto *et al.*, 2007)	Transgenic mice expressing the OVA protein specific receptor	Oral	1.5 -1.8 mg/kg approximately 2 weeks (mice between 8	↘ in IL-2 and - 4 and INF-γ secretion and ↗ in IGA and IgG2a secretion After administration of the antigen, ↗ in IFN-γ production by T cells and changes in antigen-presenting cells

			and 15 weeks old)	acting on T cells to suppress cytokine production.

6.7.4. Conclusion

In conclusion, a positive association with anti-cytomegalovirus antibodies has been found in human beings although the impact and causality of this relationship remain uncertain. No conclusion can therefore be drawn from the only study available examining the effects of BPA on the immune system.

T lymphocyte induction accompanied by overproduction of cytokines in animals is deemed to be a clear effect. The observed displacement of the immune response is mostly in favour of Th2s.

It is not known at this stage whether these findings can be extrapolated to humans.

6.8. Effects on the intestine

6.8.1. Human data

No studies were found in human beings.

6.8.2. Animal data

Braniste *et al.* studied the impact of BPA on intestinal barrier function in ovariectomised female rats and in young rats after perinatal exposure (Braniste *et al.*, 2010). These studies were conducted on two groups of animals.

The first group of animals was ovariectomised female Wistar rats (5 to 15 females) fed on a standard chow, which were administered BPA in corn oil by gavage for 15 days (0, 0.05 and 5 mg/kg bw/d; positive control: 0.6 mg/kg oestradiol benzoate in corn oil). During the last 5 days of administration the group of rats which were receiving 5 mg/kg bw/d BPA and the group being given oestradiol benzoate were treated with a daily subcutaneous injection of an oestrogen antagonist (2 mg/kg of an ER antagonist (ICI 182.780)). The animals were ovariectomised in order to remove basal oestrogen levels. A dose-response study of intestinal permeability was also performed using 5 doses of BPA (0.5 µg to 5 mg/kg bw/d) administered orally for 15 days to ovariectomised female rats (Braniste *et al.*, 2010).

Another group of animals (8-13 female rats) were treated daily with 5 mg of BPA by gavage from GD15 until weaning on PND21. After weaning, the offspring were fed with standard chow.

Measurements of intestinal permeability (Ussing chamber) and gastro-intestinal inflammation (measurement of myeloperoxidase, MPO) and the pro-inflammatory cytokine MIF (macrophage migration inhibitory factor) were performed on the offspring at adulthood (PND70). Sensitivity to visceral pain was also measured by electromyography in female ovariectomised rats subjected to colorectal distension (colorectal distension causes abdominal contractions: the motor visceral response was used to estimate visceral pain).

This study demonstrated a dose-dependent fall in intestinal permeability in the ovariectomised females (based on 5 doses of BPA tested). The dose which produced 50% maximum inhibition was 10 µg/kg bw/d. This effect, which is similar to that produced by oestrogens, is attributed to tightening of the tight intestinal epithelial junctions through an effect on ERβ. Again in adults, BPA at a dose of 5 mg/kg reduced the severity of gastro-intestinal inflammation caused by intra-colonic administration of trinitrobenzene sulphonic acid (TNBS) but increased sensitivity to visceral pain. Oestradiol has previously been shown to affect intestinal epithelial permeability (Braniste *et al.*, 2009), but also has an effect on the inflammatory response and on visceral pain. The oestrogen-mimicking activity of BPA is examined using these 3 parameters, intestinal permeability being the most sensitive (LOEL = 5 µg/kg bw/d).

Reduced intestinal permeability and increased inflammatory response were seen in adulthood only in the female offspring of mothers which had been treated with a dose of 5 mg/kg (perinatal exposure.

It should be noted that a study on malignant colonic cells showed BPA to have an antagonistic effect on cell apoptosis (Bolli *et al.*, 2010)**.** The same inhibition of apoptosis is also seen with oestradiol.

Table XII: Studies examining the effects of bisphenol A on the intestine: summary table

Reference	Species/ strain	Route	Dose Exposure period	Effects NOAEL/LOAEL
(Braniste *et al.*, 2010)	Adult ovariectomised Wistar rat	Oral	0 - 0.05 - 5 mg/kg bw/d administered for 15 days in adulthood	0.05 and 5 mg/kg: ↘ dose-dependent intestinal permeability (analogous effect to that produced by oestrogens, attributed to tightening of the tight intestinal epithelial junctions). 5 mg/kg: ↘ severity of gastro-intestinal inflammation and ↗ visceral pain sensitivity.
	Wistar rat	Oral	5 mg/kg bw/d administered from GD15 to PND21 Examination of the offspring at adulthood (PND70)	Measurements of intestinal permeability, gastro-intestinal inflammation and the pro- inflammatory cytokine MIF on the offspring at adulthood (PND70) 5 mg/kg: ↘ intestinal permeability and ↗ inflammatory response (only seen in ♀).

6.8.3. Conclusion

No studies were found in human beings.

In adult animals which had received acute treatment with BPA, the pro-nociceptive and anti-inflammatory effects of BPA were of the same type as those produced by oestradiol and appear to occur through an effect on oestrogen receptors. On the other hand, a pro-inflammatory effect was found in the female offspring of treated mothers and would appear to be due to defective immune system maturation. **BPA is suspected to have an effect on intestinal permeability and inflammation in animals (one single study.**

6.9. Effects on the prostate

In its 2008 report, the NTP-CERHR stated that insufficient data were available to consider BPA to be carcinogenic on the prostate in rodents or to be hazardous to human beings with respect to the prostate gland, although it recommended that additional studies be conducted in order to clarify the effects of BPA on prostate and urinary tract development (NTP-CERHR, 2008).

In 2010 the FAO/WHO expert panel considered that conventional carcinogenesis studies on BPA in rodents using doses in the region of 75 to 150 mg/kg bw/d showed no effect or a very weak one. The panel questioned, however, whether the carcinogenic potential of BPA had been correctly investigated in these studies as the animals were not exposed during the prenatal period. Some studies do show that perinatal exposure to BPA (at oral doses ranging from 10 to 250 µg/kg bw/d) could cause squamous prostatic epithelial metaplasia in the F1 offspring. BPA exposure during specific susceptibility windows could have an effect on prostate development and make it more susceptible to development of neoplastic or pre-neoplastic lesions following exposure to potent tumour initiators or promoters (FAO/WHO, 2010). These studies, however, have weaknesses in their protocol limiting how they can be interpreted. It also reported that a carcinogenic study in rodents was ongoing at the NTP. Oral exposure is understood to begin from the period of foetal life. A study to monitor internal levels of free and conjugated BPA is expected.

INSERM considers that the foetal or neonatal period appears to be a critical phase during which exposure to BPA could damage prostate development and predispose to the development of malignant lesions (with doses of 10 to 20 µg/kg bw/d) (INSERM, 2011). Defective organ development caused by BPA appears to predispose to hormone-dependent carcinomas (breast or prostate.

Three aspects will be considered in succession: hypertrophy, pre-neoplastic lesions and prostate cancer. The aim of this analysis above all is to assess the relevance of the harmful effects seen and whether these effects seen in animals can be transposed to human beings.

The question of xenobiotics with hormonal properties (grouped under the generic term endocrine disruptors) is relevant to the prostate gland because of its hormone-dependency during its development and growth under physiological and pathological conditions.

Vom Saal's group was one of the first to examine the effects of bisphenol A on the prostate in this context. This team began working in 1975 on the involvement of steroid hormones during foetal life and reproduction in the mouse (gestation, behaviour and sexual development). In 1993, Vom Saal co-authored a document with Soto and Colborn (Colborn *et al.*, 1993) considered to be the founding stone of the "endocrine disruptor" concept developed in the work carried out at the W.

Alton Jones Foundation and the World Wildlife Fund, and the document expressing the opinion of the panel on BPA which met at Chapel Hill in 2007 (vom Saal *et al.*, 2007).

6.9.1. Prostatic hypertrophy

- **Rodent data**

The first reference associating (prenatal) exposure of mice (CF1) to natural oestrogens (oestradiol) or xenobiotics with oestrogenic properties (DES) and prostate development (vom Saal *et al.*, 1997) was published in 1997. Exposure of gestating females to DES at doses of between 0.02 and 2.0 µg/kg bw/d produced an increase in prostate weight in adulthood (8 months). This increase in volume and weight is due to hyperplasia.

In the same year, the same team showed that prenatal oral exposure to BPA at doses of 2 and 20 µg/kg bw/d produced a 30 and 35% increase in prostate weight respectively in 6 month old mice (CF1 (Nagel *et al.*, 1997). The same authors used a similar prenatal BPA administration protocol (10 µg/kg bw/d) in 2005 and found epithelial proliferation of the primitive prostate gland ducts in mice (CF1) at birth demonstrated by an increase in the number of these ducts in the dorso-lateral part of the gland and in the total volume of the gland, which was still poorly differentiated (Timms *et al.*, 2005). The authors considered that these doses should be taken into account in risk assessment calculations, particularly as they appeared to be consistent with exposure levels in human populations.

These findings, however, have not been reproduced by other authors. Ashby *et al.* exposed gestating mice (CF1) to doses of 2 and 20 µg/kg bw/d of BPA. The offspring were humanely killed and examined at 6 months of age (Ashby *et al.*, 1999). Cagen *et al.* exposed gestating mice (CF1) to doses of 0.2, 2, 20 and 200 µg/kg bw/d of BPA. The offspring were humanely killed and examined at 3 months of age (Cagen *et al.*, 1999a). Cagen *et al.* exposed female Wistar rats to BPA in drinking water (at doses of 0.01, 0.1, 1 and 10 ppm) from the 10th week postnatally until mating with unexposed males and monitored exposure during gestation and lactation (Cagen *et al.*, 1999b). The male rat offspring which were not subsequently exposed were humanely destroyed at 90 days old. No changes in prostate weight (absolute or relative, i.e. as a proportion of body weight) or morphological abnormalities were found in any of these studies.

In a three-generation study in rats (SD) exposed to doses of 0.001, 0.02, 0.3, 5, 50, or 500 mg/kg bw/d of BPA, Tyl *et al.* found no changes in (absolute or relative) prostate weight in adulthood (Tyl *et al.*, 2002). The same authors exposed two generations of mice (CD1) to doses of 0.003, 0.03, 0.3, 5, 50 and 600 mg/kg bw/d of BPA and found no changes in (absolute or relative) prostate weight (ventral lobe, dorso-lateral lobe) in adulthood (Tyl *et al.*, 2008).

In a study which exposed adult rats (Wistar) to oral doses of 0.2, 2 and 20 µg/kg bw/d of BPA for 5 weeks Chitra found in particular a significant increase in relative ventral prostate weight which was dose-dependent (see also Section **Erreur ! Source du renvoi introuvable.**) (Chitra *et al.*, 2003).

Herath *et al.* also studied the effects of BPA in rats (Wistar) over the same exposure period in young adults (D52) for 5 weeks although using a different administration route (subcutaneous) and a dose of 3000 µg/kg bw/d (Herath *et al.*, 2004). These authors also found an increase in relative ventral prostate weight.

Recently, in an experimental model using administration of testosterone followed by BPA to adult (SD) rats which had previously been surgically castrated, Wu *et al.* found an increase in prostate weight at doses of 0.01 mg/kg bw/d of BPA for 4 weeks (Wu *et al.*, 2011). This increase was no longer seen at doses of 0.03 mg/kg bw/d and 0.09 mg/kg bw/d.

Beyond the debate raised by the methodologies, lack of reproducibility of results and possible inconsistent dose-response relationships, questions may arise as to the pathological implications of increased prostate size (hyperplasia) and as to whether the effects found can be transposed from animals to human beings.

- **Extrapolation to human beings: interpretation issues**

In human beings, prostatic hypertrophy is a benign tumour consisting of fibromuscular and glandular hyperplasia (adenomyofibroma). It is produced by an increase in the number (hyperplasia) and volume (hypertrophy) of cells in the gland. The disease develops with age particularly around the male andropause, reflecting the hormone dependency of growth of the gland. Its main clinical consequences are pollakiuria (an abnormally frequent need to pass urine). Apart from age, no risk factors have yet been clearly established. In the current state of knowledge, benign prostatic hypertrophy is not an established risk factor for developing prostate cancer in men (Miller and Torkko, 2001).

In rodents the prostate differentiates mostly after birth, whereas in humans this stage occurs during intrauterine life.

The rodent studies cited above describe an increase in prostate weight in the region of 30-35%. This is a modest increase compared to those seen in humans with prostatic hypertrophy (100, 200, up to 1000%).

Firstly, rodents are born in a relatively immature state compared to humans and their development continues after birth. In order to cause similar developmental effects, exposure would need to take place during the neonatal period in the rodent and prenatal period in the foetus. The newborn rodent would therefore be more vulnerable to this exposure than the foetus which is still protected

by the placental barrier. For example, **prostate differentiation** occurs around birth in rodents (mostly after birth), whereas this takes place during the intrauterine life in humans. In addition, unlike humans and dogs, rodents do not spontaneously develop prostatic hypertrophy; in contrast, rather, their prostate tends to atrophy with age (Maini *et al.*, 1997), reflecting a difference in composition of the hormonal environment and regulatory processes between species in terms of signals which stimulate prostate development.

6.9.2. Pre-neoplastic lesions

In an initial study, Ho *et al.* administered oestradiol (0.1 µg/kg bw) or BPA (10 µg/kg) subcutaneously in the postnatal period on days 1, 3 and 5 to 20 to 30 rats per group (Sprague-Dawley, SD) (Ho *et al.*, 2006). In half of the animals treated with oestradiol or BPA, oestradiol, which is known to induce prostatic tumour formation in a third of SD rats, was administered (at a final concentration of 75 pg/mL in blood) together with testosterone (final concentration 3 ng/mL in blood) at the age of 90 days (treatment at 90 days), to prevent the hypothalamic-testicular feedback due to prostatic involution produced by oestradiol alone. The animals were humanely killed at the age of 28 weeks (~ 6 months) and the prostate gland was examined.

Postnatal administration of BPA without treatment with the combined oestradiol-testosterone mixture at 90 days produced no changes in the prostate, either in prostate weight or development of prostatic intraepithelial neoplasia (PIN) lesions.

When the animals were treated at 90 days with the combined oestradiol-testosterone mixture the authors found PIN (all grades) in the dorsal prostate in 4 of the 10 control animals (40%; not exposed to either oestradiol or BPA), in all 10 of the 10 animals exposed to BPA (100%) [Fisher's exact $p = 0.0108$] and in 5 of the 10 animals exposed to oestradiol (50%) [Fisher's exact $p > 0.99$]. In terms of distinction by PIN grade of lesion the difference was borderline significant for high grade lesions [Fisher's exact $p = 0.0698$] and clearly insignificant for low grade lesions [Fisher's exact $p > 0.99$].

It should be noted that the neonatal dose of oestradiol administered (0.1 µg/kg bw) was chosen to be similar (0.15 µg/kg bw) to the dose reported to cause an increase in relative dorsal prostate weight in the rat at 90 days (Putz *et al.*, 2001).

In a second study, Prins *et al.* used a similar protocol to the one used in the initial study by Ho *et al.,* who belonged to the same team as Prins, with 15 to 25 animals per group, although with a few differences (Prins *et al.*, 2011; Ho *et al.*, 2006):

- The line of SD rats was different (from a different supplier, as the supplier which provided the animals for the first study had ceased trading)
- The mean final circulating oestradiol concentration at 90 days was 164 pg/mL, compared to 75 in the first study, and the mean testosterone concentration was 1.72 ng/mL compared to 3 ng/mL in the first study. BPA was administered at the same dose (10 µg/kg) subcutaneously and also orally. This study was carried out to respond to NTP criticisms

about the validity of the subcutaneous route as a relevant administration route in humans, who are mostly exposed orally.

- The PIN lesions were graded into three scores (I to III) instead of two (low grade versus high grade).

Postnatal administration of BPA, without treatment at 90 days with the combined oestradiol-testosterone mixture, did not produce any changes in the prostate gland (28 weeks), either in prostate weight or development of PIN lesions.

When the animals were treated with the combined oestradiol-testosterone mixture at 90 days the authors found that PIN (all grades) developed in the prostate in 18, 64 and 33% of controls in the ventral, lateral and dorsal prostate respectively. Subcutaneous BPA exposure produced PIN lesions (with no distinctions in grade) in 40% (ventral), 100% (lateral) and 47% (dorsal) of animals. When BPA was administered orally the corresponding figures were 40, 90 and 66% respectively. Although the publication does not state the number of animals involved the authors reported a significant difference (Fisher's exact $p > 0.01$ and < 0.02) in the prevalence of PIN lesions between the group exposed to BPA and the control group for the lateral prostate, regardless of administration route.

At the end of their studies the authors concluded that exposure to BPA at doses consistent with human exposure, particularly in young children, is liable to cause precursor prostatic lesions to adenocarcinoma. They also justified the difference in the zone of the prostate concerned (dorsal in the first study, lateral in the second) by the difference in SD rat lines, although provided no evidence to support this.

- **Extrapolation to humans: interpretation issues**

The effect of BPA following postnatal exposure was studied by Prins' team. This exposure period was chosen on the basis that unlike humans, the critical stages of prostate differentiation and development in rodents take place after birth.

The effects of BPA are not produced following simple postnatal exposure in the rodent. In order for the effects to be seen in adulthood it appears that the rodents need to be conditioned by prior oestrogen exposure. This artefact (neonatal oestrogenisation of rodents predisposing to the development of prostatic dysplasia) is meaningful in a mechanistic experimental context but has no meaning in a model intended to be used to assess risks in humans.

The authors described PIN lesions as being precursors of an adenocarcinoma, which has not so far been demonstrated either in animals or in humans. Investigation of histological lesions which predict development of a prostate tumour is a clear field of interest and the initial lesions were described in 1926. The term PIN was introduced in relation to humans in 1987 to define pre-neoplastic prostatic lesions. Three grades were initially described (I, II and III) and then subsequently renamed as "low grade" lesions (type I) and "high grade" lesions (types II and III). In this PIN the glandular architecture is maintained but the basal cell layer is ruptured with varying degrees of severity (forming the basis of the distinction between low grade and high grade). No

stromal invasion however is seen. Cytokeratin immunolabelling in the basal layer is used to differentiate PIN (labelling present) from adenocarcinoma (labelling absent). PIN however does display morphological nuclear and nucleolar abnormalities, neovascularisation and genetic instability with variations in DNA content (aneuploidy). We note that unlike adenocarcinoma, PIN does not contribute to a rise in Prostate-Specific Antigen (PSA).

Although PIN has molecular or structural abnormalities and features in common with adenocarcinoma, its long-term outcome and the nature of its relationship with adenocarcinoma, particularly as a precursor, is still uncertain and indeed controversial. Autopsies have shown PIN lesions to be prevalent, that their prevalence increases with age and that they occur early compared to the development of adenocarcinoma. There are, however, as many individuals with PIN who do not develop adenocarcinoma as there are individuals with adenocarcinoma which has not been preceded by PIN. Following recent studies, such as the study conducted in five European countries (European Randomised Study of Screening for Prostate Cancer) on biopsy series from 56,553 individuals with prostatic dysplasia, the clinical approach to PIN lesions (independently of other risk factors) is currently conservative (Laurila *et al.*, 2010). Patients with PIN are monitored, but no more than those with other objectively recognised risk factors (family history of prostate cancer, sub-Saharan African origin, etc.) with no other intervention (i.e. regular biopsies).

In terms of extrapolating rodent PIN lesions to humans, whilst both species share clear morphological and molecular similarities, additional studies are needed to confirm that dysplasia in rodents is similar to human PIN, particularly as its relationship with adenocarcinoma is uncertain.

The classification into three grades, I, II and III, and then into low and high grades, used by Prins' team is not strictly identical to the classification used in humans, which itself is relatively difficult for anatomical pathologists to reach agreement upon.

6.9.3. Prostate cancer

Rodents (unlike dogs or monkeys) are a poorly suited model to study prostate cancer because of the rarity of spontaneous prostate cancer in this species. There are however several ways of artificially creating prostatic epithelial cell dysplastic lesions or adenocarcinomas in some lines of mice or rats, for example: administering a known carcinogen (methylnitrosourea) followed by high doses of testosterone in the Lobund Wistar rat; association of testosterone with oestrogens to produce dysplastic lesions in the Noble rat; transgenic mice (C3(1)/TAG) which generate dysplastic lesions and then prostatic carcinomas which appear to develop from PIN lesions. Under these experimental conditions, the fundamental tumour molecular processes, or in other words the mechanisms, liable to be common to other species including humans, can be studied and identified using rodents. As these are not spontaneous models however, it is reasonable to continually question their relevance in a risk assessment.

6.9.4. Adenocarcinoma: Extrapolation to humans – interpretation issues

Prostate cancer is the commonest tumour in men in most Western countries and has increased greatly in incidence in the last few decades. This is explained, if not entirely at least to a large extent, by the increasing use of blood PSA measurement as an early diagnostic tool. The increasing incidence of prostate cancer is often used as an argument to support the potentially harmful effects of endocrine disruptors (vom Saal *et al.*, 2007) although no study has provided a realistic estimate of the contribution from the chemical environment and even less from endocrine disruptors. The influence of individual early diagnosis of the disease makes it extremely difficult to demonstrate the possible contribution of other factors (dietary behaviour, xenobiotics etc.) to the prolonged increase in incidence.

Based on autopsy findings, the presence of prostate tumours (adenocarcinomas) increases with age (its prevalence is of the same order of magnitude as age expressed in decades). The disease, however, is only clinically manifested in a small number of people from the 6th decade of life onwards, and increases continuously during subsequent decades. One of the criticisms of the widespread use of PSA is that it detects indolent tumours and triggers effective treatments in terms of reducing mortality but leads to considerable morbidity (impotence, urinary incontinence) which affects quality of life. Age, a family history of a first degree relative with prostate cancer and population ethnic origins are the only clearly identified risk factors for the disease (clinical expression). These reflect the existence of genetic susceptibility factors interacting with endogenous hormonal factors.

Table XIII: Studies examining the effects of bisphenol A on the prostate: summary table

Reference	Species/ strain	Route	Dose Exposure period	Effects NOAEL/LOAEL
(Nagel *et al.*, 1997)	CF1 mice	Oral	2 and 20 µg/kg bw/d Prenatal exposure from GD11 - 17	Prostate weight in mice at adulthood: ↗ prostate weight from: - 30% at a dose of 2 µg/kg bw/d - 35% at a dose of 20 µg/kg bw/d
(Timms *et al.*, 2005)	CF1 mice	Oral	10 µg/ kg bw/d Prenatal exposure from GD14 – 18	Primitive prostate gland duct epithelial proliferation found at birth
(Ho *et al.*, 2006)	Sprague-Dawley rats	Sub-cutaneous	10 µg/kg bw/d PND1, PND3 and PND5	Irreversible damage to the expression of around 30 genes through mechanisms involving DNA methylation BPA irreversibly continues expression of 4D phosphodiesterases by blocking methylation of the promoter for this gene Development of prostatic intra-epithelial neoplasia in those exposed to BPA
(Tyl *et al.*, 2008)	Mice	Oral	0.003 - 0.03 - 0.3 - 5 - 50 and 600 mg/kg bw/d Exposure for 10 weeks before mating until adulthood	No significant difference in prostate weight
(Tyl *et al.*, 2002)	Rats	Oral	0.001 - 0.02 - 0.3 - 5 - 50 and 500 mg/kg bw/d Exposure for 10 weeks before mating until adulthood	Conclusion**:** In the wide range of doses studied and specifically at doses compatible with human exposure, BPA had no effect on reproduction. Effects were seen at the highest doses (not relevant to human exposure).
(Prins *et al.*, 2011)	Sprague-Dawley rats	Sub-cutaneous and oral	10 µg/kg bw/d for both routes	- Administration of BPA alone postnatally did not cause changes in the prostate - Administration of BPA + oestradiol + testosterone at PND90 caused: - an increase in ventral, dorsal and lateral prostate weight - PIN lesions for both routes, with different percentages

6.9.5. Conclusion

In humans, no epidemiological studies designed to identify any association between BPA exposure and prostate disease have been published to date.

In animals, the studies by Tyl *et al*, conducted on several generations in the mouse and rat do not show any effect on prostate weight (Tyl *et al.*, 2002; Tyl *et al.*, 2008). Conversely, other studies such as Chitra *et al.* and Herath *et al.* show an increase in relative ventral prostate weight in the rat only after exposure in adulthood (Chitra *et al.*, 2003; Herath *et al.*, 2004) or an increase in prostate weight after prenatal exposure in the mouse (Nagel *et al.*, 1997). The effects on prostate weight are controversial. When histological examination was performed this increase in weight was associated with hyperplasia. Neonatal exposure to BPA (rodents) under induction conditions showed development of PIN lesions but not prostatic adenocarcinoma. The effects seen under these experimental conditions are suspected. In light of all of these results the experts considered that **the effects on the animal prostate are controversial.**

6.10. Effects on the breasts

According to the NTP-CERHR report, rodent studies have shown BPA to have an effect following exposure by subcutaneous perfusion at doses ranging from 0.0025 mg/kg bw/d to 1 mg/kg bw/d during gestation and support an increased susceptibility to developing mammary tumours (Durando et al, 2007; Murray et al, 2007) (NTP-CERHR, 2008). Although these lesions were described as pre-neoplastic no evidence was provided of their progression to invasive carcinoma. As a result, it cannot be concluded that BPA carries a risk of breast cancer. Similarly, no effects have been reported in rodents after exposure during adulthood.

The EU RAR report cites three studies referring to investigation for pre-neoplastic lesions. The first study by Durando *et al.* (2007) used Wistar rats exposed *in utero* between GD8 and GD23 to subcutaneous administration of 25 µg/kg bw/d (EC, 2010b). The study showed that BPA disrupts the histological structure of the mammary gland and increases its susceptibility to a carcinogen (N-nitroso-N-methylurea) administered 50 days after the end of BPA treatment. The second study by Murray *et al.* (2007) involved foetal exposure to BPA (0.025 and 1 mg kg bw/d) which induced development of pre-neoplastic and neoplastic mammary lesions. The last study cited by Colerangle and Roy (1997) assessed mammary gland growth in Noble rats treated subcutaneously with 0.1 and 54 mg/kg bw of BPA. The authors found a significant increase in conversion of immature into mature structures, a reduced average number of terminal ductules and terminal buds and an increase in the average number of lobules. The conclusions of the EU RAR report (2008) however criticised these 3 studies because of their methodological limitations.

In 2010, the FAO/WHO expert panel deemed that the conventional carcinogenesis studies on BPA in rodents using doses in the region of 75 to 150 mg/kg bw/d did not demonstrate any effects or showed only very weak effects. The panel questioned, however, whether the carcinogenic potential of BPA had been correctly investigated in these studies because the animals were not exposed during the prenatal period. Some studies have shown that perinatal exposure to BPA (at oral doses of between 10 and 250 µg/kg bw/d) can cause mammary duct epithelial proliferation in the F1 generation. BPA exposure during specific susceptibility windows may have an effect on the development of the mammary gland and make it more susceptible to the development of neoplastic or pre-neoplastic lesions after exposure to potent tumour initiators or promoters. These studies, however, have protocol weaknesses which limit their interpretation. The expert panel also reported that a carcinogenesis study in rodents was ongoing at the NTP in which oral exposure would begin from the foetal life period. This study intends to monitor internal free and conjugated BPA levels (FAO/WHO, 2010).

According to the INSERM report, many studies consistently show that foetal or perinatal exposure to BPA changes the structure of the mammary gland in adulthood in rodents (INSERM, 2011). The report cites the work by Vanderberg *et al.* (2008) which reports an increase in density, branching

and number of ducts and alveoli and terminal duct hyperplasia in mice. It also cites the work by Markey *et al.* (2001; *in utero*, mouse), Munoz-de-Toro *et al.* (2005, *in utero* and neonatal, mouse), Murray *et al.* (2007, foetal, rat), Moral *et al.* (2008, *in utero*, rat) which report accelerated maturation of the adipose cushion, delayed lumen formation and increased density of terminal duct structures.

INSERM describes studies in which exposure to BPA either was or was not shown to be related to a risk of developing breast tumours (INSERM, 2011). The study by Murray *et al.* (2007) in animals suggested an increased risk of mammary tumours in rats. INSERM also describes studies showing increased susceptibility of mammary cells exposed *in utero* to low doses of BPA to malignant change, notably the studies by Munoz-de-Toro *et al.* (2005), Durando *et al.* (2007), Wadia *et al.* (2007) and Jenkins *et al.* (2009). INSERM (2011) describes one published epidemiological study by Yang *et al.* (2009), which found no clear difference in blood BPA concentrations between cases (women diagnosed with breast cancer) and controls. INSERM ultimately concluded that although the data in rodents appeared to be convincing there was at present no study demonstrating BPA to have any developmental effects in humans.

6.10.1. Human data

Only one epidemiological study has examined the relationship between BPA exposure and the risk of breast cancer (Yang *et al.*, 2009). In this cross-sectional study, 152 Korean women (70 cases with breast cancer diagnosed between 1994 and 1997 and 82 controls recruited in the same hospital, matched for age) completed a questionnaire and had a blood BPA measurement (the biomarker of exposure used was the conjugated form). BPA levels did not differ between the cases and controls (p=0.42).

The major methodological limitations of this study, such as lack of statistical power (low numbers), undetectable BPA in half of the subjects with no details about any possible differences between cases and controls, a non-standardised questionnaire inappropriate for the question being asked (measurement of BPA, a substance which does not persist, after the diagnosis of breast cancer), prevent any conclusions being drawn about the association between BPA and breast cancer.

6.10.2. Animal data

In most of the reproductive toxicology studies performed with females exposed *in utero* to BPA, it can be seen that either the authors did not analyse the mammary glands or the histological examinations were not suitable for showing carcinogenic effects. Also, studies analysing reproductive toxicity did not follow the animals for long enough after prenatal exposure to detect carcinogenic effects in adulthood.

Apart from the studies of Moral *et al.*, Jenkins *et al.* and Betancourt *et al.* which were performed using the oral route, all other studies were performed using subcutaneous pumps with doses ranging from 0.000025 to 10 mg/kg bw/day (Betancourt *et al.*, 2010b; Betancourt *et al.*, 2010a; Jenkins *et al.*, 2009; Moral *et al.*, 2008). There are 12 studies in rodents (7 in mice and 5 in rats) covering this type of effect. Nine studies covered prenatal exposure, 3 were postnatal studies, including a few studies from 2 laboratories.

6.10.3. Prenatal and perinatal exposure

Several *in utero* studies showed neoplastic and non-neoplastic effects on the mammary glands. This is particularly true of *in utero* exposure as is shown here by several studies in rodents.

Munoz de Toro *et al.* looked at the extent to which perinatal exposure to BPA between GD9 and PND4 in CD-1 mice was able to induce a change in mammary gland development in F1s (Munoz del Toro *et al.*, 2005). Using an Alzet osmotic pump, the authors exposed the mothers to concentrations of 25 and 250 µg of BPA/kg bw/day (BPA diluted in 50% DMSO). The mammary glands were sampled then analysed at 30 days. The analyses show that perinatal exposure to BPA significantly increases the response to oestrogens by increasing the number and size of breast buds and increases the expression of progesterone receptors. The authors suggest that this increase could be a precursor to an increase in the secondary branching of mammary ducts observed at 4 months and a significant increase in the percentage of mammary alveoli at the age of 6 months. Consequently, these correlations suggest that exposure to BPA in particular increases susceptibility to the development of cancer in the mammary glands.

In 2007, Murray *et al.* examined the extent to which prenatal exposure to BPA is sufficient to induce the development of preneoplastic lesions in the mammary gland in the absence of any additional carcinogenic treatment. They exposed pregnant Wistar-Furth rats to doses of 2.5, 25, 250 and 1000 µg/kg bw/day between GD9 and PND1 using an Alzet osmotic pump (Murray *et al.*, 2007). The anatomical and histological observations were made in females at puberty (PND50) and on PND95. The results suggest that prenatal exposure to BPA significantly increases the number of hyperplastic ducts in the mammary gland for all doses at puberty (PND50), whereas on PND95 the incidence of hyperplastic ducts is not significantly greater than that of the controls at the lower dose of 2.5 µg/kg bw/day. On PND50, the authors observed 1 case in 4 of CIS at the two BPA doses of 250 and 1000 µg/kg bw/day (1/4 at 250 µg/kg bw/day and 1/4 for 1000 µg/kg bw/day) and report that this incidence “increased” on PND95 with an incidence of 2 cases in 6 (nonsignificant difference). The structures observed were of the “cribriform” type regarded as intraductal carcinomas (CIS) according to the criteria described earlier by two authors (Russo and Russo, 1996; Singh *et al.*, 2000). In both rodents and humans, intraductal hyperplasia is regarded as a precursor of CIS (Singh *et al.*, 2000). Several methodological limitations must be noted: the

small number of animals used and the lack of information about the incidence of CIS in the controls. No investigation was made after PND95. It should be noted that the strain of rat used is very sensitive to chemical carcinogens.

Vandenberg *et al.* published two articles in 2007 and 2008 about the mammary gland and BPA (Vandenberg *et al.*, 2007b; Vandenberg *et al.*, 2008). In the first study of 2007, a single concentration of 250 ng of BPA/kg bw/day administered by continuous infusion from a subcutaneous pump was used between days GD8 and GD18 in CD-1 mice aged 8 weeks. In the foetus on GD18, BPA altered the general organisation of the mammary gland for all the morphological criteria studied. To validate these observations, these same authors performed a second study in 2008, in which mice were exposed to BPA (0, 0.25, 2.5 and 25 µg BPA/kg bw/day) from GD8 to PND16. The authors studied the characteristics of the mammary glands of the neonates at 3, 9 and between 12 and 15 months after birth. The results confirm the previous observations according to which exposure to BPA alters the morphology of the mammary glands in adult mice. The effects observed are hyperplasia, with the appearance of “polished” ducts with all doses of BPA at 9 months, but not at 12-15 months. The question of the reversibility of these effects was raised by the authors in their conclusion.

Doherty *et al.* propose a new mechanism to explain the effects of endocrine disrupters on mammary development (Doherty *et al.*, 2010). These authors exposed pregnant CD-1 mice to 10 µg of BPA/kg bw/day between GD6 and GD21. *In utero* exposure to BPA produced an increase in the expression of the histone “enhancer of zeste homologue 2” (EZH2), which suggests that BPA could be involved in the development of mammary lesions in adults. Expression of EZH2, a risk biomarker which is said to be involved in the development of breast cancer, was assessed 6 weeks after birth. It should be noted that this protein is involved in stem cell renewal and is said to be activated by the mutant BRCA1 gene (Kunju *et al.*, 2011). Durando *et al.* performed a prenatal study in Wistar rats exposed to 25 µg of BPA/kg bw/day by subcutaneous infusion between GD8 and GD23 (Durando *et al.*, 2007). The low doses of BPA produced ductal hyperplasia, desmoplasia and the presence of mastocytes in the stroma, which suggests an increased risk of developing cancer, even 50 days after the end of exposure to BPA. This is in perfect agreement with other published results quoted above.

Moral *et al.* exposed female Sprague-Dawley rats to 25 or 250 µg of BPA/kg bw/day by gavage from GD10 to GD21 (Moral *et al.*, 2008). The female neonates were sacrificed and the mammary glands sampled on PND21, 35, 50 and 100 to observe the morphological changes, and to assess gene expression and the cell proliferation index. An increase in the number of undifferentiated epithelial structures and changes in gene expression were observed. The results suggest that the effects on the mammary gland depend on both the dose and the period of exposure and that BPA

affects the susceptibility of the mammary gland to undergo changes towards undifferentiated structures.

Betancourt *et al.* studied the susceptibility to developing a mammary gland tumour after *in utero* exposure to BPA followed by postnatal exposure to a carcinogenic agent (dimethylbenzanthracene = DMBA) (Betancourt *et al.*, 2010a). The authors mention that the changes in the mammary glands are not accompanied by clinical signs such as premature vaginal opening or a variation in oestrogens and progesterone, which would, according to the authors, indicate that the changes are epigenetic alterations acting directly on the target organ. The highest dose of BPA (250 µg/kg bw/day) increased the incidence of breast tumours and changed the window of the mammary gland's susceptibility to DMBA, which moved from PND50 to PND100. In addition, the authors made a proteomic analysis in female rats treated by gavage with doses of 25 or 250 µg/kg bw/day of BPA during gestation (GD10-GD21) (Betancourt *et al.*, 2010b). The change in the expression of certain proteins that regulate cell proliferation which was observed on PND21 (weaning) and PND50 (puberty) could increase the susceptibility of the mammary gland to tumour development.

A study by Wadia *et al.* sought to show whether perinatal exposure to BPA between GD8 and PND2 could induce mammary gland sensitivity to oestradiol in adulthood in CD-1 and C57B16 mice (Wadia *et al.*, 2007). The authors wanted to compare the sensitivity of each of these 2 strains of mice. Pregnant mice were exposed to 250 ng of BPA/kg bw/day from GD8 to PND2. On PND25 the neonates were ovarectomised, implanted with an oestradiol pump, exposed to concentrations of 0.5 or 1.0 µg of E2/kg bw/day for 10 days and sacrificed on PND35. The 2 strains showed a similar response. However, perinatal exposure to BPA altered several parameters in the 2 strains, and these effects were slightly more pronounced in the CD-1 strain. The results suggest that perinatal exposure to BPA alters the response to oestradiol at puberty in both strains, even though the effects are more pronounced in the CD-1 mice.

6.10.4. Postnatal and/or pubertal exposure

Only a few recent studies look at postnatal exposure. The study of Jenkins *et al.* shows that female rats whose mothers were treated with BPA at a dose of 25 and 250 µg/kg bw/day during lactation (PND2 to PND20) develop more breast tumours and show a reduction in the latency period until the onset of those cancers after treatment by gavage on PND50 with a carcinogen, dimethylbenzanthracene (DMBA) (Jenkins *et al.*, 2009). The type of tumours is not specified in the article. The highest dose of BPA produced an increase in cell proliferation and a reduction in apoptosis in the mammary glands on PND50 (but not on PND21) combined with overexpression of the proteins regulating cell proliferation. The time to the appearance of the tumours was significantly shorter in the group exposed to the highest dose. The authors conclude that BPA plays an amplifying role in the onset of mammary tumours after exposure to DMBA in the female

offspring. This suggests that the effect of BPA could act via epigenetic mechanisms. This mode of action was recently demonstrated by (Yang *et al.* 2009).

Jones *et al.* assessed the impact of the loss of the function of the BRCA1 gene on cell proliferation induced by BPA (Jones *et al.*, 2010). This study is open to criticism and interpretation of the results is difficult. It is a mechanistic study which cannot be used directly for the assessment of risk. Another study, that of Colerangle and Roy, assessed the growth of the mammary gland in female Noble rats treated subcutaneously with BPA at doses of 0.1 and 54 mg/kg bw/day (Colerangle and Roy, 1997). They noted a significant increase in the conversion of immature structures into mature structures, a reduction in the number of ductal buds and an increase in the mean number of lobules. The authors also noted an alteration in the cell cycle which was said to be an important factor in the development of genetic instability such as nucleotide errors in the synthesis of DNA.

6.10.5. Exposure in adulthood

As reported in the NTP study report, a study of carcinogenesis via the oral route in female rats (BPA: 74 and 135 mg/kg bw/day) and mice (BPA: 650 to 1300 mg/kg bw/day) did not show any neoplastic or non-neoplastic effect on the mammary gland (see Section **Erreur ! Source du renvoi introuvable.**) (NTP, 1982).

6.10.6. Transposition to humans: interpretation issues

Precancerous lesions of the breast are atypical epithelial proliferations which develop within the lactiferous duct tree and are of two types: ductal and lobular. These two types differ not in their location but in the type of their constituent cells. Histological diagnosis of precancerous lesions is difficult and inter-pathologist reproducibility is mediocre, as is shown by a number of studies. The classification of precancerous lesions in humans is based on the terms for ductal (DIN) or lobular (LIN) intraepithelial neoplasia. Ductal carcinoma *in situ* (DCIS) is a preinvasive cancerous lesion. In the United States, DCIS accounts for almost 20% of the cancers picked up in screening (1 case of DCIS per 1300 screening mammographies) (Ernster *et al.*, 2002).

When left in place, a preneoplastic or precancerous lesion can turn into a preinvasive carcinoma or an *in situ* carcinoma which can itself turn into an invasive carcinoma. The theory about the existence of a continuum between the normal mammary gland and invasive breast cancer, even if it may appear too simplistic, is based on direct and indirect arguments (Antoine *et al.*, 2010). Recent epidemiological studies have shown that women with a history of benign breast lesions had an increased risk of breast cancer.

Similarly, the natural development of low-grade ductal carcinomas *in situ* (DCIS) was determined by long-term follow-up studies in women who had undergone a diagnostic biopsy without any other

treatment. After 10 years' follow-up, 14 to 60% of these women had a diagnosis of invasive cancer in the same breast (Page *et al.*, 1995). The natural development of high-grade DCIS or of clinically palpable DCIS, on the other hand, is not well characterised since, in most cases, the tumour is removed in its entirety by surgery which is also the case with atypical ductal hyperplastic (ADH) lesions.

The substantial increase in the number of biopsies performed on the basis of infra-clinical images and recent data provided by molecular study of the lesions have shed new light on the risk of hyperplastic lesions becoming cancerous.

When hyperplastic lesions turn into cancers *in situ* then into invasive cancers, imbalances are observed at chromosomal level with loss of heterozygosity in 40% of cases of hyperplasia and more than 70% of high-grade carcinomas *in situ* (Aubele *et al.*, 2000). Molecular markers of tumoral transformation in the breast such as the oestrogen receptor, expressed by normal epithelial breast cells, are expressed by more than 70% of ductal carcinomas *in situ* (DCIS) and the proto-oncogene HER2/neu is overexpressed in half the cases of DCIS but not in atypical hyperplasias (Allred *et al.*, 1992).

Rodents, i.e. rats and mice, have been widely used to study mammary carcinogenesis, in models of either spontaneous or induced tumours. The main advantage of the rat model is that the carcinoma most resembles human breast cancer; breast cancer in mice is often of viral and hormone-dependent origin (Cardiff *et al.*, 2000; Gould 1995). In CD-1 mice, spontaneous non-neoplastic and neoplastic lesions are not very common (less than 5%: (Gad 2007)).

The different strains of rats used have shown differing sensitivities to neoplasms induced chemically or by radiation, Sprague-Dawley or Wistar being more susceptible than the Fisher rat. In Sprague-Dawley rats, the incidence of spontaneous tumours is close to 50% in chronic studies (example, historical data (NTP, 2010)). Certain strains, such as Wistar-Furth, show increased susceptibility to mammary carcinogenesis via chemical carcinogens (Gould 1995).

The factors of mammary gland susceptibility include, in addition to genetic factors, the degree of differentiation of the breast tissue at the time of exposure, physiological and hormonal status, and diet. Susceptibility is increased in prepubertal females during the mammary development period: the ducts end in terminal buds (TEBs) which will progressively differentiate into alveolar buds and alveolar lobules. The greatest number of tumours was induced in female SD rats at between 40 and 46 days, the period of most active differentiation of the TEBs regarded as the target of chemical carcinogens (Russo and Russo, 1996). Breast carcinomas were induced in rats by chemical agents or ionising radiation. The most commonly used chemical carcinogens include the polycyclic aromatic hydrocarbon dimethylbenzanthracene (DMBA) or the alkylating agents N-ethyl-N-nitrosourea (ENU) and N-methyl-N-nitrosourea (NMU). After a single dose of DMBA or NMU,

adenocarcinoma develops in 20 days in young rats. These cancers sometimes invade the surrounding tissue but rarely metastasise to distant sites (Gould 1995). A short-term carcinogenesis protocol involving the injection of NMU at 21 days made it possible to describe the chronology of the induction of preneoplastic and neoplastic breast lesions (Thompson *et al.*, 1998), and to compare these lesions with those observed in humans (Singh *et al.*, 2000). Thus, certain similarities were described regarding the lesions observed in humans and those induced in rodents.

- The similarities include:
  - Development in a multistage process
  - Most of the cancers induced by DMBA or NMU are hormone-dependent
  - A similar morphological pattern: hyperplasia, intraductal hyperplasia regarded as preneoplastic, adenomas/adenocarcinomas. Ductal carcinomas *in situ* (DCIS) are regarded as a morphological progression towards breast carcinoma from intraductal proliferative lesions.

Table XIV: Comparison of the histopathological preneoplastic and neoplastic lesions of the mammary gland induced in prepubertal rats with those described in humans (Singh *et al.*, 2000)

	Humans	Rats
Benign lesions	Fibroadenomas that can exhibit carcinomas *in situ* (CIS) and atypical ductal hyperplasias (ADH)	No ADH or CIS in the fibroadenomas
Hyperplasia	Possible atypical hyperplasias	No atypical hyperplasias
Carcinomas *in situ*	Lobular carcinomas *in situ* (LCIS) and ductal carcinomas *in situ* (DCIS) may be observed. Several histological subtypes. Possible microcalcifications.	Less histological diversity. DCIS are observed, particularly cribriform and papillary ones. No microcalcifications of the DCIS.
Invasive carcinomas	Elastosis and possible calcifications. Several types. Lymph nodes involved.	No elastosis or microcalcifications Absence of lobular carcinomas, etc. Much less histological diversity No lymph-node metastasis

- The differences include:
  - The morphology of most breast tumours in mice does not resemble that of human breast cancers (Cardiff *et al.*, 2000);

- In rats, similarity of the histological lesions has been described, with less diversity than in humans: for example, no atypical hyperplasia, no microcalcifications, no lobular form of CIS or invasive lobular carcinoma have been described in the model of short-term carcinogenesis induced by the carcinogen NMU (Singh *et al.*, 2000).
- The regional lymph nodes are not invaded in rats as compared to humans.

Table XV: Studies examining the effects of bisphenol A on breast cancer: summary table

Reference	Species/ strain	Route	Dose Exposure period	Effects NOAEL/LOAEL
(Betancourt *et al.*, 2010a)	Sprague-Dawley Rats	Oral	0 – 25 - 250 µg BPA/kg F0: Exposure in mothers to BPA from GD10 to GD21 followed by single dose of DMBA on PND50 or PND100 . F1: exposure not checked	**Effects observed:** - *In utero* exposure to 250 µg/kg of BPA **associated with a single exposure to DMBA** at 100 days postnatally (but not on PND50), produced an increase in the incidence of mammary tumours and a shorter latent time compared to the control group. - **Without DMBA**, an increase in cell proliferation and overexpression of some proteins involved in cell proliferation was observed. **Critical effect:** - Amplification of breast tumour development (number/rat and time to occurrence) in a DMBA model - Expression of proteins involved in cell proliferation - Changes in proteins which influence cell proliferation on PND100 (250 µg/kg) - ERα, PR-A, Bcl-2, steroid receptor coactivators, (SRCs), EGFR, IGF-1R, and phospho-c-Raf. **Doses** are not known in the offspring and are possibly less than: **NOAEL 25 µg/kg bw/d** **LOAEL 250 µg/kg bw/d**
(Betancourt *et al.*, 2010b)	Rats	Oral	0 – 25 - 250µg BPA/kg GD10 - GD21. Female descendants were humanely killed on PND21 and PND 50.	↗ phospho-AKT, ↗ c-Raf, phospho-ERKs-1 and 2, ↘ TGF-β in breast tissues at 50 days postnatally Important signalling pathways are disrupted by BPA. Prenatal exposure to BPA results in deterioration of expression of proteins in breast glands postnatally.
(Doherty *et al.*, 2010)	CD1 Mice	Intra-peritoneal	0 - 10 µg/kg-5 m/kg GD9 to GD26	↗ histone H3 trimethylation ↗ of EZH2 (2X) expression in mammary tissues compared to the control
(Durando *et al.*, 2007)	Female Wistar rats	Sub-cutaneous pump	25 µg/kg GD8 to GD23	↗ proliferation/apoptosis ratio ↗ ductal hyperplasia ↗ sign of desmoplasia ↗ neoplastic lesion

				No NOAEL/LOAEL
(Jenkins *et al.*, 2009)	Female Sprague Dawley rat pups	Oral	0 - 25 and 250 µg/kg bw/d, 5 d/week Administered to lactating mothers from PND 2 to PND 202 (equivalent to 15 administrations/mother). The female baby rats were treated with a single dose of DMBA on PND50.	↗ tumour incidence at high dose **NOAEL 25 µg/kg bw/d** **LOAEL 250 µg/kg bw/d**
(Jones *et al.*, 2010)	BRCA1 deleted mice	Sub-cutaneous pump	250 ng BPA/kg bw/d	Difficult to interpret (transgenic mice) BRCA1 deletion followed by BPA exposure stimulates mammary glands leading to hyperplasia compared to the control
(Moral *et al.*, 2008)	Sprague-Dawley rats	Gavage	25 et 250 µg/kg pc GD10 à GD21	Increase in the number of undifferentiated epithelial structures (TEB and TD). No effects on proliferation; BPA exposure changes the gene expression signature: - altered gene expression, maximal at 100 d with the high dose (genes up-modulated at the two doses, including a cluster related to immune response; underexpressed genes including differentiation-linked genes at high dose). - At low dose, the expression profile is changed most at 50 d.
(Munoz del Toro *et al.*, 2005)	CD1 mice	Sub-cutaneous pump	25 - 250 ng/kg bw dissolved in DMSO GD9 to PND4	↗ response to oestrogens ↗ expression of progesterone receptors.
(Murray *et al.*, 2007)	Wistar-Furth rats	Sub-cutaneous pump	2.5 – 25 – 250 – 1000 µg/kg bw GD9 to PND1	↗ number of intraductal hyperplasia in mammary gland at all doses (more pronounced at PND50 compared to PND95). CIS present in mammary glands of animals exposed to the highest doses at puberty and at 3 months.
(Vandenberg *et al.*, 2007b)	Female CD1 mice	Sub-cutaneous pump	250 ng BPA/kg bw/d GD8 to GD18	↗ ductal area ↘ cell size Delay in lumen formation Adverse changes in mammary gland phenotype
(Vandenberg *et al.*, 2008)	Female CD1 mice	Sub-cutaneous pump	0 - 0.25 - 2.5 - 25 µg/kg bw/d GD8 to PND16	Deterioration in development of mammary glands ↗ proliferation indexes compared to control group
(Wadia *et al.*, 2007)	Outbred CD-1 mice	Sub-cutaneous	0 - 250 ng/kg bw/d	Perinatal exposure to BPA does not adversely affect the uterine response to E2

	Inbred C57B16 mice	pump	Mixed exposure BPA and E2 GD8 to PND2	administered from PND25 to PND35 but does adversely affect the uterine response of the mammary gland.

6.10.7. Conclusion

Most of the literature on the association between BPA and breast cancer is less than 10 years old and is changing, with many recent references.

No conclusions can be drawn about the relationship between BPA exposure and breast cancer **in humans,** because of methodological limitations in the only epidemiological study found.

In animals, although the studies are somewhat heterogeneous, some of the effects observe converge and the working group has therefore concluded that:

- structural maturation of the mammary gland at adulthood accelerated as a result of prenatal or perinatal exposure to BPA is a **recognised effect in animals**;
- development of intraductal hyperplastic lesions from perinatal or prenatal exposure to BPA is a **recognised effect in animals**;
- development of neoplastic lesions (CIS: intraductal carcinoma *in situ*) after perinatal exposure to BPA is **a suspected effect in animals**;
- increased susceptibility of mammary glands to develop subsequent mammary tumours (following co-exposure with a carcinogen) from prenatal or perinatal exposure to BPA is a **suspected effect in animals**.

➢ **The effect of BPA on the mammary gland is taken into consideration for the risk assessment.**

7. Information from ecotoxicological studies

The aim of this section is to provide information about knowledge on the effects of bisphenol A on a group of wild and laboratory species. Examination of the ecotoxicological effects of bisphenol A supplements the data obtained from toxicology studies, either by confirming the results of these studies conducted in mammals or by providing an understanding of other types of effects or other mechanisms of action of bisphenol A.

Different environmental risk assessments relating to bisphenol A were conducted by the European Union in 2003 and 2008 and formed the basis of this work. Recent studies have been examined and this section presents either new data or important data from previous assessments which justify particular attention or being re-examined in light of current knowledge (EC, 2003; EC, 2010a). Whilst studies are available in different aquatic and terrestrial taxonomic groups including reptiles, crustaceans, insects, earthworms, birds and also plants, not all taxonomic groups are examined. In contrast, the emphasis has been placed on fish and molluscs which are the best documented groups and/or the groups in which the effects are controversial. Studies on amphibians and the antagonistic effect of BPA on the thyroid system are presented in Section **Erreur ! Source du renvoi introuvable.** of this report.

7.1. Exposure data

Calculations of concentrations in water and sediments, based on the physico-chemical properties of BPA and available production data, show a predicted water concentration (PECwater [19]) of 0.032 µg/L and predicted sediment concentration (PECsediment) of 0.52 µg/kg, dry weight (EC, 2010a). Monitoring data, however, often describe higher concentrations. Findings on surface water accepted by the European Union document show a median value of 0.01 µg/L, a mean value of 0.13 µg/L and a 95th percentile of 0.35 µg/L (EC, 2010a). Analysis of the literature shows that internationally bisphenol A is found regularly in surface water at concentrations of between tens of ng/L and tens of µg/L (Belfroid *et al.*, 2002; INERIS, 2010a; Kolpin *et al.*, 2002).

It should be noted that bisphenol A is not monitored in the aquatic environment surveillance systems in France, and because of this limited data are available on the environmental concentrations of this compound.

[19] PEC = Predicted Environmental Concentration, PNEC = Predicted No Effect Concentration

Bisphenol A is found in the sedimentary compartment at concentrations of between tens and hundreds of µg/kg dry weight (INERIS, 2010a). The European Union indicates median, mean and 95th percentile values of 16, 60 and 256 µg/kg respectively (EC, 2010a).

7.2. Bioconcentration

The capacity for BPA to be bioconcentrated in the tissues of exposed organisms is relatively low and the bioconcentration factor calculated from its chemical properties (log Kow) is 155 in fish (EC, 2003). The values found are 67 in carp (*Cyprinus carpio*), 94 in salmon (*Salmo salar* (Honkanen *et al.*, 2004)) compared to 147 in the frog (*Rana temporaria*) and 144 in the mollusc *Pisidium amnicum* (Heinonen *et al.*, 2002; Koponen *et al.*, 2007).

7.3. Toxicokinetic data

The only data available are on fish. BPA penetration in the salmon (*Salmo salar*) and brown trout (*Salmo trutta*) is in the region of 1 L/kg/h (Bjerregaard *et al.*, 2007; Honkanen *et al.*, 2004). BPA has a short half-life in the trout of 3.7 hours after intra-peritoneal injection (Lindholst *et al.*, 2001) and less than 1% of an orally administered dose remains in the liver or muscles 5 or 24 hours after exposure ends (Bjerregaard *et al.*, 2007). BPA is mostly metabolised by being converted into BPA glucuronide and to a lesser extent into BPA sulphate. This conversion only appears to actually occur (and then become gradually more effective) after 7 days post-fertilisation (Bjerregaard *et al.*, 2008). It appears therefore that BPA cannot or can only poorly be metabolised in the very early stages of development.

7.4. Acute toxicity

Acute toxicity values range from 1 to 20 mg/L BPA in vertebrates and invertebrates. More specifically, several acute toxicity studies in fish are available and provide information about the ecotoxic nature of bisphenol A. Lethal concentrations 50 measured between 48 and 96 hours are between 4.6 and 17.9 mg/L (Table XVI).

Table XVI: Some acute toxicity data on bisphenol A in fish

Species	Duration (h)	LC_{50} (mg/L)	Comment	Reference
Fathead minnow (*Pimephales promelas*)	48	4.6	Continuous ASTM	(Alexander *et al.*, 1998)
	72	4.6		
	96	4.6		
Atlantic silverside (*Medinia medinia*)	48	11	Continuous ASTM	
	72	9.4		
	96	9.4		
Medaka (*Oryzias latipes*)	96	13	OECD 203	(Yokota *et al.*, 2000)
Swordfish (*Xiphophorus helleri*)	96	17.9	Non-standard method	(Kwak *et al.*, 2001)

7.5. Effects in fish

Oestrogenic activity is confirmed by measuring induction of vitellogenin (vtg), a protein whose synthesis depends on transcription activity via binding to the oestradiol receptor. Measurement of oestrogenic potential produces an LOEC of 95 µg/L (Lindholst *et al.*, 2000) and 19 mg/kg bw/day (Bjerregaard *et al.*, 2007) in rainbow trout (*Oncorhynchus mykiss*). These values, however, vary greatly between species. Vtg induction has been found from 59 µg/L in the cod (*Gadus morhua*) (Larsen *et al.*, 2006), whereas no effect on this parameter was seen on bream (*Abramis brama*) up to 22.8 mg/L (Rankouhi *et al.*, 2004). This difference may be explained by species-specific differences in oestrogen receptor affinity or by differences in metabolism.

BPA may also have specific mechanisms of action. Whilst some studies document that the compound has weak affinity for oestrogen receptors compared to oestradiol, recent studies using transcriptome approaches have identified and compared the modified hepatic gene network in the zebrafish *(Danio rerio*) and carp (*Cyprinus carpio)* after exposure to BPA and other oestrogens (Kausch *et al.*, 2008; Moens *et al.*, 2006; Moens *et al.*, 2007). These studies showed that BPA regulates different specific genes to those regulated by oestrogens. This is a particularly striking result in the zebrafish in which 47 genes are regulated by BPA compared to 211 by oestrogens, the common genes encoding vitellogenin. The transcriptome profiles therefore suggest that BPA acts through a different mechanism to that of the other xeno-oestrogens. A detailed analysis of the

transcriptome profiles produced by BPA and oestrogens is ongoing in order to better characterise the mechanism of action of BPA in fish.

Major changes in the reproductive organs and in adult and developing organisms have been reported in various studies. Sex ratio changes have been found (LOEC) at 355 µg/L in the medaka (*Oryzias latipes;* (Yokota *et al.*, 2000)) and from 1000 µg/kg in the zebrafish (Drastichova *et al.*, 2005). Effective gametogenesis changes producing inter-sex individuals (with reproductive organs containing both male and female cells) have been found from 32 and 22 µg/L in the medaka (O*ryzias latipes*) and the carp (*Cyprinus carpio*) respectively (Bowmer and Borst, 1999; Metcalfe *et al.*, 2001). Lahnsteiner *et al.* report falls in sperm quality and mobility (of less than 10% and more than 50% respectively, with a dose-response relationship) at concentrations of 1.75 and 2.4 µg/L at the beginning of the reproductive season in the brown trout, *Salmo trutta* (this effect was no longer significant 2 to 4 weeks later) (Lahnsteiner *et al.*, 2005). Haubruge *et al.* also reported a fall in spermatozoa count in guppy (*Poecilia reticulata*) gonads with an LOEC of 387 µg/L (Haubruge *et al.*, 2000). Finally, spermatogenesis is affected in the fathead minnow, *Pimephales promelas* (spermatozoa count -40%) from 16 µg/L (Sohoni *et al.*, 2001) although a second experiment by Caunter *et al.* (2006) reported in the European assessment report (EC, 2010a) failed to confirm this result at equally low concentrations. Inhibition of male and female gonadal growth is seen after exposure to 640 µg/L for 164 days and egg production is inhibited at 1280 µg/L in the fathead minnow (Sohoni *et al.*, 2001). Defective oviduct development has been reported with an NOEC of 16 µg/L in the carp (*Cyprinus carpio*) (Bowmer and Gimeno, 2001). In the fario trout (*Salmo trutta fario*), a BPA concentration of 1.75 µg/L delayed egg laying by two weeks but had no effect on egg quality (egg mass or fertility (Lahnsteiner *et al.*, 2005)) although (Thomas and Sweatman, 2008) found delayed oocyte maturation probably due to BPA binding to the oocyte maturation progestogen membrane receptor in the Atlantic croaker (*Micropogonius undulatus*). Similarly, exposure to BPA produces a reduced egg fertilisation rate in the zebrafish with an LOEC of 5.7 µg/L at 14 days (Bayer, 1999). This fall in fertility may be due to the ability of BPA to reduce (from 23 µg/L) spermatozoa mobility as was reported in the Atlantic croaker (*Micropogonius undulatus*) after stimulation with a progestogen (Thomas and Doughty, 2004).

In 2008, Ramakrishnan and Wayne reported a fall in the embryonic development time between 1 and 3 days post-fertilisation (Ramakrishnan and Wayne, 2008). They also found a fall in eclosion mass and early sexual maturation. Similar growth results were also seen medaka larvae exposed to BPA at eclosion (Zha and Wang, 2006). This result suggests that BPA may have an effect on genes which control the developmental clock via a thyroid receptor-mediated pathway. Anti-thyroidal activity (compensated by adding T3) has also been reported by Gilbert *et al.* during

development (50 hpf[20]) of otoliths[21] in the zebrafish (*Danio rerio*) (Gilbert *et al.*, 2011). A recent *in vitro* study on john dory hepatocytes transfected with a growth hormone receptor (GHR) expression vector showed that bisphenol A influences the expression of genes which encode GHR at concentrations of between 10^{-6} and 10^{-9} M (0.23-230 µg/L). Kwak *et al.* found a reduction in tail length, an important sexual characteristic in the swordfish (*Xiphophorus helleri*) at BPA concentrations of 2 and 20 µg/L (Kwak *et al.*, 2001).

Exposure of parent fish to BPA may also have effects on the next generations. This is suggested by the results of the multi-generation study conducted by Sohoni *et al.* in which it reduced eclosion of eggs from the fathead minnow (*Pimephales promelas*) exposed to 160 µg/L in the F2 generation (Sohoni *et al.*, 2001).

Exposure to 50 µg/L induced micronuclei in turbot (*Scophthalmus maximus*) erythrocytes (Bolognesi *et al.*, 2006). Bisphenol A can produce teratogenic effects in the fish. This was shown by Honkanen *et al.* who reported oedema in the vitelline sac and haemorrhage in salmon (*Salmo salar*) alevins exposed to 1 g/L for 6 days (Honkanen *et al.*, 2004). More recently, McCormick *et al.* also reported haemorrhage and oedema in zebrafish exposed to a similar concentration (McCormick *et al.*, 2010).

7.6. Effects in molluscs

The effects of BPA on molluscs are the most widely discussed by the scientific community as according to the studies these seem to be the most susceptible organisms to BPA and those in which BPA produces major effects.

The effects of BPA have been assessed in the snail, *Marisa cornuarietis*. From a dose of 0.25 µg/L, the compound produces a “superfemale” syndrome in which the females exhibit hypertrophy of the accessory sex glands, malformations of the oviduct and oocyte overproduction leading to oviduct rupture and increased mortality. These effects have been reported repeatedly by the same team (Oehlmann *et al.*, 2000; Oehlmann *et al.*, 2006; Schulte-Oehlmann *et al.*, 2001). The authors calculated the EC10 to be 13.9 (Schulte-Oehlmann *et al.*, 2001) or 14.8 ng/L (Oehlmann *et al.*, 2006) based on oocyte production.

Forbes *et al.* did not find related effects at exposures of 0, 0.1, 1, 16, 160 or 640 µg/L of BPA (Forbes *et al.*, 2007), although no positive control was used in this study. Specifically, there is no indication that the test used can identify any adverse effect on reproductive organs such as those

[20] Hpf = hour post-fertilisation; Dpf = day post-fertilisation

[21] Otolith: a mineral structure (calcium carbonate) contained in the balance organ

described by Oelhmann *et al.* (Oehlmann *et al.*, 2000; Oehlmann *et al.*, 2006). No histological examination was performed on the reproductive organs.

The "superfemale" phenotype (excluding the oviduct malformations) at the same exposure doses (1-100 µg/L) has been seen in the marine species *Nucella lapillus* (Oehlmann *et al.*, 2000). The males also displayed reduced penis and prostrate size and a reduction in sperm concentration. A dose-dependent increase in oocyte production and number of young were found in *Potamopyrgus antipodarum*, following exposure to 1 µg/kg of BPA in sediment (Duft *et al.*, 2003). Similar results have been reported following exposure through water. Induction of egg production and increased young was found from 1 µg/L and was dose-dependent up to 25 µg/L followed by a negative effect at 100 µg/L of BPA (Jobling *et al.*, 2004).

The studies carried out by Oehlmann's group appear to show that the effects are similar to those produced by oestrogenic compounds in vertebrates (Oehlmann *et al.*, 2000; Oehlmann *et al.*, 2006). This is supported by the antagonistic effect of anti-oestrogens (in vertebrates). The importance of sex steroids such as oestradiol in mollusc physiology is still however debated. It should be noted particularly that the equivalent oestradiol receptor present in molluscs does not bind to oestradiol.

Zhou *et al.* carried out work on embryonic development in the abalone (*Haliotis diversicolor supertexta*) at BPA exposure doses of 0.05 to 10 µg/L (Zhou *et al.*, 2010; Zhou *et al.*, 2011). Significant increases in malformations were seen from 0.2 µg/L at different stages in development rising to more than 40% of embryos at 2 µg/L. Only 10% of trochophore larvae convert into veliger larvae at 10 µg/L whereas a significant effect (40% failure) is seen from 0.2 µg/L. Metamorphosis is also affected. Concentrations of 0.2 and 2 µg/L reduce the process by factors of 3.2 and 3.6 and almost no larvae undergo metamorphosis at 10 µg/L. The effects of BPA are associated with major changes in the proteome affecting different proteins involved in oxidative equilibrium, homeostasis, hormone metabolism and immunity.

7.7. Conclusion

The ecotoxicological studies show that:

- Bisphenol A is liable to cause major physiological changes through its oestrogenic activity. The presence and involvement of oestradiol in a large number of zoological groups from the cnidarians upwards suggests that a very large number of species may be disrupted by this mechanism although the specificity, distribution and sensitivity of hormone receptors may vary greatly depending on the group in question.

  Developmental reports suggest that oestrogenic activity is not the only pathway by which BPA interacts with the physiology of organisms. In particular the thyroid axis appears to be sensitive to the compound and depending on the zoological groups and/or doses it may have agonist or antagonist effects.
- Apart from the mechanisms of action, ecotoxicological studies suggest that very considerable effects, particularly on reproduction and development, may affect wild species and that these effects are seen at concentrations which are liable to be encountered in the environment.

8. Discussion - conclusion

8.1. Methodological limitations

The working group considered that the data currently available in humans on the link between BPA exposure and effects on health are very limited and insufficient to be used as the main approach to a quantitative risk assessment. Results from studies of an acceptable quality and those without major methodological limitations were however used to classify the effects of BPA based on human data.

There is a relatively rich literature on the toxicity of BPA in terms of animal data. These studies were conducted either in a context of regulatory assessment in which case they followed official guidance notes and were conducted in accordance with Good Laboratory Practice, or for research purposes using appropriate models for the hypotheses tested. Most of the second type of study examined the hypothesis of endocrine disruption at low doses whereas the former type more specifically examined critical effects such as malformations, growth, survival of litters, ano-genital distance, age at puberty, oestral cycle etc. The working group considered all of these studies, regardless of origin, although analysed them in terms of the criteria defined in the methodology section in this report. The experts sought to identify a set of evidence for a given effect allowing the type of effects of BPA to be classified.

For the same target organ the results of these studies often disagree. The main evaluations prior to this work had already highlighted this finding which was confirmed by the ANSES working group experts. This can be explained by various reasons, particularly:

- **Influence of administration route:** the main route of BPA exposure in the general population is generally considered to be oral. Experimental studies using oral administration are considered to be the most relevant by the expert bodies to assess the risks in humans, particularly associated with dietary exposure (EFSA, 2010; FAO/WHO, 2010; Hengstler *et al.*, 2011). However, most recent studies demonstrating the effects of low dose BPA have exposed the animals either subcutaneously, by injection or by osmotic pumps implanted beneath the skin. Although this route of exposure is not a natural means of BPA exposure in humans the working group did not exclude the results of these studies. In order to use them in a risk assessment, the levels of systemic exposure achieved in these studies in animals must however be linked to systemic oral or cutaneous exposure in humans. Certain authors stress the lack of a first pass hepatic effect to explain the differences in effects seen between oral and subcutaneous administration (Hengstler *et al.*, 2011). **A detailed analysis of BPA toxicokinetic findings by species and by exposure routes is currently ongoing in**

order to establish the equivalence between these different studies and the levels of exposure in humans.

- **Lack of a control** in a **BPA contamination study** or the **presence of phyto-oestrogens** in the diet, which may introduce bias. Several "old" studies did not take account of exogenous BPA intake from drinking bottles or cages, for example, or from phyto-oestrogens in the diet. Lack of controls for these factors may influence the interpretation of the study results. Cages, litter, diet and water can lead to uncontrolled "parallel" exposure and therefore change oestrogenic activity. In addition, the studies used BPA exposure at increasingly small doses and therefore increasingly closer to baseline noise. Most of the studies, however, included a control group in which the animals were housed and fed under the same conditions as the treated group and which therefore only differed in terms of the doses of BPA administered. **The experts did not *a priori* exclude this type of study but considered them on a case-by-case basis depending on the differences found between the treated animals and the control animals.**

- **The lack of a positive control or an inappropriate positive control** can also make interpreting a study difficult. The working group also considered, however, that in some studies the choice of positive control supported a hypothesis about the mechanism of action of BPA (for example if a positive control is DES or 17-β oestradiol, an oestrogenic mechanism of action is implicitly assumed to be involved). We now know that the effects of BPA may be mediated by other pathways than oestrogens and therefore that failure of a "positive" control is not strictly speaking a factor indicating that the study is of poor quality. **The experts did not *a priori* exclude this type of study but considered them on a case-by-case basis.**

- **Inadequate numbers of animals** can be a methodological limitation of a study. A study on a small number of animals which does not show BPA to have an effect may not have sufficient power to identify the effect being investigated. Conversely, lack of effect in a sample of sufficient size can be considered as evidence with a high level of confidence. A sufficient number of animals, however, cannot be established for each type of effect. For effects which do not vary greatly a minimum of 6 animals is generally considered to be sufficient. If the effect being investigated is highly variable (hormone measurements, number of spermatozoa per ejaculate etc.), a larger number of animals is required.

- Following the same concept, statistically it is important that studies which examine effects on future generations take account of the **"litter effect"**. This involves giving preference to a statistical analysis which does not count the total number of animals in the future generations independently of the mother to which they are born but which considers the number of litters affected. This limitation is seen fairly often in the older studies and leads to an over-estimate of the statistical significance of the effects which are found.

For all of these reasons the group set out to list the methodological limitations identified in the studies and to decide whether or not to include the results of these studies in the evaluation. An important factor in this analysis is the reproducibility of the studies: an effect reported in only one publication or by only one group using the same experimental model must be reproduced by another group under similar conditions in order to be deemed to be a clear effect. Most of the reported effects associated with BPA exposure come from studies which were either not reproduced, in which case the effect is deemed at most to be "suspected" if the study did not contain major methodological bias, or the studies were repeated but produced diverging results: in this case the effect is deemed to be "controversial". The following decision tree was used (Figure 7)

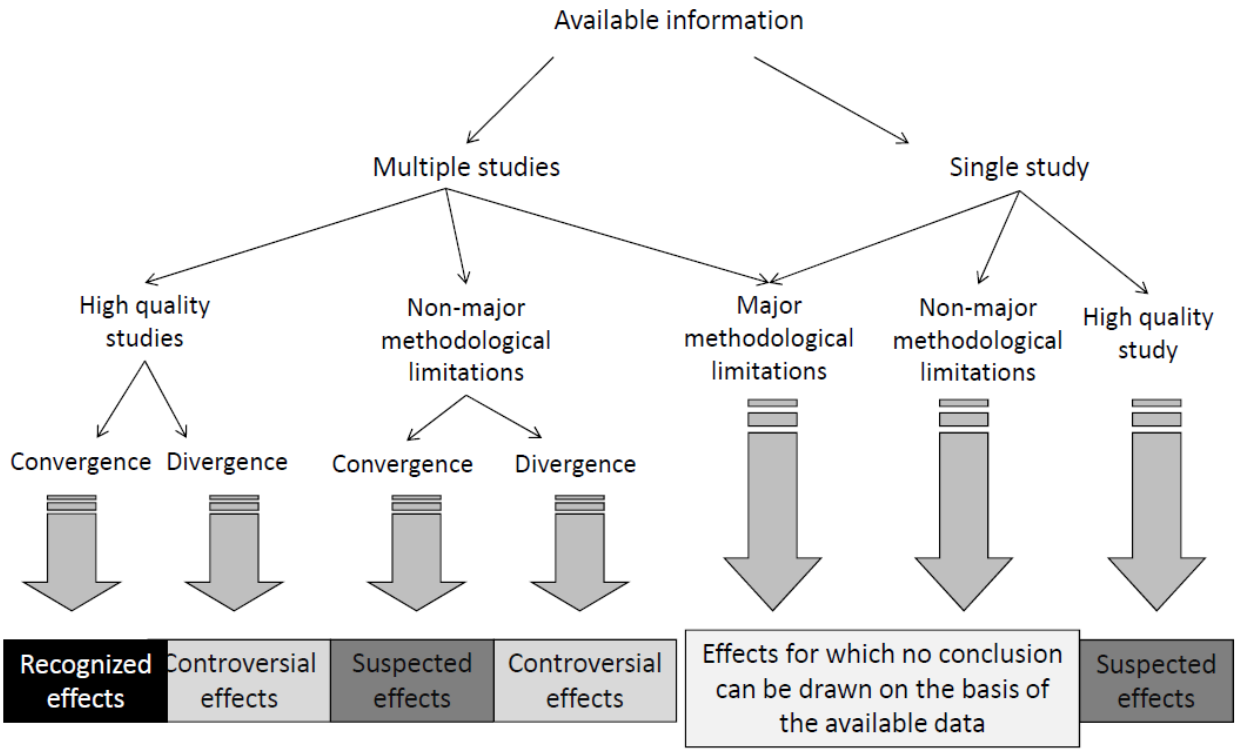


Figure 7: Decision tree

- **Mechanism of action**: in order to extrapolate an effect of BPA in animals to humans, knowledge of the mechanism of action is an important factor which must be taken into account. BPA is considered to be a weak oestrogen α and β receptor agonist. Not all BPA's mechanisms of action are yet fully understood. BPA may also bind to other cell receptors including the androgen receptor, the aromatic hydrocarbon receptor, the oestrogen γ receptor, the transmembrane oestrogen receptor and thyroid hormone receptors, etc. As a result it would be overly simplistic to interpret the effects of BPA only from the perspective of an oestrogen-mimicking effect. The involvement of several of these systems following

BPA exposure could explain certain effects observed at low doses, due to possible synergy, but it could also explain the non-linear dose-response relationships reported in some studies. Pronounced responses at low doses on a given hormone pathway may trigger feedback effects which are well known for some hormones, with less pronounced effects at higher doses of BPA. Finally, other mechanisms of action than those involving binding to hormone receptors have been described, such as activating the expression of specific embryonic genes, modulating second messenger systems, or hormone synthesis or degradation pathways.

8.2. Details of the assessment of the toxicological effects of BPA

8.2.1. Experimental methods

It should be pointed out that the OECD regulatory documents are not always appropriate to assess the effects of substances with these types of mechanism of action.
For example, the OECD carcinogenesis study protocols do not require animals to be exposed *in utero* and followed up throughout their entire life. Similarly, fertility studies do not stipulate that successive litters from the mothers exposed *in utero* should be followed up. A recent study on BPA found reduced fertility in mice exposed *in utero* to BPA several generations later (Cabaton *et al.*, 2010). It is also important to highlight that many academic and/or governmental studies examine effects such as developmental neurotoxicity (MacLusky *et al.*, 2005), changes in the prostate gland (Prins *et al.*, 2011) and the breast (Durando *et al.*, 2007; Murray *et al.*, 2007) whereas the current regulatory toxicology tests do not presently include such in-depth investigations for these organ types. These investigations are not therefore as detailed as those carried out in academic studies. Furthermore, another major difference between the “regulatory” and “academic” toxicology tests is the animal treatment period and post-treatment examinations. This factor must be put in perspective with the susceptibility windows (see Section **Erreur ! Source du renvoi introuvable.**).

8.2.2. Susceptibility window

For several years the exposure period has been considered to be a factor which can largely influence the toxicity of a substance. Exposure during a “susceptibility” period may cause greater toxicity than exposure to the same dose level during another period. In addition, exposure during the developmental period may result in toxicity with effects which may appear several years or even several decades later. These findings notably give rise to diseases of “foetal” or “developmental” origin.

In this report the experts have sought to clearly distinguish between effects seen after prenatal, perinatal or adult exposure.

Finally, extrapolations must be made over time in order to compensate for differences in lifespan. The earliest stages of spermatogenesis begin shortly after birth in rodents and are completed at 6-8 8 weeks old whereas these events occur around the age of 12-15 in boys (

Figure 8). Similarly, the organs which form the hypothalamus-pituitary axis which regulate the oestral cycle are mature at 15 days in rodents, whereas this occurs at 10-12 years in girls. The periods of ovarian differentiation also differ between mammals (Figure 9). It should also be pointed out that the development periods for the prostate gland are different in humans and rodents (Figure 10).

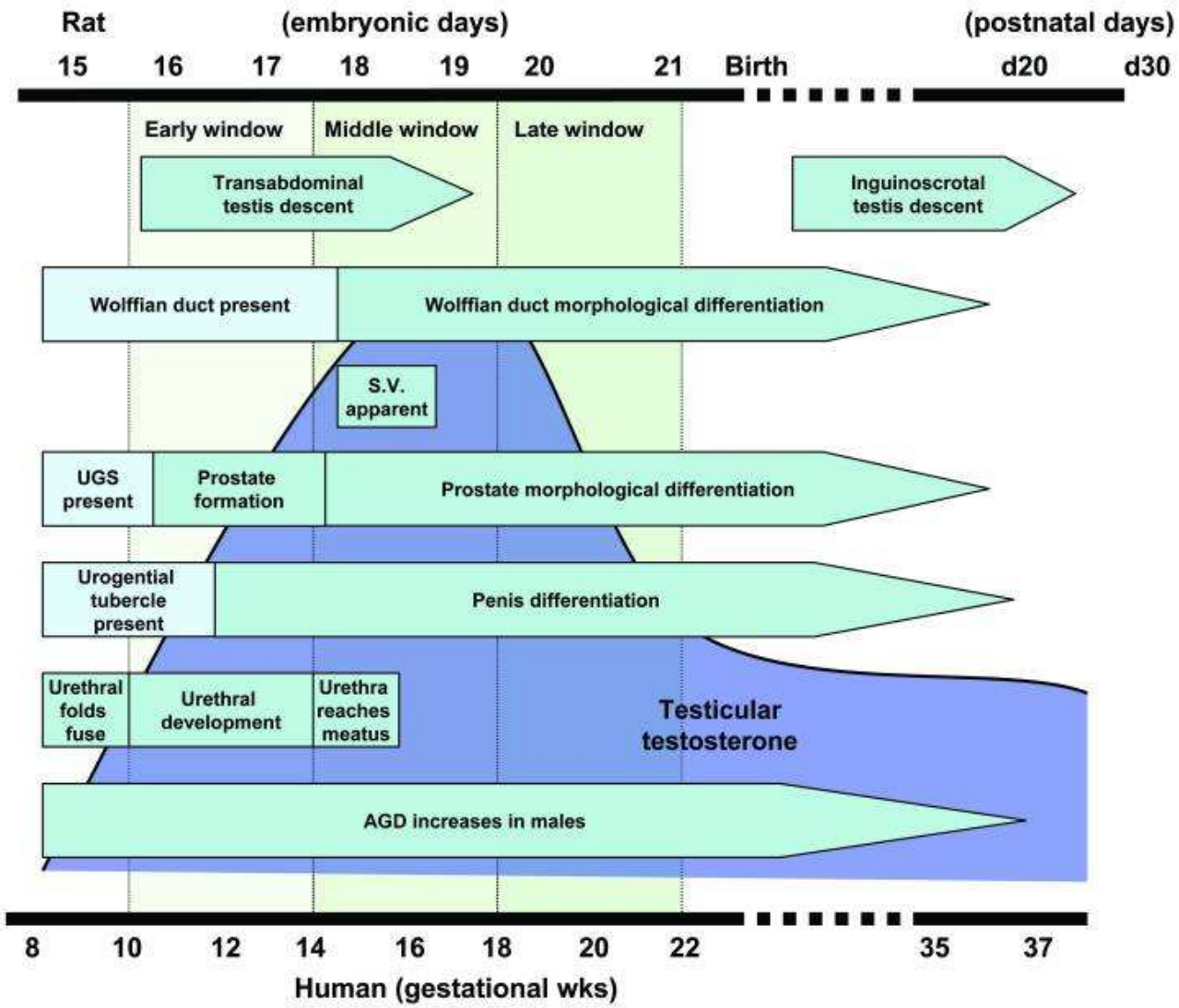


Figure 8: Diagram showing the main developmental periods of the male genital tract in humans and rats in relation to the level of testosterone production (Welsh *et al.*, 2008)

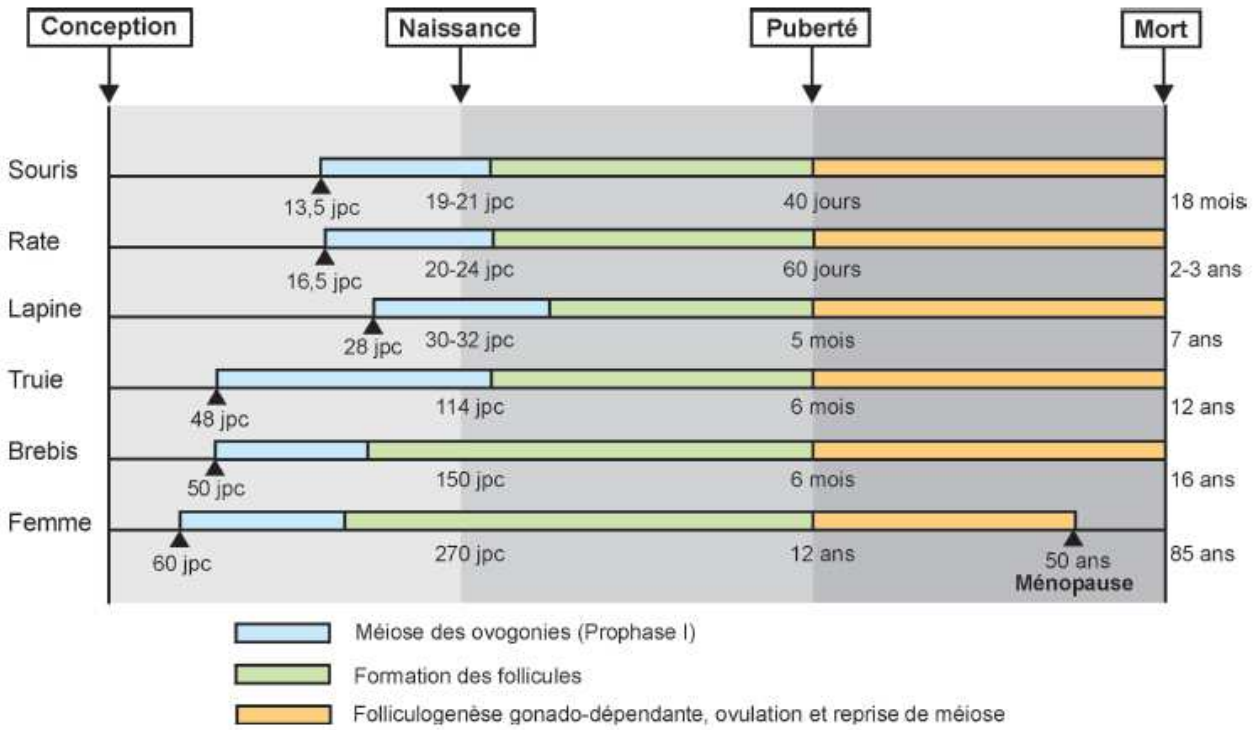
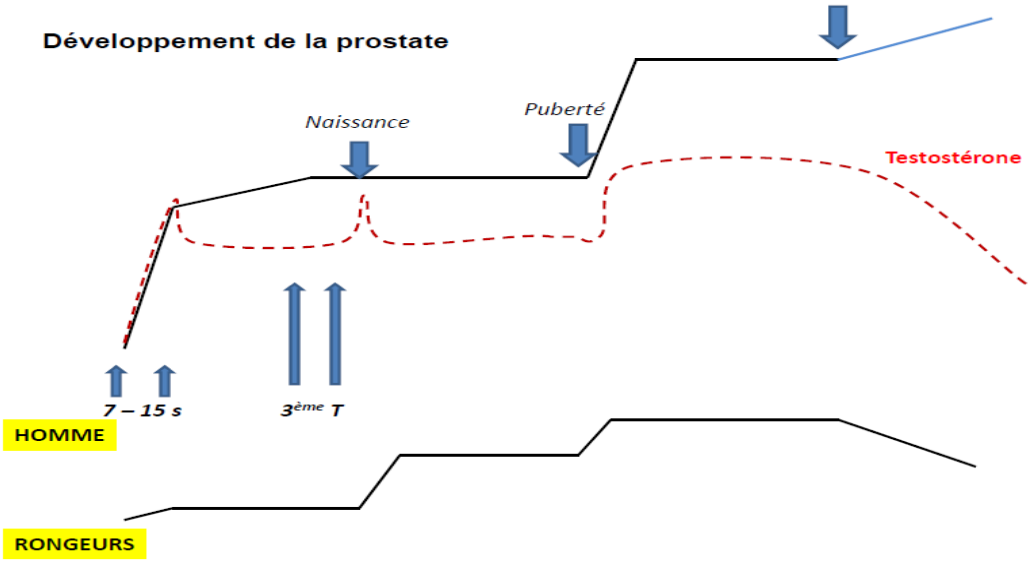


Figure 9: Comparison of ovarian differentiation periods in various mammals (INSERM, 2011)

FRANCAIS	ANGLAIS
conception	conception
naissance	birth
puberté	puberty
mort	death
souris	[female] mouse
rate	[female] rat
lapine	[female] rabbit
truie	sow
brebis	ewe
femme	woman
mois	months
ans	years
ménopause	menopause
méiose des ovogonies (prophase I)	meiosis in oogonia (prophase I)

formation des follicules	follicle formation
folliculogenèse gonado-dépendante, ovulation et reprise de méiose	gonad-dependent folliculogenesis, ovulation and resumption of meiosis



Key: Dashed red line: testosterone level
Black solid line: weight (or volume) of the prostate.
Blue arrow: highlights the different periods in life: birth, puberty, climacteric.

Figure 10: Schematic representation of prostate developmental periods in humans vs. rodents

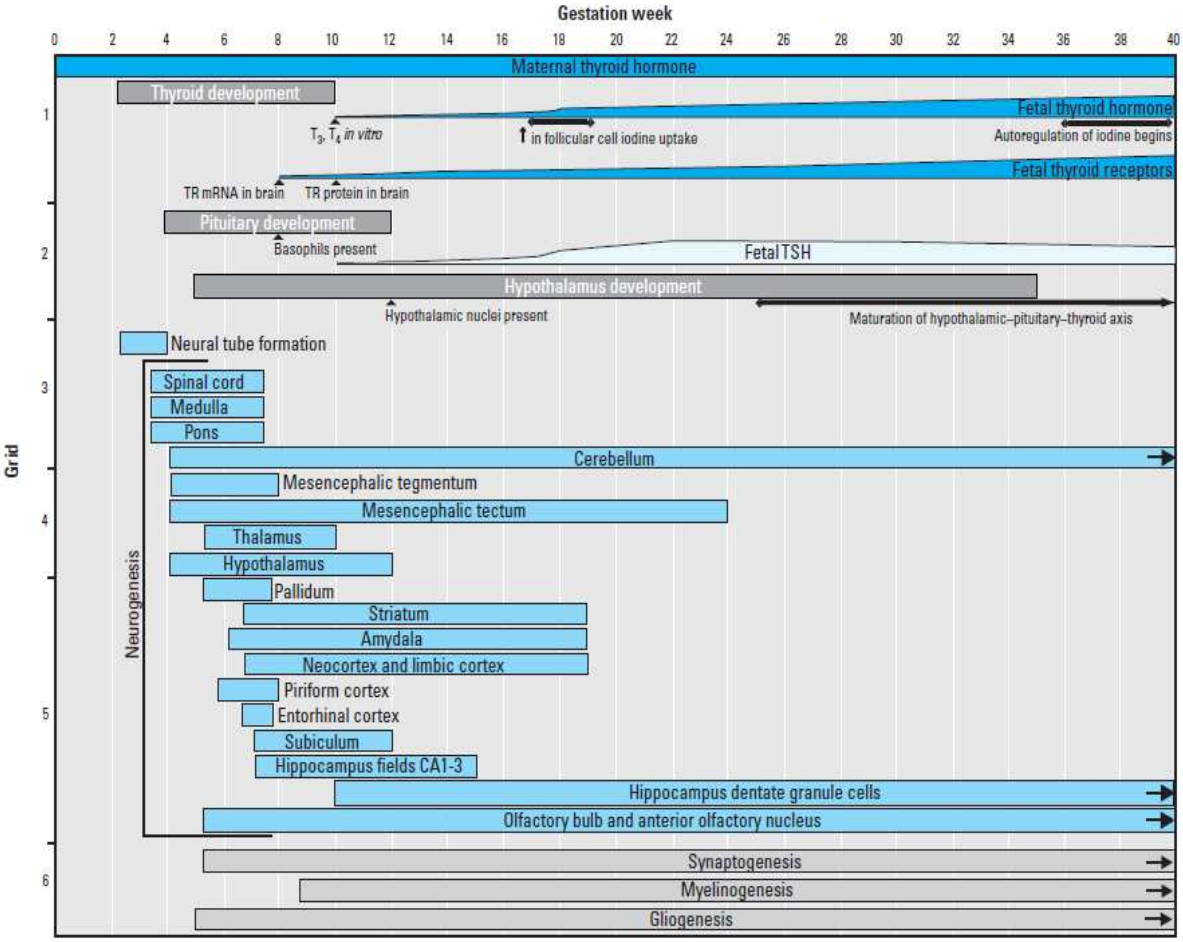


Figure 11: Thyroid and brain developmental periods from conception to birth (Bayer *et al.*, 1993; Howdeshell 2002)

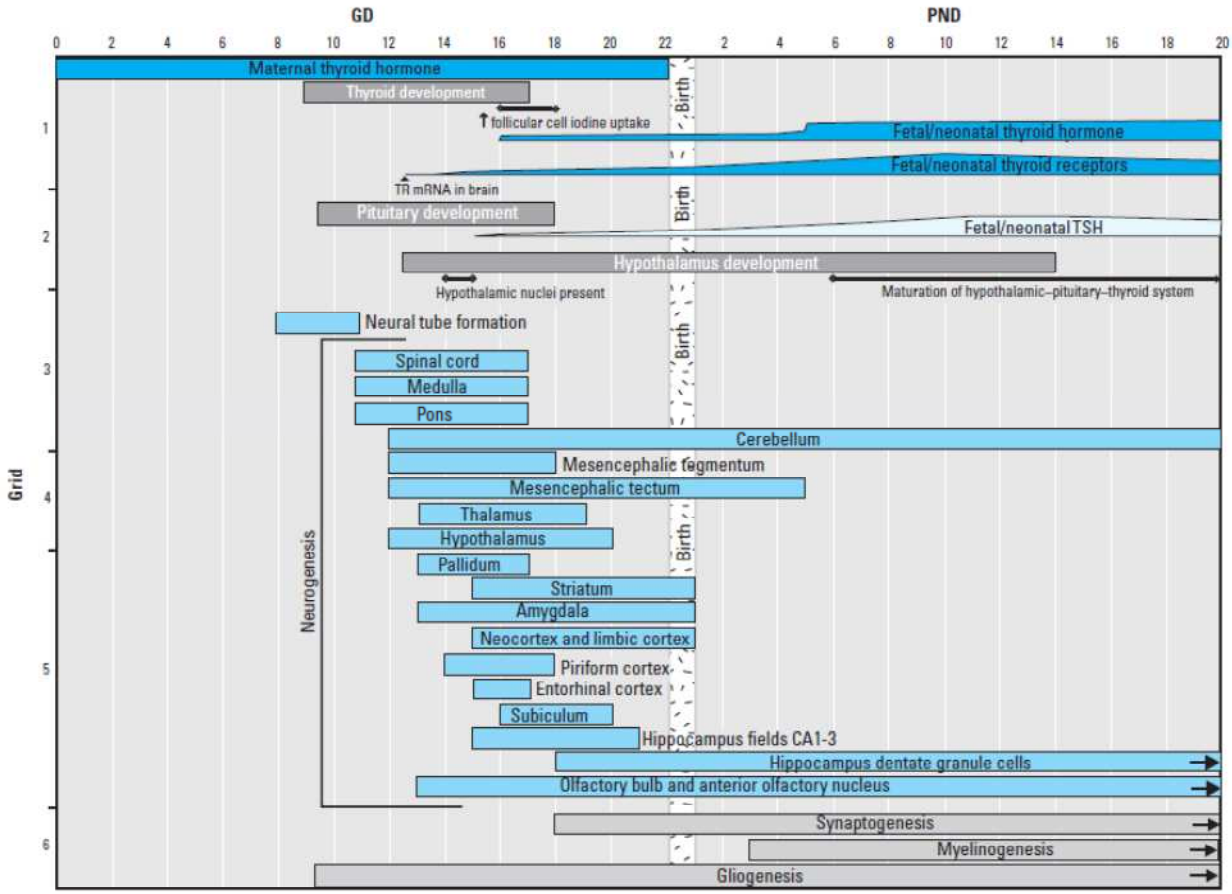


Figure 12: Thyroid and brain developmental periods from conception to PND20 in rats. GD0 indicates the date of conception, and PND1 indicates the date of birth

(Bayer *et al.*, 1993; Howdeshell 2002)

This is why it is important, in assessing the health effects of BPA, to ensure that the window of susceptibility for a given effect has been carefully covered when selecting the exposure period and the time at which the effects are investigated.

Moreover, the same effect may be initiated by various mechanisms of action that will not necessarily be disrupted by the same factors. For example, masculinisation of the hypothalamic-pituitary-gonadal (HPG) axis occurs at about the time of birth in male rodents and is partially mediated by oestradiol produced locally in the brain from circulating testosterone. In humans, on the other hand, this stage of development is initiated in the 3rd trimester of pregnancy and is controlled essentially by the androgens, without oestrogen involvement.

8.2.3. Transposition from animal data to humans

In the most favourable scenario, the data observed in humans may allow an effect, or even a mechanism of action, to be determined. In this case, there is no need to discuss transposing from animal data to humans.

The following factors should be considered in discussing transposition from a specified mechanism of action in animals:

- Toxicokinetics: Taking into account bioavailability and metabolic data in animals and humans: similarities, noteworthy differences?
- Effects: Are they identical in animals and humans? Can one conclude that effects observed in animals may occur in humans in the absence of human data or evidence ruling out transposition from effects observed in animals to humans (species-specificity, differences among laboratory species if several are tested, etc.) ?
- Mechanisms of action: Are they specific to animals, or can they initially be considered similar in animals and humans?

Concerning BPA, the most useful data in terms of assessing health effects are from animal studies. Therefore, the results observed in animals must take into account the differences among the species that may place limitations on pure and simple transposition from results in rodents to humans.

The implications of transposing from these results of animal studies to human subjects take on increased relevance here, namely because this entails considering all or a portion of data observed in animals to be valid for humans. The mechanisms of action leading to these effects are additional factors to be taken into consideration when this type of information is available. Nevertheless, failure to identify a mechanism of action does not mean that an effect can be eliminated from consideration when assessing the impact on health.

Finally, differences in developmental period are to be taken into account: the earliest stages of spermatogenesis in rodents are initiated shortly after birth, and the process is completed at 6-8 weeks, while these same phenomena occur in boys at the age of approximately 12-15. In the same manner, the maturation of the organs forming the HPG axis, which regulates the oestrus cycle, is completed at 15 days in rodents, while this occurs at the age of 10-12 years in girls (Figure 9).

This is why it is important to take these differences into account when transposing from effects observed in rodents to humans.

8.2.4. Issues with non-monotonic dose-response relationships

Toxicology has long been based on the concept of a monotonic dose-response relationship. According to this hypothesis, it is the dose that controls the severity of the effects observed, and exposure to a given dose "n" is considered to cause greater toxicity than exposure to a lower dose. Specifically, demonstration of a non-monotonic dose-response relationship is a criterion contributing to assessment of causality. Multiphasic dose-response curves may sometimes be disqualified by a regulatory assessment committee as "contradicting the universal principles of toxicology".

In order to assess whether non-monotonic dose-response relationships are observed with BPA, experts have examined the studies they have to analyse in order to find those for which effects at low doses are greater than those observed at higher doses, thus constituting a non-monotonic dose-response relationship for a given effect.

- For example, in **the study on the effects of BPA on anxiety**, it was found that the results obtained by Poimenova *et al.* appeared to indicate increased anxiety in F1 females born to mothers exposed to dietary BPA at 40 µg/kg of body weight/day during pregnancy and nursing (Poimenova *et al.*, 2010). On the other hand, looking at the studies conducted by Tian *et al.* using doses of BPA of 100 and 500 µg/kg of body weight/day in mice, in prenatal and postnatal exposure (from GD7 to PND36), BPA was found to have an anxiolytic effect at 100 µg/kg of body weight/day, in contrast to the anxiogenic effect reported by Cox *et al.* at a dose of 8 mg/kg of body weight/day (Cox *et al.*, 2010; Tian *et al.*, 2003). The question therefore remains of determining whether a difference in dose of a factor of 80 can explain the differing anxiogenic/anxiolytic effects of BPA. Nevertheless, the studies by Tian *et al.* should be regarded with caution, because the experimental groups of individuals included only two mothers (Tian *et al.*, 2010). Moreover, the periods of exposure in these 3 studies are not directly comparable, which places a limitation on interpretation of the differences observed.

- Regarding **effects on lipogenesis**, in the study by Sargis *et al.*, the action of BPA on induction of adiponectin shows a bell curve, with a visible effect beginning at 10 nM and peaking at 100 nM and a visible effect at 1 µM (Sargis *et al.*, 2010). An identical dose-response relationship is observed with dexamethasone. The negative effects on the expression of adiponectin observed by Kidani *et al.* are therefore not surprising, as they occur at concentrations greater than 1 µM (Kidani *et al.*, 2010). Therefore, BPA appears to induce the expression of adiponectin at low doses and inhibit it at higher doses (which are already low).

- Regarding **effects on the thyroid**, Xu *et al.* consider a non-monotonic dose-response relationship to have been observed on behavioural tests (see Section **Erreur ! Source du renvoi introuvable.**) (Xu *et al.*, 2007).

- Non-monotonic relationships of BPA with respect to the **male reproductive system** have been reported in several studies:

  - On **human sperm cells**. At high concentrations, BPA exerts an inhibitory effect *in vitro* on cells derived from a human seminoma cell line. This effect appears via activation of the oestrogen receptor ERβ (the affinity of BPA for this receptor is 1000 times lower than that of oestradiol, which neutralises non-genomic effects mediated by membrane signalling pathways, leading for example to an influx of calcium). At low concentrations (10^{-9}), this genomic effect disappears, which leads to the expression of non-genomic effects mediated by a GPCR (G-protein-coupled non-classical membrane ER), an ncmER for which BPA shows a strong affinity. When simultaneously present at low concentrations, BPA and oestradiol thus show antagonistic effects (Bouskine *et al.*, 2009). This could explain the U-shaped curves described by several models (Maffini *et al.*, 2006; vom Saal and Hughes, 2005; Welshons *et al.*, 2006), which could be due to two different types of ER receptors and to genomic and non-genomic mechanisms (Bouskine *et al.*, 2009).

  - According to Gupta, low doses of BPA (50 µg/kg of body weight/day) administered to mice during gestation caused an increase in prostate size in the F1 offspring associated with an increase in androgen receptor activity (Gupta 2000). No effect on the size or weight of the testicles was observed. As for DES, it shows comparable effects at the dose of 100 ng/kg of body weight/day, while at a higher dose (200 µg/kg of body weight/day), reductions in prostate weight, activation of androgen receptors, and anogenital distance are observed. These effects were reproduced *in vitro* in cultures of the foetal urogenital tract, which would appear to indicate a direct effect of these compounds on the developing reproductive organs.

  - In mice exposed to doses of BPA of 2 µg/kg of body weight/day or 20 µg/kg of body weight/day from GD11 to GD17, an increase in preputial gland size and a decrease in epididymal size were observed at the lowest dose. At a dose ten times higher, a decrease in daily sperm cell production per gram of tissue was observed (20% less than the control group) (vom Saal *et al.*, 1998).

In conclusion, among studies showing the effects of BPA at low doses, a certain number emphasise non-monotonic dose-response relationships. Nevertheless, in order to correctly understand the dose-response relationship of BPA, it is necessary to follow the same effect in the same study model and over a sufficient dose range. The majority of studies of BPA involve either high dose ranges, or in the most recent studies, very low doses, but only a few studies have involved exposing the animals to a range covering a sufficient number of log doses (for example, 0.1 – 1 – 5 – 10 – 50 – 100 – 500 - 1000).

8.3. Summary of the conclusions of the report on health effects

8.3.1. Recognised effects

- In humans, the data available are insufficient to allow us to conclude that there are any recognised effects.

- In animals (rodents or sheep), the following effects are considered as recognised:
  - On the male reproductive system: reduction in sperm production after exposure only in adulthood;
  - On the female reproductive system,
    - Increase in the occurrence of ovarian cysts in adulthood due to pre- and postnatal exposure;
    - Changes in the endometrium (hyperplasia) in adulthood due to pre- and/or postnatal exposure; effects on the hypothalamic-pituitary axis causing variations in gonadotropic hormone levels and changes in sex steroid receptor expression due to prenatal exposure; advanced puberty on exposure (early pre- and postnatal period).
  - On brain development in connection with pre- and/or postnatal exposure: changes in the profile of neurodifferentiation associated with sexual dimorphism, impairment of the aminergic and glutamatergic NMDA systems, changes in oestrogen α and β receptor expression and in the number of neurons sensitive to oxytocin and serotonin;

- On the mammary glands:
  - Acceleration of structural maturation of the mammary gland and development of intraductal hyperplasia following pre- or perinatal exposure.
  - Increased susceptibility of mammary glands to subsequent development of mammary tumours (in co-exposure with a carcinogenic agent) associated with foetal or perinatal exposure;
- On lipogenesis: adipocyte hypertrophy, predisposition to obesity, elevated cholesterol levels and triglyceride levels, overexpression of lipogenic proteins following pre-and perinatal exposure in adults.

8.3.2. Non-recognised effects

- **Controversial effects**
  - In humans, based on the available data, effects of BPA on the male reproductive system are considered to be "controversial".

  - In animals, the following effects are considered to be controversial:
    - On the male reproductive system due to exposure during the prenatal, neonatal and postnatal (nursing) exposure periods: organ weight, spermatogenesis, sperm production, hormonal assays, puberty, etc.;
    - On the female reproductive system (pregnancy outcomes and expression of α oestrogen receptors and progesterone receptors in the genital tract) following exposure in adults;
    - On anxiety in young animals and adults, exploratory behaviour, and behavioural sexual dimorphism associated with pre- or perinatal exposure;
    - Advanced vaginal opening or age of first oestrus (markers of sexual maturity) following pre- and/or postnatal exposure
    - On glucose metabolism in young animals and adults following pre- and perinatal exposure;
    - Increased prostate weight and volume following prenatal, perinatal, or adult exposure;
    - Preneoplastic lesions of the PIN type following neonatal exposure.

- **Suspected effects**
  - In humans, based on the available data, the following effects are considered as **"suspected":**
    - Anomalies in oocyte maturation in cases of medically-assisted procreation;
    - Cardiovascular diseases and diabetes (one cross-sectional study).

  - In animals, the following effects are considered as suspected:
    - On the male reproductive system (reduction in plasma testosterone levels, changes in sexual behaviour) due to a single exposure during puberty;
    - Changes in maternal behaviour (reduced time spent with their young (nursing) and reduced nesting behaviour) in connection with postnatal exposure;
    - On the thyroid: changes in free and total T4 varying with the stage of postnatal development following prenatal or neonatal exposure;
    - Development of mammary tumours (ductal carcinoma *in situ,* DCIS) following perinatal exposure;
    - On the intestines: increased inflammatory response and reduction in intestinal permeability in adult females exposed in the perinatal period.

8.3.3. Effects for which the data do not make it possible to draw a conclusion

- In humans, the working group concluded that the human data currently available are insufficient to be able to draw a conclusion on the following effects:
  - On the endometrium (endometriosis, hyperplasia), polycystic ovaries and outcome of pregnancy (miscarriages and premature deliveries);
  - Behavioural disorders in children;
  - On the thyroid;
  - Breast cancer.

- In animals, no effect was classified in this category, because all of the effects were classified as either recognised, suspected, or controversial.

8.4. Conclusions according to organs or systems

These conclusions are based on the results of human data available at present, and on experimental data obtained at doses below the NOAEL of 5 mg/kg of body weight/day that were used to determine the current TDI established by EFSA.

8.4.1. Effects on the male reproductive system

The experts emphasised the difficulty of arriving at a conclusion based on epidemiological studies, because such studies are not fully comparable. It is important to note that the populations studied are not always the same (fertile and infertile men). In humans, effects of BPA on the male reproductive system are controversial.

In animals, effects on spermatogenesis (reduction in number of sperm) due to 5-week exposure of adults to BPA are recognised. In fact, the results of the studies by Chitra *et al.* of subcutaneous administration are comparable for the same exposure period (exposure limited to adults) (Chitra *et al.*, 2003; Herath *et al.*, 2004).

In animals, effects on the male reproductive system due to exposure during the prenatal, neonatal, and postnatal (nursing) periods are controversial.

In animals, effects on the male reproductive system (reduction in plasma testosterone levels, changes in sexual behaviour) due to a single exposure during puberty are suspected.

- Impairment of spermatogenesis is an effect to be taken into consideration in the health risk assessment.

8.4.2. Effects on the female reproductive system

There have been relatively few epidemiological studies investigating a link between exposure to BPA and effects on reproduction in women. The studies are subject to methodological limitations (size of study population, selection of participants, statistical analyses, etc.), which make them difficult to interpret. Correlations among populations (including numerous possible confounding factors) could only be conclusive if they were based on a very large number of observations, and regardless of the statistical approach used to analyse these data. Human data should therefore be considered with extreme caution, and are by no means conclusive with respect to an effect of BPA on the parameters studied. Thus the experts have expressed reservations about the entirety of the epidemiological data and feel that under the current circumstances, human data relating to the

effects of BPA on the endometrium (endometriosis, hyperplasia), polycystic ovaries and pregnancy outcomes (miscarriages and premature deliveries) do not allow a conclusion to be reached in women.

An effect of BPA on oocyte maturation in women in cases of medically-assisted procreation is suspected based on one good-quality study (Mok-Lin *et al.*, 2010) and another with non-major methodological limitations (Fujimoto *et al.*, 2011).

In animals, based on the comparability of the results of various studies under various conditions and using various models, the following effects may be considered to be "recognised in animals" for the exposure protocols currently being developed (pre- and postnatal exposure):

- Increased occurrence of ovarian cysts,
- Hyperplastic endometrial changes,
- Advanced puberty on exposure (early pre- and postnatal phases).

Effects on the hypothalamic-pituitary-gonadal axis due to *in utero* exposure or early postnatal exposure giving rise to variations in the levels of sexual hormones, and changes in expression of the sex hormone receptors, have been observed in several studies. These effects are "recognised in animals".

In animals, the potential effects of exposure in adults are observed at doses far higher than the NOAEL set by EFSA (for example, number of implantation sites, histological reorganisation of the uterine wall, morphology of the genital tract, etc.).

➢ Effects that are "recognised in animals" should be taken into account in the health risk assessment.

8.4.3. Effects on the brain and behaviour

In humans, the working group concluded that the available human data are insufficient to allow a conclusion to be drawn on the effects of BPA on behaviour.

In animals:

- Effects associated with pre- or perinatal exposure to BPA on brain development have been confirmed by several studies that show, for example, changes in the neurodifferentiation profile, impairment of the aminergic and glutamatergic NMDA systems, and changes in the expression of ERα and ERβ receptors and in the number of neurons susceptible to oxytocin and serotonin. These changes occur in particular in regions such as the hypothalamus (more specifically, the region involved in sexual dimorphism) and in the hippocampus, the region involved in cognitive activities and anxiety, particularly those activities associated with the NMDA receptors. These neural effects could partly explain the behavioural effects

of BPA and make it possible to focus the direction of research so as to confirm or rule out effects of BPA on behavioural sexual dimorphism and on anxiety and exploratory behaviour, thus providing a focus for future research. These histological changes in neurogenesis are assessed by the working group to be effects "recognised in animals".

- Studies concerning a link between pre- or perinatal exposure to BPA and anxiety were carried out with exposure levels that are not directly comparable. BPA was found to have no effect in the study of Stump *et al.,* an anxiogenic effect in the study of Poimenova *et al.* and Cox *et al.*, and an anxiolytic effect in the study of Tian *et al.* (Cox *et al.*, 2010; Poimenova *et al.*, 2010; Stump *et al.*, 2010; Tian *et al.*, 2010). Therefore, taking into account these results and results prior to 2010, effects on anxiety caused by pre- or perinatal exposure to BPA are controversial in animals, as are those on exploratory behaviour and behavioural sexual dimorphism.
- Changes in maternal behaviour associated with pre- or postnatal exposure to BPA are suspected effects.

➢ These histological changes concerning neurogenesis are effects to be taken into account in the health risk assessment (HRA).

8.4.4. Effects on metabolism and the cardiovascular system

In a cross-sectional study in humans, correlations were observed between the highest urinary levels of BPA and cardiovascular disease (coronary disease) and diabetes (Melzer *et al.*, 2010). The working group judged these effects to be suspected.

In animals, reviewed studies concerning effects on enzymatic activity, growth and metabolism suggest that rodents exposed as adults or during gestation undergo metabolic changes in various organs such as the liver, adipose tissue and pancreas. Moreover, some authors have noted changes in expression of the genes encoding the proteins involved in the cell signalling pathways of lipogenesis and carbohydrate metabolism. A tendency towards observation of *in vitro* effects on lipogenesis is seen. Mechanistic *in vitro* studies support these observations.

Nevertheless, the lack of repeatability of the reported effects does not allow effects on carbohydrate metabolism to be confirmed.

In animals, therefore, BPA induces increases in lipid levels, a tendency towards obesity, and activation of lipogenesis. Effects on lipogenesis following prenatal and perinatal or adult exposure are considered as recognised (*in vivo* and *in vitro* data). Effects on glucose metabolism following prenatal and perinatal exposure to BPA are controversial.

- These metabolic changes in lipid metabolism are effects to be taken into account in the HRA.

8.4.5. Effects on the thyroid

In humans, the data are considered to be limited and fairly inconclusive, because on the one hand, the protocol appears poorly suited for assessment of thyroid function, and on the other, the effects observed (negative correlation between BPA and TSH) depend on the model used to analyse the data (Meeker *et al.*, 2010a). The working group cannot draw any conclusions based on these data in humans.

In animals, data on the metamorphosis of amphibians in response to T3 show a potential effect of BPA as a thyroid hormone antagonist in these vertebrates. This effect is assessed as recognised in amphibians and could be attributed to mechanisms described in *in vitro* studies. Although the amphibian model is useful in terms of screening and studying mechanisms of action, it is nevertheless unsuitable for characterising risk in humans.

In rodents, experimental data are based on relatively similar approaches (developmental exposure, spontaneous oral administration, etc.) and tend to show an effect of BPA on thyroid function for a period corresponding to final maturation of the hypothalamic-pituitary-thyroid axis. **Effects on the thyroid associated with neonatal exposure to BPA are considered as suspected in rodents.**

8.4.6. Effects on the immune system

In conclusion, in humans, a positive association with anti-cytomegalovirus antibodies has been observed, but the extent and causality of this reaction remain uncertain. The only study available on the effects of BPA on the immune system therefore does not allow a conclusion to be drawn.

In animals, induction of T lymphocytes accompanied by overproduction of cytokines is considered to be a recognised effect. The displacement of the immune response observed tends to be in favour of a Th2 response.

At this stage, there has not been any extrapolation from these observations to humans.

8.4.7. Effects on the intestine

No study in humans has been identified.

In adult animals, a pro-inflammatory effect and a decrease in intestinal permeability were observed in the female offspring of mothers exposed to BPA. An effect of BPA on intestinal inflammation and permeability is suspected (based on a single study) in animals.

In adult animals that have undergone acute treatment with BPA, the pro-nociceptive and anti-inflammatory effects of BPA are of the same type as those caused by oestradiol, and appear to take place via an action on the oestrogen receptors. On the other hand, among animals born to treated mothers, a pro-inflammatory effect is observed in the female offspring and appears to take place due to a maturation defect in the immune system. An effect of BPA on intestinal inflammation and permeability is suspected (based on a single study) in animals.

8.4.8. Effects on the prostate

In humans, there have been no reports published to date of epidemiological studies intended to demonstrate a possible association between exposure to BPA and prostate disease.

In animals, the studies of Tyl *et al.* conducted on several generations in mice and rats do not show any effect on prostate weight (Tyl *et al.*, 2002; Tyl *et al.*, 2008). On the other hand, other studies, such as that of Chitra *et al.,* show an increase in weight relative to the ventral prostate in rats after exposure only in adulthood (Chitra *et al.*, 2003; Herath *et al.*, 2004) or an increase in prostate weight after prenatal exposure in mice (Nagel *et al.*, 1997). Effects on prostate weight are controversial. When a histological examination was conducted, this weight increase was found to be associated with hyperplasia.

Neonatal exposure to BPA (rodents) under controlled conditions showed lesions of the PIN type, but no occurrence of prostatic adenocarcinoma. Effects observed under these experimental conditions are suspected. In light of all these results, the experts assess effects on the prostate in animals as controversial.

8.4.9. Effects on the breasts

Most of the literature on the association between BPA and breast cancer has appeared within the last ten years, and this field of research is evolving, with numerous references appearing recently.

In humans, the methodological limitations of the only epidemiological study available do not allow conclusions to be drawn on the link between BPA and breast cancer.

In animals, although studies show a certain degree of uniformity, the common occurrence of certain effects has led the working group to the following conclusions:

- Acceleration of structural maturation of the mammary glands in adults associated with prenatal or perinatal exposure to BPA is an effect that is "recognised in animals";
- Development of intraductal hyperplastic lesions associated with perinatal or prenatal exposure to BPA is an effect that is "recognised in animals";

- Development of lesions of the neoplastic type (CIS; ductal carcinoma) following perinatal exposure to BPA is a suspected effect;
- Increased susceptibility of the mammary glands to subsequent development of mammary tumours (in co-exposure with a carcinogenic agent) associated with prenatal or perinatal exposure to BPA is a suspected effect.

➢ The effect of BPA on the risk of occurrence of breast cancer is to be taken into account in the HRA.

8.4.10. Information from ecotoxicological studies

Apart from the mechanisms of action, ecotoxicological studies suggest that very considerable effects, particularly on reproduction and development, may affect wild species and that these effects are seen at concentrations which are liable to be found in the environment.

Ecotoxicological studies show that:

- Bisphenol A is liable to cause major physiological changes through its oestrogenic activity. The presence and involvement of oestradiol in a large number of zoological groups from the cnidarians upwards suggests that a very large number of species may be disrupted by this mechanism although the specificity, distribution and sensitivity of hormone receptors may vary greatly depending on the group in question.

  Developmental reports suggest that oestrogenic activity is not the only pathway by which BPA interacts with the physiology of organisms. In particular the thyroid axis appears to be sensitive to the compound and depending on the zoological groups and/or doses it may have agonist or antagonist effects.
- Apart from the mechanisms of action, ecotoxicological studies suggest that very considerable effects, particularly on reproduction and development, may affect wild species and that these effects are seen at concentrations which are liable to be encountered in the environment.

8.5. Effects considered for the health risk assessment

In the absence of data in humans, the working group considered that the effects observed in animals can be transposed to humans, except in cases where it has been demonstrated that these effects observed in animals are specific to the species in question.

On completion of this analysis, the experts will first take into account effects assessed to be "recognised" (no recognised effect has been seen in humans to date) and suspected in humans for conducting the health risk assessment. Nevertheless, they reserve the possibility, depending on

the relevance and plausibility of the effects, to investigate in a second step effects assessed to be "suspected" or "controversial" for conducting the HRA. Moreover, the experts will take into consideration any new toxicological or human data available in cases where such data may cause the classification to be modified.

The working group will therefore consider for their risk assessment effects evaluated as:

- **"recognised in animals":**
  - increased incidence of ovarian cysts on pre- and postnatal exposure,
  - hyperplastic changes in the endometrium on pre- and postnatal exposure,
  - advancement of the age of puberty on early pre- and postnatal exposure,
  - impairment of sperm production on exposure in adults,
  - histological changes in neurogenesis on pre- and perinatal exposure,
  - effects on lipogenesis following prenatal, perinatal, or adult exposure,
  - effects on the mammary gland: acceleration of structural maturation of the mammary gland in adults and development of hyperplastic intraductal lesions associated with pre- or perinatal exposure to BPA.

- **"suspected in humans"**:
  - effects on oocyte maturation in women in the case of medically-assisted procreation,
  - effects on cardiovascular disease (coronary disease) and diabetes.

9. Research recommendations

Some initial research recommendations were proposed by the working group. These recommendations may be supplemented in the future after expert appraisals have been completed.

Taking into account the importance of the exposure period on the expected impact of endocrine disruptors and the late onset of many of these effects, the possibility should be investigated of conducting a combined toxicity study on development and carcinogenesis using, after making suitable modifications, the principles and procedures described in OECD TG 414 and TG 451, for example. The preferred administration route should be oral administration because of the predominance of this exposure route in humans, and the study should cover the period from conception in the parents to adulthood in the offspring in order to reflect the chronic nature of BPA exposure.

Knowledge of exposure to BPA should be improved, and matrices of the use/exposure type should be developed in epidemiological studies.

The concept of TDI is not suitable for this type of compound. A threshold concept would be more suitable.

10. Prospects

On completion of this analysis, the tasks of the working group will be as follows:

- To conduct a more detailed investigation of studies likely to demonstrate non-monotonic dose-response relationships;
- To assess the severity and reversibility of critical effects included in the health risk assessment;
- To identify effects to be included in the health risk assessment. In order to do this, the experts from the working group plan to classify the effects in order to integrate all of the human and animal data. A classification grid has been proposed. This grid has not been finalised, and will be reviewed by the working group in future research.
- To conduct a supplementary analysis of toxicokinetic data in order to allow assessment of noteworthy similarities or differences (qualitative or quantitative) between animal species and humans and determination of bioavailability of BPA in humans;
- To determine dose bioequivalence based on solid toxicokinetic data in order to allow use of the results of subcutaneous studies in the health risk assessment;
- To propose the development of a tool (PBPK model) allowing the active dose in the target organ of animals to be determined, after which one could extrapolate from this data to humans. This tool would also make it possible to integrate currently available and future biomonitoring data;
- To identify or establish one or more toxicity reference value(s) (TRVs) if appropriate;

On completion of this expert appraisal, the feasibility and relevance of conducting a health risk assessment (HRA) will be studied, taking into account all routes of exposure and uses, the most susceptible populations, and the corresponding windows of exposure.

In addition, ANSES will identify possible substitutes and will list the data available on the toxicity of these substitutes, without necessarily going as far as to assess the risks connected with use of these substitutes.

Finally, discussions on endocrine disruptors in general are in progress. A series of questions have been submitted by internationally recognised experts and/or involved parties. The results of these discussions will be taken into account, provided that they allow documentation of the method of assessing endocrine disruptors or raise new concerns with respect to this type of compound.

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ANNEXES

Annexe 1: Lettres de saisine

Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE
Ministère de la santé et des sports

Le Directeur général de la santé
EA4 N° 220

Paris, le - 4 JUIN 2009

COURRIER REÇU LE
0 8 JUIN 2009
2186

Monsieur le directeur général
Agence française de sécurité sanitaire
de l'environnement et du travail
253, avenue du Général Leclerc
94701 Maisons-Alfort Cedex

Objet : Substances reprotoxiques et perturbateurs endocriniens.
N/Réf : 090018 *(numéro de dossier à rappeler dans toute correspondance)*

Les données scientifiques semblent mettre en évidence dans les pays industrialisés une baisse de la fertilité chez l'homme depuis une vingtaine d'années. Ces évolutions préoccupantes sont souvent attribuées à la pollution chimique de notre environnement.

L'action de certaines substances chimiques sur la reproduction ou sur les organes de la reproduction peut être due soit à une action directe reprotoxique qui peut affecter l'adulte, le jeune enfant, l'embryon ou le fœtus lors de son développement prénatal, soit à une action hormono-mimétique (*« oestrogen- ou androgen-like »)* lorsque ces substances sont des perturbateurs endocriniens.

Dans ce contexte de préoccupations tant pour le public que pour les autorités sanitaires, j'ai saisi l'INSERM pour la réalisation d'une expertise collective visant notamment à répertorier sous six mois les substances reprotoxiques préoccupantes, à décrire les différents effets reprotoxiques sur le développement ou la fertilité connus et leurs mécanismes, à analyser les facteurs de risque chez l'enfant et l'adulte en identifiant en particulier les périodes de la vie les plus sensibles en termes d'exposition et à analyser les tests *in vitro* et *in vivo* actuellement mis en œuvre, au niveau réglementaire ou au stade de la recherche, pour détecter ces effets. L'AFSSET est associée à cette expertise.

Sur la base de la liste des substances reprotoxiques établie dans le cadre de cette expertise de l'INSERM, je souhaite que vous poursuiviez dans votre champ de compétence ces travaux d'expertise afin de déterminer s'il existe aujourd'hui des produits destinés au grand public contenant de telles substances, de quantifier leurs utilisations, les niveaux d'exposition qui en résultent et de procéder à une évaluation bénéfice/risque. L'AFSSA et l'AFSSAPS seront saisies de la même manière dans leur champ de compétence.

Je souhaite par ailleurs que votre agence assure la coordination générale des travaux de ces différents organismes afin de produire une évaluation globale des expositions et des risques et

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de proposer, si nécessaire, le renforcement de certaines préconisations d'usage. A cet effet, vous voudrez bien en particulier, parmi ces produits :

- Identifier ceux d'entre eux contenant ces substances ou susceptibles d'être concernés ;
- Sélectionner ceux à étudier en priorité ;
- Analyser et si possible quantifier des voies d'exposition de la population générale à ces substances en précisant les sources directes et indirectes, et incluant des populations vulnérables et les personnes en milieu de travail ;
- Envisager des substitutions.

S'agissant du domaine des résidus de médicaments dans les eaux, je vous indique qu'il a déjà fait l'objet de ma part d'une demande d'expertise auprès de l'AFSSA et de l'AFSSAPS. Par ailleurs, un plan d'actions relatif aux résidus médicamenteux dans les milieux est en cours d'élaboration conjointement par la Direction Générale de la Santé et la Direction de l'Eau et de la Biodiversité du MEEDDAT. Si une saisine est nécessaire sur ce thème, elle sera élaborée en concertation entre les deux ministères en cohérence avec le plan d'action sus mentionné.

Le Directeur Général de la Santé,

Pr Didier HOUSSIN

Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE

COURRIER REÇU LE
22 FEV. 2010
660

→ Acat
Signalé.

MINISTERE DE L'ECOLOGIE, DE l'ENERGIE, DU DEVELOPPEMENT DURABLE ET DE LA MER, en charge des Technologies vertes et des Négociations sur le climat

Direction générale de la prévention des risques

Paris le 18 FEV. 2010

Le Directeur général de la prévention des risques

à

Monsieur le Directeur général de
l'Agence Française de Sécurité Sanitaire
de l'Environnement et du Travail
253 Avenue du Général Leclerc
94701 Maisons-Alfort

Objet : Bisphénol A
Copie : DGS, DGAL, DGT, DGCCRF, AFSSA, INERIS

Le bisphénol A fait l'objet d'une attention particulière au niveau international en raison de la publication régulière de nouvelles études relatives aux effets sur la santé dans des revues spécialisées. La sensibilité des enfants et des femmes enceintes et le caractère perturbateur endocrinien du bisphénol A suscitent ainsi des inquiétudes au sein de la population et de la communauté scientifique.

Au vu de ces informations scientifiques complémentaires et des démarches engagées par les autorités de divers pays (Japon, Norvège, Canada), il nous semble pertinent d'approfondir la réflexion sur les risques encourus pour la santé et l'environnement.

Le bisphénol A est utilisé pour la fabrication de polycarbonates utilisés dans de nombreux plastiques, dans la fabrication de différentes résines et d'ignifugeants. De plus le bisphénol A est utilisé, entre autres, en tant qu'additif dans les retardateurs de flammes et comme révélateur dans les papiers thermiques.

Dans ce cadre, je vous demande d'engager des travaux portant sur les risques sanitaires résultant d'une exposition de l'homme au bisphénol A via l'environnement. Je vous demande ainsi de bien vouloir, en lien avec l'Afssa :

- transmettre une synthèse des dangers présentés par le bisphénol A à partir notamment d'une revue des études publiées depuis le rapport d'évaluation des risques présenté au niveau communautaire par Le Royaume-Uni en février 2008 dans le cadre du règlement

793/93 et le dossier Annexe XV transitoire soumis au 1er décembre 2008, de faire un bilan des connaissances multidisciplinaires en la matière et identifier les éventuelles lacunes; pour réaliser ce travail, vous pourrez utilement prendre l'attache du président du comité scientifique du programme de recherche sur les perturbateurs endocriniens pour identifier les scientifiques qui peuvent utilement y contribuer ;

- identifier les usages conduisant à une exposition humaine et caractériser ces situations d'exposition ;
- identifier les substituts au bisphénol A ainsi que les données disponibles sur les dangers présentés par ces substituts.

Vous pourrez également vous rapprocher de l'Ineris qui, à la demande de l'Onema, a engagé un travail sur les données de production et d'utilisation du bisphénol A, les rejets et le devenir de cette substance dans l'environnement, ainsi que les perspectives de substitution et de réduction des émissions.

En fonction des informations recueillies lors de cette première phase, vous voudrez bien analyser la faisabilité et la pertinence d'une évaluation des risques sanitaires liés aux différents modes d'exposition et à partir des données disponibles.

Il me paraît souhaitable que cette molécule fasse l'objet d'une attention particulière de votre agence lors des travaux sur les perturbateurs endocriniens engagés au niveau national avec l'Inserm.

A la lumière de ces travaux, vous formulerez si nécessaire des recommandations, notamment pour la prise en compte du bisphénol A dans le cadre du règlement (CE) n°1907/2006 dit règlement REACh. Une analyse de la procédure la plus pertinente (inscription dans le premier plan d'action communautaire pour évaluation, préparation d'un dossier de restriction, de classification et d'étiquetage harmonisés ou d'identification de substance extrêmement préoccupante en vue de son inscription à l'annexe XIV) devra notamment être conduite en tenant compte des outils de gestion prévus par d'autres réglementations sectorielles.

Enfin, je vous remercie de bien vouloir dresser un bilan des recherches en cours concernant le bisphénol A ou ses substituts et d'en tirer d'éventuelles recommandations relatives aux domaines à investiguer en priorité.

Le Directeur général de la prévention des risques

Laurent MICHEL

French Ministry of Health and Sports

The Director General for Health

EA4 No. 220

Paris, 4 June 2009

The Director General

French Agency for Environmental and Occupational Health Safety

253 avenue du Général Leclerc

94701 MAISONS-ALFORT CEDEX

MAIL RECEIVED

08 JUNE 2009

2186

Subject: Reprotoxic substances and endocrine disruptors.

Ref. /No.: 090018 *(file number to be referenced in all correspondence)*

The scientific data seem to show a fall in male fertility in industrialised countries over the past twenty or so years. These worrying changes are often attributed to chemical pollution in our environment.

The action of certain chemicals on reproduction or on the reproductive organs can be due either to a direct reprotoxic action affecting the adult, young child, embryo or foetus during its prenatal development, or to a hormone-mimetic action (oestrogen- or androgen-like) when these substances are endocrine disruptors.

In this context of concern, both for the public and the health authorities, I requested that INSERM conduct a collective expert appraisal aiming primarily to draw up, within six months, a list of the reprotoxic substances of concern, to describe the different known reprotoxic effects on development or fertility and their mechanisms, to analyse the risk factors in children and adults by identifying in particular the most susceptible periods in life in terms of exposure, and to analyse the *in vitro* and *in vivo* tests currently used, for regulatory or research purposes, to detect these effects. AFSSET has been contributing to this expert appraisal.

Based on the list of reprotoxic substances compiled as part of INSERM's expert appraisal, I would like you to pursue this expert work in your sphere of competence in order to determine whether there are currently products intended for the general public that contain such substances, to quantify their use and the associated exposure levels, and to conduct a benefit/risk assessment. AFSSA and AFSSAPS will likewise receive solicited requests relating to their respective spheres of competence.

I would also like your agency to coordinate overall the work of these organisations in order to make a general assessment of exposure and risk and, if necessary, to propose the reinforcement of certain recommendations for use. Accordingly, with respect to these products I would be grateful if you would:

- identify those containing these substances or likely to be affected;
- select those to be studied as a priority:
- analyse and if possible quantify the routes by which the general population are exposed to these substances, specifying direct and indirect sources, and including vulnerable populations and people exposed in occupational environments;
- consider substitutes.

With respect to drug residues in water, I can inform you that I have already requested an expert appraisal on this topic from AFSSA and AFSSAPS. Moreover, an action plan relating to drug residues in different environments is currently being prepared jointly by the Directorate General for Health and the Directorate for Water and Biodiversity of the MEEDDAT [Ministry of Ecology, Energy, Sustainable Development and Land Planning]. If a solicited request is needed on this theme, it will be prepared in consultation with both Ministries to ensure coherence with the abovementioned action plan.

Director General for Health

[Signature]

Pr Didier HOUSSIN

MAIL RECEIVED
22 February 2010
660

MINISTRY OF ECOLOGY,
ENERGY,
SUSTAINABLE DEVELOPMENT
AND THE SEA
responsible for Green technologies
and Negotiations on the climate

Directorate General for Risk Prevention

Paris, 18 February 2010

The Director General for Risk Prevention
to:
The Director General
French Agency for Environmental and Occupational Health Safety
253 avenue du Général Leclerc
94701 MAISONS-ALFORT CEDEX

Subject: Bisphenol A
cc: DGS, DGAL, DGT, DGCCRF, AFSSA, INERIS

Bisphenol A has been the focus of particular attention at the international level due to the regular publication in specialised journals of new studies relating to the health effects. The susceptibility of children and pregnant women and the endocrine-disrupting nature of bisphenol A have thus led to concerns being raised within the population and the scientific community.

In view of this complementary scientific information and the steps taken by the authorities in various countries (Japan, Norway, Canada), it seems appropriate to us to examine in greater depth the risks posed to health and the environment.

Bisphenol A is used to manufacture polycarbonates used in many plastics, and in the manufacture of different resins and fire retardants. In addition, bisphenol A is used, among other things, as an additive in flame retardants and as a developer in thermal papers.

In this context, I would like you to undertake work focusing on the health risks resulting from human exposure to bisphenol A via the environment. I would also be grateful if you would, in conjunction with AFSSA:

- issue a summary of the hazards posed by bisphenol A, mainly using a review of the studies published since the risk assessment report presented at European level by the United Kingdom in February 2008 in the context of Regulation (EEC) 793/93 and the transitional Annex XV dossier submitted on 1 December 2008, to review the multidisciplinary knowledge on the subject and identify any gaps; to conduct this work you may find it useful to consult the Chairman of the scientific committee of the research programme on endocrine disruptors in order to identify any scientists who may be able to help you identify;
- the uses leading to human exposure and characterise these exposure situations;
- substitutes for bisphenol A as well as the available data on the hazards posed by these substitutes.

You may also contact INERIS, who at ONEMA's request has undertaken work on data on bisphenol A production and use, discharges and fate of this substance in the environment, as well as prospects for substitution and emissions reduction.

Depending on the information gathered during this first phase, I would then like you to analyse the feasibility and relevance of conducting a health risk assessment on the different methods of exposure and based on the available data.

It seems worthwhile for your agency to focus on this compound while work on endocrine disruptors is undertaken at the national level with INSERM.

In light of this work, you should if necessary formulate recommendations, particularly on the inclusion of bisphenol A in the scope of Regulation (EC) no. 1907/2006 (REACh Regulation). An analysis of the most relevant procedure (inclusion in the first EU action plan for assessment, preparation of a dossier for restriction, harmonised classification and labelling or identification of a substance of very high concern with a view to its inclusion in Annex XIV) should in particular be conducted, taking into account the management tools stipulated by other sector regulations.

Finally, please produce a review of current research on bisphenol A or its substitutes and make the necessary recommendations concerning the areas to investigate as a priority.

Director General for Risk Prevention

[Signature]

Laurent Michel

Annex 2: Definitions of endocrine disruptors

- EPA: repeat of the definition by Kavlock in 1996: "an exogenous agent that *interferes* with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body which are responsible for the maintenance of homeostasis, reproduction, development and or behavior";

- European Commission: Weybridge (1996): "An endocrine disruptor is an exogenous substance or mixture that *alters* function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations"; or

- "exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function" see European Workshop on the Impact of Endocrine Disruptors on Human Health and Wildlife, Weybridge, United Kingdom (1996);

- The WHO definition (2002) distinguishes between two categories: "**endocrine disruptors"** and "**potential endocrine disruptors"**, expressing the difference between a definition that gives primacy to an exclusively pathogenic mechanism of action, and one that includes endocrine disruption in a potentially pathogenic mechanism of action, or, to use the terminology of the EPA and Weybridge between *interference* and *alteration:*

  - An *endocrine disruptor* is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations.

  - A *potential endocrine disruptor* is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations.

- This WHO definition is used by the OECD working groups which also take into account a definition of what is an **"adverse effect"**:

  - "Change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences". Note: there is no statutory definition for "impairment". (Descriptions of Selected Key Generic Terms used in Chemical Hazard/Risk Assessment – OECD Series on Testing and Assessment No. 44, mentioned in ENVIRONMENT DIRECTORATE, JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY, Advisory Group on Endocrine Disrupters Testing and Assessment (EDTA) of the Test Guidelines Programme, Workshop on Endocrine Disrupters Testing and Assessment (EDTA) 22-24 September, Copenhagen, Denmark, ENV/JM/TG/EDTA/RD(2009)1)

- Endocrine Society:
  - "An endocrine-disrupting substance is a compound, either natural or synthetic, which through environmental or inappropriate developmental exposures alters the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment", Diamanti-Kandarakis *et al.*, Endocrine Reviews (2009) .
- The REACh architecture lists endocrine disruptors within the category of substances of very high concern, but in a separate group to CMRs, PBTs (Persistent, Bioaccumulative, Toxic substances) or vPvBs (very Persistent and very Bioaccumulative). Under REACh, endocrine disruptors are substances giving rise to the same level of concern as the above three classes of substances.

Annexe 3: Effets sur le développement autres que des atteintes du système reproducteur mâle et femelle

Même s'il n'y a aucune preuve chez l'Homme d'un effet reprotoxique du BPA, le NTP-CERHR a conclu en 2008 que les études chez l'animal de laboratoire montrent qu'une exposition à de fortes doses de BPA pendant la gestation ou la lactation peut entraîner une réduction de la survie des fœtus chez le rat et la souris (> 500 mg/kg bw/d), du poids des petits à la naissance et de la croissance néonatale chez le rat et la souris (> 300 mg/kg bw/d) ainsi qu'un retard de la puberté chez les mâles (souris) et femelles (rats et souris) (> 50 mg/kg bw/d). Ces effets sont observés aux doses auxquelles des baisses de poids corporels sont également observés chez les mères traitées. Ces doses sont très largement supérieures aux doses d'exposition humaine.

Dans son évaluation des risques (EC, 2003; EC, 2010b), les effets sur la fertilité et les performances de reproduction ont été évalués. Cette évaluation s'est appuyée notamment sur la base des résultats des études de Tyl *et al.* (2000), Chemical Compound Safety Research Institute (2000) et du NTP (1985b). Ces études étaient jugées de bonne qualité. Il y a à la fois une étude de 2 générations et une étude 3 générations toutes deux conduites chez le rat (Chemical Compound Safety Research Institute, 2000 et Tyl *et al.*, 2000, respectivement) et une étude de reproduction continue chez la souris (NTP, 1985b).

Dans l'étude rat 2-générations, aucun effet néfaste sur la fertilité n'a été observé jusqu'à la dose maximale testée de 200 µg/kg bw/d. On peut néanmoins noter que cette dose est relativement faible.

Dans l'étude rat 3-générations, utilisant des doses de 0,001 à 500 mg/kg bw/d, une réduction statistiquement significative de la taille de la portée a été observée dans les trois générations à la dose la plus élevée (F1: 11,5 petits vivants par portée par rapport à 14,3 pour les contrôles; F2: 10,8 contre 14,6; F3: 10,9 contre 14,8). Cette réduction n'était pas corrélée à une augmentation du nombre de petits morts par portée. A la dose de 500 mg/kg bw/d une toxicité générale a été observée chez les adultes dans toutes les générations (réduction du gain de poids > 13% chez les deux sexes et dégénérescence des tubules rénaux chez les femelles seulement).

Dans l'étude souris, des réductions significatives du nombre de portées produites par paire F0 et de la taille des portées (petits vivants par portée) ont été observées aux doses médiane et forte (environ 600 et 1300 mg/kg bw/d). Ces diminutions de taille des portées ont été observées après chaque accouplement et ce de manière dose-reliée mais n'étaient pas liées à une augmentation des petits mort-nés. Aucun effet n'a été observé à la dose la plus faible (environ 300 mg/kg bw/d).

Des diminutions de la taille des portées ont également été observées dans d'autres expériences de "cross-over" dans lesquelles les cas où seules les femelles étaient traitées engendraient les effets les plus importants. A la forte dose, des effets de toxicité générale dans le foie et les reins des parents F0 ont été décrits mais non gradés (cf. RAR ESR pour plus de détails). Aucun effet indésirable sur la fertilité n'a été observé dans la seule portée testée par dose à la génération F1 dans cette étude.

Ces données ont servi de base à la proposition de classification et d'étiquetage du bisphenol A en tant que composé Toxique pour la reproduction catégorie 2, H 361f- Toxicité spécifique pour certains organes cibles.

Le panel d'experts réuni sous l'égide de la (FAO/WHO, 2010) a pris en compte les études publiées depuis 2008. Sur la base des études chez les rongeurs après administration de faible dose de BPA par voie orale ou sous-cutanée (inférieures à 1 mg/kg bw/d), le panel d'experts a jugé:

- suffisantes les preuves en faveur de:
  - l'absence d'effets tératogènes du BPA
  - l'absence d'effet sur la survie des nouveaux nés et le poids à la naissance
  - l'absence d'effet sur la croissance des nouveaux nés et leur survie pendant la lactation
  - l'absence d'effet sur la distance ano-génitale chez les males et femelles
  - l'absence d'effet morphologique masculinisant chez la femelle ou démasculinisant chez le mâle

- suffisantes (mais avec un certain degré d'incertitude) les preuves en faveur de:
  - l'absence de diminution du taux d'implantation, l'absence d'effet sur la fertilité ou la fécondité;

- controversés les effets sur:
  - une modification de l'âge de la puberté
  - des modifications du poids ou de l'histologie des organes reproducteurs chez les males et femelles exposés
  - des modifications des taux hormonaux chez les males et femelles exposés ainsi que chez leur descendants
  - des modifications du poids, de l'histologie des organes reproducteurs ou des paramètres spermatiques chez les descendant males.

Les experts estiment importantes les incertitudes quant aux effets du BPA chez les rongeurs à des doses inférieures à 1 mg/kg bw/d par voie orale ou cutanée sur les cibles conventionnels en lien avec la reprotoxicité ou le développement.

Les experts soulignent le manque de données sur:

- les effets d'une exposition néonatale au BPA sur le développement de la fonction de reproduction
- les effets du BPA sur des modèles animaux non rongeurs: lapins, primates non-humains, … qui pourraient être plus pertinents pour certains types d'effets (prostate par exemple).

Annexe 4: Effets chroniques

D'après l'UE-RAR 2003 et 2010 (EC, 2003; EC, 2010b), seules quatre études de toxicité chronique **par inhalation** sont disponibles chez le rat: Nitschke *et al.* (1985 et 1988; études non publiées financées par Dow Chemical), Stasenkova *et al.* (1973; article en russe) et Gage (1970). Ces études chez le rat ont mis en évidence:

- des modifications réversibles de l'épithélium nasal: une coloration brunâtre autour du nez des rats à partir de 50 mg/m^3 et une inflammation faible et une hyperplasie de l'épithélium à 50 et 150 mg/m^3 [Nitschke *et al.*, 1985 et 1988];

- une incontinence périnéale à 150 mg/m^3 [Nitschke *et al.*, 1985 et 1988];

- une diminution du gain de poids corporel [Nitschke *et al.*, 1988; Stasenkova *et al.*, 1973];

- des effets sur les reins et le foie: modification du poids absolus des reins et du foie (diminution à 150 mg/m^3 chez les rates dans l'étude de Nitschke *et al.*, 1988 et augmentation dans l'étude de Stasenkova *et al.*, 1973), diminution de l'acide ascorbique dans le foie et les reins et de l'excrétion de l'acide hippurique dans l'urine [Stasenkova *et al.*, 1973].

Nitschke *et al.* (1988) ont mis en évidence un NOAEC de 10 mg/m^3 sur lequel le SCOEL s'est basé pour construire sa VLEP 8h [SCOEL, 2004]. En 2008, la FDA a examiné les 2 études de Nitschke *et al.* et les a considéré robustes pour l'identification d'organes cibles potentiels mais ne les considère pas utiles pour évaluer les effets suite à une exposition par voie orale (FDA, 2008).

Par **voie orale**, l'UE-RAR 2003 et 2010 (EC, 2003; EC, 2010b) recense différents effets pour des doses très supérieures à 5 mg/kg bw/d, NOAEL ayant servi à dériver la DJT actuelle:

- une diminution du gain de poids corporel chez le rat (NTP ,1982: 2 semaines, 90 jours, 2 ans; Takahashi et Oishi, 2001; Tyl *et al.*, 1978) et la souris (NTP, 1982 et 1985b);

- une diminution poids corporel chez le rat (> 4000 ppm chez mâle et 8000 ppm chez femelles) (General electric, 1976);

- une diminution significative de gain de poids d'organes chez la souris (> 650 mg/kg bw/d) ();

- des effets hépatiques: une diminution du poids (absolu et relatif) du foie (> 466 mg/kg) chez le rat (Takahashi et Oishi 2001), une augmentation du poids relatif du foie chez le chien (270 mg/kg) (General Electric, 1976), des modifications de la taille et de l'état de nucléation des hépatocytes chez la souris (NTP, 1982; Furukawa *et al.*, 1994);

- une diminution du poids (absolu et relatif) de la glande préputiale (> 235 mg/kg) et de la prostate (> 950 mg/kg) chez le rat (Takahashi et Oishi 2001) et une augmentation du poids relatif du cerveau, des reins (femelles), des testicules à 2500 ppm chez le rat (Tyl *et al.*, 1978), une augmentation du poids absolu et relatif des reins (> 2600 mg/kg) et des ovaires (> 1300 mg/kg) chez les souris femelles (Furukawa *et al.*, 1994);

- une hypertrophie du caecum chez le rat (Tyl *et al.*, 1978; NTP 1982, 90 jours);

- des modifications hématologiques chez la souris: diminution du nombre d'érythrocytes, de l'hémoglobine (mâle) et de l'hématocrite (4800 mg/kg chez les mâles et 650 mg/kg chez les femelles) (Furukawa *et al.*, 1994).

Selon l'UE-RAR (2010) (EC, 2010b), aucune étude de toxicité chronique n'est disponible pour la voie cutanée.

Annexe 5: Effets cancérogènes

Une seule étude de cancérogénicité a été menée en 1982 par le NTP. Des rats F344 et des souris B6C3F1 âgés de 5 semaines à l'initiation du traitement ont été exposés par voie orale (administration dans la nourriture) pendant 103 semaines (25 mois). Une augmentation de l'incidence du nombre de leucémies chez les rats mâles à la dose de 148 mg/kg bw/d, et une augmentation des tumeurs au niveau des cellules de Leydig, ainsi qu'une augmentation de l'incidence des leucémies chez les rats femelles ont été observées. Cependant ces résultats perdent leur significativité lors de l'ajustement sur la mortalité due à l'espérance de vie. De plus, les tumeurs des cellules de Leydig sont reconnues être très fréquentes chez cette souche de rat âgé (environ 88%), ce modèle n'est donc pas approprié pour mettre en évidence ce type de cancer. Chez les souris, l'augmentation du nombre de leucémies n'est pas significative. L'étude présente des limites au niveau de son protocole: nombre limité d'animaux par groupe de dose, observations cliniques limitées, manque de données sur le poids des organes,… De ce fait, les résultats sont débattus: Health Canada (2008) a jugé ces données comme non convaincantes, le CFSAN'S cancer assessment committee (2009) considère que les cancers observés ne seraient pas liés à l'exposition au BPA, l'UE-RAR en 2008 conclut que le BPA ne produit pas d'augmentation significative du nombre de tumeurs dans cette étude qu'ils estiment néanmoins bien conduite (EC, 2010b).

Annexe 6: Génotoxicité

Les études de génotoxicité *in vitro* ne montrent pas d'effet mutagène ni clastogène chez des cellules de bactérie, de champignon ou de mammifère UE-RAR, 2002 (EC, 2003; EC, 2010b). Cependant, la formation d'adduits à l'ADN (Tsuisui *et al.*, 1998) ainsi que des dommages sur des cellules MCF-7 et MDA-MB-231 (Iso *et al.*, 2006) ont été reportés. Par ailleurs, un effet aneugène du BPA est décrit, avec la formation de micronoyaux en l'absence d'activation métabolique dans des cultures de cellules embryonnaires du hamster de Syrie et des cultures de cellules du hamster chinois V79, respectivement (NTP, 2007; BESC, 2003; Haighton *et al.*, 2002). Le BPA perturberait la formation de microtubules dans des cellules et des systèmes cellulaires (Pfeiffer *et al.*, 1996 et 1997).

In vivo, un grand nombre d'études normalisées de génotoxique n'ont pas montré de pouvoir mutagène et clastogène. Les études mettant en évidence un effet aneugène sont néanmoins plus controversées. En effet, Hunt *et al.* (2003) observent une augmentation de l'échec de la congression (capture des chromosomes par les fibres kinétochoriennes et orientation au niveau de la plaque équatoriale en métaphase) et une augmentation des anomalies méiotiques. Cependant, cette étude présente des faiblesses méthodologiques et les effets n'ont pas été confirmés par Pacchietrotti et al, 2008 qui ont étudié l'effet aneugène sur les cellules somatiques de souris exposées au BPA par voie orale.

Bien que l'effet aneugène reste débattu, les différents organismes, (EC, 2010b; FAO/WHO, 2010; Health Canada, 2008) concordent sur l'absence d'effet mutagène et clastogène du BPA.

Annexe 7: Tableau récapitulatif des études épidémiologiques analysées dans le cadre de ce rapport

Effets sur le sperme								
Référence **Titre de l'article**	**Type d'étude**	**Population investiguée**	**Mesure de BPA**	**Méthode analytique**	**Ajustements**	**Résultats/discussion**	**Qualité de l'étude**	**Chapitre(s) concerné(s)**
(Li *et al.*, 2011) Urine bisphenol-A (BPA) level in relation to semen quality	Étude de cohorte	Population investiguée: travailleurs N total = 218 → Effectif suffisant	Urinaire (BPA libre et conjugué)	HPLC	Age: oui Sexe: s/o Médicaments: maladies chroniques Tabac: oui IMC: oui Autres contaminants: oui (exposition antérieure à des métaux lourds et autres substances chimiques Autres: alcool, créatinine, éducation, histoire professionnelle, état marital, âge des 1ères relations sexuelles, lieu de l'étude	Résultats: Corrélation statistiquement significative entre les concentrations urinaires élevées en BPA et la diminution de la qualité du sperme (concentration, motilité, vitalité, numération spermatique), Pas de corrélation entre BPA urinaire et volume spermatique et morphologie ou volume Dans un sous-échantillon (n=87) de sujets non exposés au travail, une corrélation négative entre le BPA et la concentration et la numération spermatique Commentaires: Un certain nombre de sujets ont été perdus de vue.	Études de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.2 Effets sur le système reproducteur mâle
(Meeker *et al.*, 2010b) Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic	Eude transversale nichée dans une étude (2000-2004)	Population investiguée: hommes inféconds (18-55 ans) N = 190 hommes	Urinaire (BPA libre et conjugué) (<36,4 ng/mL)	HPLC/MS/MS	Age: oui Sexe: s/o Médicaments: s/o Tabac: oui IMC: oui Autres contaminants: Non Autres: gravité spécifique,	Résultats: association entre une diminution de la concentration spermatique et pourcentage de formes typiques, une altération des caractéristiques du sperme, une augmentation de la fragmentation de l'ADN nucléaire du spermatozoïde, et la concentration urinaire en BPA Commentaires:	Études de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.2 Effets sur le système reproducteur mâle

						Un seul échantillon d'urine pour la moitié des hommes investigués et un seul prélèvement de sperme pour tous les participants. Il est important de noter que certains paramètres de l'analyse du sperme n'ont pas été réalisés en respectant les recommandations de l'OMS (OMS, 1999).		
(Mendiola *et al.*, 2010) Are Environmental Levels of Bisphenol A Associated with Reproductive Function in Fertile Men?	Étude transversale	Population investiguée: hommes fertiles (hommes ayant déjà eu au moins un enfant, partenaires de femmes enceintes) N = 375 hommes → Effectif suffisant	Urinaire - BPA libre et conjugué) (<0,4 -6,5 ng/mL)	HPLC/MS/MS	Age: oui Sexe: s/o Médicaments: non Tabac: oui IMC: oui Autres contaminants: Non Autres: ethnie, créatinine urinaire et moment de prélèvement	Résultats: - Relation inverse entre concentration urinaire de BPA et l'index de testostérone libre (FAI) et le rapport FAI/LH - Association positive significative entre les concentrations urinaires en BPA et SHBG - Pas de association entre concentrations urinaires de BPA et caractéristiques spermatiques Ces résultats peuvent refléter un effets oestrogénomimétiques du BPA. Commentaires: - population sélectionnée = hommes fertiles seulement	Études de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.2 Effets sur le système reproducteur mâle

Effets sur les hormones sexuelles								
Référence **Titre de l'article**	**Type d'étude**	**Population investiguée**	**Mesure de BPA**	**Méthode analytique**	**Ajustements**	**Résultats/discussion**	**Qualité de l'étude**	**Chapitre(s) concerné(s)**
(Cha *et al.*, 2008) Influence of occupational exposure to bisphenol A on the sex hormones of male epoxy resin painters	Étude cas témoins	Population investiguée: population professionnelle (homme) N = 25 cas (peintres résine époxy) vs 25 témoins (non peintres) →Effectif faible	Urinaire	HPLC	Age: oui Sexe: s/o Médicaments: non Tabac: oui IMC: non Autres contaminants: Non	Résultats: Augmentation significative de LH et FSH Diminution significative de testostérone	Études non retenues car présentant des limites méthodologiques majeures Cette étude a été écartée compte tenu des faiblesses méthodologiques suivantes: - faible effectif, - exposition potentielle à plusieurs solvants organiques, - observations non extrapolables à la population générale, - trop grande proportion de fumeurs et de consommateurs de boisson alcoolisée.	6.1 Apport des études épidémiologiques
(Galloway *et al.*, 2010) Daily Bisphenol A Excretion and Associations with Sex Hormone Concentrations: Results from the InCHIANTI Adult Population Study	Étude transversale nichée dans l'étude italienne prospective InCHIANTI	Population investiguée: population générale adulte (20-74 ans) N = 715 → Effectif suffisant	Urinaire (BPA libre et conjugué)	HPLC/MS/MS	Age: Oui Sexe: oui Médicaments: non Tabac: oui IMC: oui Autres contaminants: Non Autres: nombre d'année d'étude, circonférence abdominale, créatinine	Résultats: Chez les hommes (n=307): association significative entre excrétion BPA journalière et les concentrations en testostérone totale (fortement significative après ajustement). Chez les femmes, pas d'association entre BPA et les concentrations en testostérone totale ou d'œstradiol. Chez tous les sujets: association significative entre l'excrétion journalière de BPA et les mesures d'adiposité (BMI et circonférence abdominale) Commentaires:	Études de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.2 Effets sur le système reproducteur mâle

						- Urine de 24h dans les bouteilles en plastiques. - Pas de mesure de la FSH.		
(Meeker *et al.*, 2010a) Urinary Bisphenol A Concentrations in Relation to Serum Thyroid and Reproductive Hormone Levels in Men from an Infertility Clinic	Étude transversale	Population investiguée: hommes consultant pour des problèmes de fertilité N = 167 cas vs 190 témoins	Urinaire (BPA libre et conjugué) (0,4 à 36,4 ng/mL)	HPLC/MS/MS	Age: Oui Sexe: s/o Médicaments: s/o Tabac: oui IMC: oui Autres contaminants: Non Autres: saison, moment de prélèvement, gravité spécifique	Résultats: - Association positive entre concentrations urinaires en BPA et la FSH et le ratio FSH/inhibine B - Association négative entre concentrations urinaires en BPA et le ratio œstradiol/testostérone, TSH, inhibine B et index de testostérone libre et œstradiol. Commentaires: Des réserves peuvent être émises concernant cette étude: - elle porte sur une population particulière d'hommes consultant pour des problèmes d'infertilité dans le couple.	Études de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.2 Effets sur le système reproducteur mâle 6.6 Effets sur la thyroïde
(Mendiola *et al.*, 2010) Are Environmental Levels of Bisphenol A Associated with Reproductive Function in Fertile Men?	Étude transversale	Population investiguée: hommes fertiles (hommes ayant déjà eu au moins un enfant, partenaires de femmes enceintes) N = 375 hommes → Effectif suffisant	Urinaire - BPA libre et conjugué) (<0,4 - 6,5 ng/mL)	HPLC/MS/MS	Age: Oui Sexe: s/o Médicaments: non Tabac: oui IMC: oui Autres contaminants: Non Autres: ethnie, créatinine urinaire et moment de prélèvement	Résultats: - Relation inverse entre concentration urinaire de BPA et l'index de testostérone libre (FAI) et le rapport FAI/LH - Association positive significative entre les concentrations urinaires en BPA et SHBG - Pas d'association entre concentrations urinaires de BPA et caractéristiques spermatiques Ces résultats peuvent refléter un effets oestrogénomimétiques du BPA. Commentaires: - population sélectionnée = hommes fertiles seulement	Études de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.2 Effets sur le système reproducteur mâle
(Takeuchi *et al.*, 2004) Positive	Étude transversale	Population investiguée: population	Sérique	ELISA (validation de la méthode de	Age: non Sexe: s/o	Résultats: corrélation entre concentrations plasmatiques en testostérone	Études non retenues car présentant des limites méthodologiques majeures	6.1 Apport des études épidémiologiques

relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction		générale: femmes N = 7 patientes souffrant d'hyperprolactiné mie, 21 avec une aménorrhée hypothalamique, 19 avec SOPK (13 non obèses et 6 obèses) vs 26 témoins (7 obèses ou 19 non obèses) -> Effectif faible		dosage par HPLC)	Médicaments: s/o Tabac: non IMC: non Autres contaminants: non	(libre et totale) et BPA d'une part, et concentration en BPA et indice de masse corporelle d'autre part: les taux sont significativement augmentés chez les femmes ayant un SOPK (obèses ou non) et les obèses sans trouble de l'ovulation. Commentaires: Les résultats restent difficilement interprétables en l'état, à cause de l'imprécision du plan d'échantillonnage, du manque d'information sur les critères d'inclusion et de l'absence de prise en compte dans les résultats des pathologies des témoins.	Cette étude a été écartée compte tenu des faiblesses méthodologiques suivantes: - faible effectifs, - analyse statistique peu détaillée, - la comparaison finale est faite par rapport aux femmes non obèses, sans trouble du cycle (considérées comme témoins) - absence d'ajustement à des facteurs confondants, - les dosages plasmatiques du BPA par la technique ELISA dans le plasma (limite mineure).	6.5 effets sur le métabolisme et le système cardiovasculaire

Effets sur la fonction sexuelle								
Référence **Titre de l'article**	**Type d'étude**	**Population investiguée**	**Mesure de BPA**	**Méthode analytique**	**Ajustements**	**Résultats/discussion**	**Qualité de l'étude**	**Chapitre(s) concerné(s)**
(Li *et al.*, 2010a) Occupational exposure to bisphenol A and the risk of self-reported male sexual dysfunction	Étude de cohorte (2004-2008)	Population investiguée: population professionnelle (hommes) N = 230 exposés (1 usine de production de BPA et 3 usines de production de résines) vs 404 témoins non exposés issus de «plusieurs » usines (matériaux de construction, fournisseurs d'eau, textiles, électronique, commerces, …) du même secteur géographique que usine de production de BPA (284 volontaires et 120 maris de femmes travaillant dans ces usines)	Urinaire Air: Protocole comporte des mesures de la teneur de BPA dans l'atmosphère sur les lieux de travail, l'historique des expositions, le monitoring individuel, un inventaire des équipements de protection, des mesures d'hygiène ainsi que le relevé des expositions à d'autres produits. Volontaires répartis en sous-groupes en fonction critères ci-dessus, Mesures de BPA réalisées pour chacun au poste de travail et à défaut, valeur moyenne de l'atelier retenue. Mesure d'exposition exprimée en valeur cumulée.	HPLC (air) Méthode non précisée pour les dosages urinaires	Age: oui Sexe: s/o Médicaments: pathologies chroniques Tabac: oui IMC: non Autres contaminants: co-exposition à des composés chimiques Autres: éducation, état marital, histoire professionnelle	Résultats: risque significativement augmenté de troubles sexuels (fonction érectile, fonction orgasmique, désir sexuel, satisfaction globale vie sexuelle) Commentaires: Imprécision de la mesure de l'effet: utilisation d'un questionnaire pour évaluer le risque de troubles érectiles entraîne un risque de surestimation intégrant l'International Index of Erectile Function Inventory renseignés par les enquêtés (analyse par odds ratios avec $IC_{95\%}$)	Étude de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.2 Effets sur le système reproducteur mâle

(Li *et al.*, 2010b) Relationship between urine BPA level and declining men sexual function.	Étude transversale	Population investiguée: population professionnelle (hommes) (fabrique de résine époxy) N total = 427 (173 exposés vs 254 non exposés) → Effectif suffisant	Urinaire (BPA libre et conjugué)	HPLC/FD	Age: non Sexe: s/o Médicaments: pathologies chroniques Tabac: oui IMC: non Autres contaminants: oui (autres produits chimiques ou métaux lourds) Autres: caractéristiques démographiques, alcool, histoire professionnelle	Résultats: Corrélation entre mesures BPA urinaire et diminution de la fonction sexuelle. Relation négative entre concentrations urinaires en BPA les plus élevées et la diminution du désir sexuel, une augmentation des difficultés pour avoir une érection, une diminution de la force d'éjaculation, une diminution globale de satisfaction à vie sexuelle Commentaires: L'écart très important dans la fréquence des troubles de la vie sexuelle entre les sujets exposés et non exposés mérite d'être noté, mais il pourrait aussi être attribué à un biais comme par exemple, les entretiens ne se faisaient probablement pas à l'aveugle du statut du travailleur vis-à-vis de l'exposition ou encore le choix des valeurs seuils des événements de la vie sexuelle estimés à partir d'une échelle non explicitée.	Étude de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.2 Effets sur le système reproducteur mâle

Fausse-couches/avortements spontanés								
Référence **Titre de l'article**	**Type d'étude**	**Population investiguée**	**Mesure de BPA**	**Méthode analytique**	**Ajustements**	**Résultats/discussion**	**Qualité de l'étude**	**Chapitre(s) concerné(s)**
(Sugiura-Ogasawara *et al.*, 2005) Exposure to bisphenol A is associated with recurrent miscarriage	Étude cas témoins	Population investiguée: population générale: femmes ayant eu un antécédent d'au moins 3 fausses au 1er trimestre N = 45 cas vs /32 témoins (médecins, infirmières, secrétaires dans l'école de médecine) → Effectif faible	Sérique	ELISA	Age: non Sexe: non Médicaments: non Tabac: non IMC: non Autres contaminants: Non	Résultats: - association positive avec Ac anti-nucléaire mais pas avec les autres paramètres - taux sérique de BPA supérieur chez les femmes ayant un antécédent de 3 fausses couches.	Études non retenues car présentant des limites méthodologiques majeures Cette étude a été écartée en raison des faiblesses méthodologiques suivantes: - faibles effectifs, - choix du groupe contrôle discutable (aucune preuve de tentative de grossesse dans ce groupe), - liste limitée de facteurs confondants à considérer. - méthode analytique (ELISA) qui ne discrimine pas les différentes formes de BPA, - autres facteurs confondants de fausse-couche, - sur le plan de l'analyse des résultats (niveaux sériques médians identiques dans les deux groupes) - sur les outils statistiques choisis	6.1 Apport des études épidémiologiques 6.3 Effets sur le système reproducteur femelle

Puberté et développement mammaire								
Référence **Titre de l'article**	**Type d'étude**	**Population investiguée**	**Mesure de BPA**	**Méthode analytique**	**Ajustements**	**Résultats/discussion**	**Qualité de l'étude**	**Chapitre(s) concerné(s)**
(Wolff *et al.*, 2008a) Environmental exposures and puberty in inner-city girls	Étude transversale	Population investiguée: fille de 9 ans N = 192 => 186 au final → Effectif OK	Urinaire	Non précisée	Age: oui Sexe: s/o Médicaments: oui Tabac: oui IMC: oui Autres contaminants: oui Autres: race, ethnie, créatinine urinaire, taille, complété par l'ensemble des prédicteurs identifiés par des comparaisons significatives au seuil 20 %.	Résultats: Pas de modification de l'âge de survenue de la puberté chez les filles. Commentaires: la puissance de l'étude n'est pas connue et la taille de l'étude n'est pas si grande	Études de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.3 Effets sur le système reproducteur femelle
(Wolff *et al.*, 2010) Investigation of Relationships between Urinary Biomarkers of Phytoestrogens, Phthalates, and Phenols and Pubertal Stages in Girls	Étude de cohorte prospective	Population investiguée: filles entre 6 et 8 ans N = 1151 → Effectif excellent	Urinaire	Non précisée	Age: oui Sexe: s/o Médicaments: oui (en particulier, « endocrine medical conditions excluded ») Tabac: non IMC: oui Autres contaminants: oui Autres: race/ethnie (pour les patientes de Mount Sinai School of Medicine)	Résultats: Pas de modification de l'âge de survenue de la puberté chez les filles.	Études de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.3 Effets sur le système reproducteur femelle

Effets sur la prématurité								
Référence **Titre de l'article**	**Type d'étude**	**Population investiguée**	**Mesure de BPA**	**Méthode analytique**	**Ajustements**	**Résultats/discussion**	**Qualité de l'étude**	**Chapitre(s) concerné(s)**
(Cantonwine *et al.*, 2010) Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study	Étude mexicaine, rétrospective de type cas-témoins nichée dans une étude de cohorte	Population investiguée: femmes enceintes N = 30 cas (accouchement < 37 semaines de grossesse) vs 30 témoins (accouchement >38 semaines de grossesse) → effectif limité	Urinaire	HPLC/MS/MS	Age: oui Sexe: s/o Médicaments: non Tabac: s/o (femmes non fumeuses mais tabagisme passif non pris en compte) IMC: oui Autres contaminants: oui (métabolites urinaires des phtalates) Autres: éducation maternelle, statut marital, sexe des enfants	Résultats: le groupe « prématuré » (accouchement <37 semaines de grossesse, n=12) a environ 2 fois plus de BPA que les témoins Commentaires: - Prématurité basée uniquement sur âge gestationnel à l'accouchement, pas de mesures échographiques. Compte tenu de l'hétérogénéité de ce groupe (césariennes électives, accouchement spontané, pré éclampsie, etc.), il est difficile de cerner l'effet hypothétique; - Pas de mesures de plomb et d'autres contaminants; - Une seule mesure du BPA (un seul spot urinaire), pas de mesures répétées, - Absence d'information sur le tabagisme passif et d'autres facteurs de risque de prématurité (histoire obstétricale)	Étude présentant des limites méthodologiques majeures Cette étude n'a pas été retenue en raison des limites suivantes: - tabagisme passif non pris en compte, - autres facteurs de risque de prématurité non pris en compte (histoire obstétricale) - Mode d'accouchement non précisé (césarienne ? naissances spontanées ?) - effectif trop faible pour avoir une puissance statistique suffisante pour déterminer l'effet d'une exposition environnementale à faible dose. - En effet, cet effectif est à peine suffisant à l'application des tests statistiques paramétriques tels que réalisé par les auteurs.	6.1 Apport des études épidémiologiques 6.3 Effets sur le système reproducteur femelle

Effets ovariens								
Référence **Titre de l'article**	**Type d'étude**	**Population investiguée**	**Mesure de BPA**	**Méthode analytique**	**Ajustements**	**Résultats/discussion**	**Qualité de l'étude**	**Chapitre(s) concerné(s)**
(Mok-Lin *et al.*, 2010) Urinary bisphenol A concentrations and ovarian response among women undergoing IVF	Étude de cohorte prospective	Population investiguée: femmes suivant un protocole de stimulation ovarienne dans le cadre d'une FIV (21-44 ans) N = 84 femmes (112 cycles de FIV) → Effectif suffisant	Urinaire (BPA libre et conjugué)	HPLC/MS/MS	Age: oui Sexe: s/o Médicaments: non Tabac: non IMC: oui Autres contaminants: non Autres: gravité spécifique, FSH à jour 3	Résultats: la concentration urinaire en BPA est associée: - à la diminution du nombre d'ovocytes récupérés après stimulation - à la diminution des pics d'œstradiol sérique BPA détecté de manière majoritaire chez les femmes ayant recours à une FIV Commentaires: - l'urine pour le BPA a été prélevée 2 fois, une moyenne géométrique a été calculée pour chaque sujet. - La concentration de BPA urinaire retrouvée reflète une exposition au moment du prélèvement et non durant la période de maturation folliculaire, plusieurs mois auparavant. - Il est délicat d'extrapoler à la population générale des résultats observés chez un échantillon de femmes infertiles consultant pour une FIV.	Étude de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.3 Effets sur le système reproducteur femelle
(Cobellis *et al.*, 2009) Measurement of Bisphenol A and Bisphenol B Levels in Human Blood Sera From Healthy and Endometriotic Women	Étude chez l'Homme	Population investiguée: femmes fertiles consultants dans un service de gynécologie obstétrique pour des douleurs pelviennes chroniques, des dysménorrhées	Sérique Rq: mesure du Bisphenol B également	HPLC/fluorescence	Age: non Sexe: s/o Médicaments: non Tabac: non IMC: non Autres contaminants: non	Résultats: Absence de bisphenols chez les témoins BPA chez 30 sérums (51,7%) Présence d'au moins un des 2 bisphenol a été vérifiée chez les femmes porteuses d'endométriose (63,8%)	Études non retenues car présentant des limites méthodologiques majeures Cette étude est écartée en raison: - du faible effectif (11 témoins seulement), - de la description très limitée	6.1 Apport des études épidémiologiques 6.3 Effets sur le système reproducteur femelle

		ou kystes ovariens N = 58 cas (endométriose) vs 11 témoins (même population mais sans endométriose) → Effectif des témoins faible				Commentaires: Cette étude est plutôt concentrée sur les aspects analytiques, en particulier sur les techniques de dosage mises en œuvre pour analyser le BPA sérique.	des résultats - analyse statistique descriptive simple sans ajustement	
(Fujimoto *et al.*, 2011) Serum unconjugated bisphenol A concentrations in women may adversely influence oocyte quality during in vitro fertilization	Étude de cohorte	Population investiguée: couple en cours de FIV (femmes infertiles ayant une stimulation ovarienne et leur partenaire homme) N = 58 femmes et 37 hommes	Sérique (BPA non conjugué)	HPLC/ESA coularray 5600 detector	Age: oui Sexe: non Médicaments: non Tabac: oui IMC: non Autres contaminants: non Autres: ethnie	Résultats: Association significative entre la concentration sérique en BPA des femmes et la diminution du taux de fertilisation des ovocytes Commentaires: Les patients ayant eu recours aux deux procédures de fécondation in vitro (sans et avec micro-injection de spermatozoïdes) ont été considérés comme un seul groupe. Or, la qualité des gamètes mâles est différente dans ces deux groupes.	Études de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.3 Effets sur le système reproducteur femelle
(Hiroi *et al.*, 2004) Differences in serum bisphenol a concentrations in premenopausal normal women and women with endometrial hyperplasia	Étude transversale	Population investiguée: femmes N = 19 patientes présentant une hyperplasie endométriale (2 groupe en fonction de la complexité: 10 avec hyperplasie "simple" et 9 avec hyperplasie "complexe") et 7 avec un carcinome endométriale vs 11 témoins →	Sérique	ELISA	Age: non Sexe: s/o Médicaments: non Tabac: non IMC: non Autres contaminants: non	Résultats: Corrélation inverse à celle attendue: les témoins ont plus de BPA que les cas (non significatif). Concentration sérique BPA = 2,9 ng/mL chez les femmes avec hyperplasie simple vs 1,4 ng/mL chez femmes avec hyperplasie complexe. Même relation inverse observée chez femmes porteuses d'un carcinome endométriale	Études non retenues car présentant des limites méthodologiques majeures Cette étude n'a pas été retenue en raison de limites suivantes: - effectif trop restreint, - non prise en compte de facteurs confondants.	6.1 Apport des études épidémiologiques 6.3 Effets sur le système reproducteur femelle

		Effectif limité						
(Itoh *et al.*, 2007) Urinary bisphenol-A concentration in infertile Japanese women and its association with endometriosis: A cross-sectional study	Étude transversale	Population investiguée: Patientes se plaignant majoritairement d'infertilité (endométriose, 24-43 ans) N = 140 -> Effectif suffisant	Urinaire (BPA total)	HPLC/MS	Age: non Sexe: s/o Médicaments: non Tabac: non IMC: non Autres contaminants: non Autres: créatinine	Résultats: Pas d'association significative entre le taux de BPA urinaire (non ajusté et ajusté à la créatinine) et le stade de l'endométriose Commentaires: - le dosage du BPA urinaire ne reflète pas une imprégnation au long cours mais une exposition récente. - pas de groupe témoin véritable dépourvu de pathologie, - prélèvement urinaire stocké dans un tube en plastique pendant 5 ans au congélateur	Étude de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.3 Effets sur le système reproducteur femelle
(Kandaraki *et al.*, 2011) Endocrine Disruptors and Polycystic Ovary Syndrome (PCOS): Elevated Serum Levels of Bisphenol A in Women with PCOS	Étude transversale, appariée sur l'âge et l'IMC	Population investiguée: femmes N = 71 cas (femmes avec SOPK) vs 100 témoins → Effectif suffisant	Sérique	ELISA	Age: oui (via l'appariement) Sexe: s/o Médicaments: s/o Tabac: s/o IMC: oui (via l'appariement) Autres contaminants: non Autres: via une analyse multivariée (paramètres anthropométriques, hormonaux et métaboliques)	Résultats: - Concentrations sériques en BPA significativement plus importantes chez les femmes atteintes de SOPK (obèses ou non) comparées aux femmes témoins normales. - Chez les femmes atteintes de SOPK (obèses ou non): taux de testostérone et ratio LH/FSH significativement augmentés alors que niveaux de SHBG étaient plus faibles par rapport aux témoins. - Concentrations en BPA sont significativement corrélées aux concentrations en testostérone et androstènedione et à une insulino-résistance. - Concentrations en BPA sont significativement corrélées avec l'existence de SOPK.	Études non retenues car présentant des limites méthodologiques majeures Cette étude a été écartée en raison de: - méthode analytique (ELISA) qui ne discrimine pas les différentes formes de BPA.	6.1 Apport des études épidémiologiques 6.3 Effets sur le système reproducteur femelle
(Takeuchi *et al.*, 2004) Positive	Étude transversale	Population investiguée: population	Sérique	ELISA (validation de la méthode de	Age: non Sexe: s/o	Résultats: corrélation entre concentrations plasmatiques en testostérone	Études non retenues car présentant des limites méthodologiques majeures	6.1 Apport des études épidémiologiques

relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction		générale: femmes N = 7 patientes souffrant d'hyperprolactiné mie, 21 avec une aménorrhée hypothalamique, 19 avec SOPK (13 non obèses et 6 obèses) vs 26 témoins (7 obèses ou 19 non obèses) -> Effectif faible		dosage par HPLC)	Médicaments: s/o Tabac: non IMC: non Autres contaminants: non	(libre et totale) et BPA d'une part, et concentration en BPA et indice de masse corporelle d'autre part: les taux sont significativement augmentés chez les femmes ayant un SOPK (obèses ou non) et les obèses sans trouble de l'ovulation. Commentaires: Les résultats restent difficilement interprétables en l'état, à cause de l'imprécision du plan d'échantillonnage, du manque d'information sur les critères d'inclusion et de l'absence de prise en compte dans les résultats des pathologies des témoins.	Cette étude a été écartée compte tenu des faiblesses méthodologiques suivantes: - faible effectifs, - analyse statistique peu détaillée, - la comparaison finale est faite par rapport aux femmes non obèses, sans trouble du cycle (considérées comme témoins) - absence d'ajustement à des facteurs confondants, - les dosages plasmatiques du BPA par la technique ELISA dans le plasma (limite mineure).	6.3 Effets sur le système reproducteur femelle

Effets sur le comportement des enfants								
Référence **Titre de l'article**	**Type d'étude**	**Population investiguée**	**Mesure de BPA**	**Méthode analytique**	**Ajustements**	**Résultats/discussion**	**Qualité de l'étude**	**Chapitre(s) concerné(s)**
(Braun *et al.*, 2009) Prenatal Bisphenol A Exposure and Early Childhood Behavior	Étude de cohorte prospective	Population investiguée: Mères et leurs enfants de 2 ans (incluent dans le programme Health Outcomes and Measures of the Environment Study; utilisation d'une bio banque existante, recrutement en 2003) N = 249 mères et leurs enfants de 2 ans -> Effectif suffisant	Urinaire (chez les mères à 16 et 26 semaines de gestation et à naissance) BPA libre et conjugué	HPLC/MS/MS	Age: oui (sur l'âge de la mère) Sexe: s/o Médicaments: s/o Tabac: oui IMC: s/o Autres contaminants: oui	Résultats: Association positive avec un comportement d'externalisation Commentaires: - pas de fiabilité biologique - utilisation d'une biobanque existante (recrutement en 2003) – les échantillons ont été conservés pendant 4-5 ans, *questionnaire – pas de mesures directes de BPA urinaire chez l'enfant, L'étude a fait objet d'une analyse très critique (Human Data on Bisphenol A and Neurodevelopment doi:10.1289/ehp.0901610) dont les remarques sont bien justifiées.	Étude de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.4 Effets sur le cerveau et le comportement
(Miodovnik *et al.*, 2011) Endocrine disruptors and childhood social impairment	Étude de cohorte prospective	Population investiguée: enfants de 7 à 9 ans N = 137 enfants	Urinaire (Chez les 404 mères entre la 25ème et la 40ème semaine de grossesse)	Non précisé	Age: oui (âge maternelle et âge exact de l'enfant à l'examen) Sexe: oui (sexe des enfants) Médicaments: non Tabac: non IMC: non Autres	Résultats: Aucune association significative n'a été retrouvée entre les taux urinaires de BPA et des troubles sociaux. Le BPA était corrélé positivement avec la sévérité des troubles sociaux (« Social Responsiveness Scale »), mais cette relation était non statistiquement significative.	Étude de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.4 Effets sur le cerveau et le comportement

					contaminants: non Autres: créatinine urinaire des enfants, statut marital à la date de suivi, niveau de formation des mères, race, QI des mères et des enfants			

Effets sur le métabolisme /système cardiovasculaire								
Référence **Titre de l'article**	**Type d'étude**	**Population investiguée**	**Mesure de BPA**	**Méthode analytique**	**Ajustements**	**Résultats/discussion**	**Qualité de l'étude**	**Chapitre(s) concerné(s)**
(Hong *et al.*, 2009) Community level exposure to chemicals and oxidative stress in adult population	Étude transversale	Population investiguée: population générale adulte N = 960 → Effectif excellent	Urinaire	HPLC/MS	Age: oui Sexe: oui Médicaments: non Tabac: oui IMC: non Autres contaminants: oui Autres: activité physique, histoire médicale et professionnelle, alcool	Résultats: Relation positive significative entre concentrations urinaires de contaminants chimiques, notamment phtalates et BPA, et marqueurs de stress oxydatif dans une analyse de régression simple (non significatif si analyse de régression multiple pour le BPA) Sujets avec les teneurs élevées en BPA tendaient une hyperglycémie a jeun mais pas associé avec les indices de résistance à l'insuline	Étude de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.5 Effets sur le métabolisme et le système cardio-vasculaire
(Lang *et al.*, 2008) Association of Urinary Bisphenol A Concentration With Medical Disorders and Laboratory Abnormalities in Adults	Étude transversale nichée dans l'étude NHANES (2003-2004)	Population investiguée: population générale adulte (18-74 ans) N = 1455 adultes -> Effectif suffisant	Urinaire (BPA libre et conjugué)	HPLC/MS	Age: oui Sexe: oui Médicaments: non Tabac: oui IMC: oui Autres contaminants: non Autres: créatinine urinaire, ethnie/race, éducation, ressources financières, tour de taille	Résultats: association positive entre les concentrations urinaires les plus élevées en BPA (5 et 13 ng/mL) et les maladies cardiovasculaires, le diabète et les niveaux sanguins des enzymes hépatiques Commentaires: Étude qui mérite une attention particulière car: - étude puissante avec un beau design, - les associations sont très robustes, - la taille d'échantillon est importante, - basée sur des cohortes nationales américaines, Cependant, le fait de prendre des médicaments n'a pas été pris en compte	Étude de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.5 Effets sur le métabolisme et le système cardio-vasculaire

						et l'exposition contemporaine n'est pas forcément représentative de l'exposition antérieure qui est corrélée avec l'effet observé, Remarque: Les études de Melzer et Lang ont été réalisées à 2 ans d'intervalle avec le même type de protocole.		
(Melzer *et al.*, 2010) Association of Urinary Bisphenol A Concentration with Heart Disease: Evidence from NHANES 2003/06	Étude transversale nichée dans l'étude NHANES (2003-2006)	Population investiguée: population générale adulte (18-74 ans) N = 1455 (2003/04) et 1493 (2005/06) -> Effectif suffisant	Urinaire (BPA libre et conjugué)	HPLC/MS	Age: oui Sexe: oui Médicaments: non Tabac: oui IMC: oui Autres contaminants: non Autres: créatinine urinaire, ethnie/race, éducation, ressources financières, tour de taille	Résultats: - En 2005/06: association significative entre les concentrations urinaires en BPA les plus élevées et les maladies coronariennes. Pas association entre les concentrations urinaires en BPA et le diabète. - En 2003/06: association significative entre les concentrations urinaires en BPA les plus élevées et les pathologies cardiaques, le diabète, phosphatase alcaline et lactate deshydrogénase. Commentaires: Étude qui mérite une attention particulière car: - bon design et bonne puissance (80 % pour la population des 2003/2004 et 74% pour la population 2005/2006) - les associations sont robustes, - la taille d'échantillon est importante, - basée sur des cohortes nationales américaines, Cependant, le fait de prendre des médicaments n'a pas été pris en compte et l'exposition contemporaine n'est pas forcément représentative de l'exposition antérieure qui est corrélée avec l'effet	Étude de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.5 Effets sur le métabolisme et le système cardio-vasculaire

						observé. Remarque: Les études de Melzer *et al.* et Lang *et al.*ont été réalisées à 2 ans d'intervalle avec le même type de protocole.		
(Takeuchi *et al.*, 2004) Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction	Étude transversale	Population investiguée: population générale: femmes N = 7 patientes souffrant d'hyperprolactinémie, 21 avec une amenorhée hypothalamique, 19 avec SOPK (13 non obèses et 6 obèses) vs 26 témoins (7obése ou 19 non obèses) -> Effectif faible	Sérique	ELISA (validation de la méthode de dosage par HPLC)	Age: non Sexe: s/o Médicaments: s/o Tabac: non IMC: non Autres contaminants: non	Résultats: corrélation entre concentrations plasmatiques en testostérone (libre et totale) et BPA d'une part, et concentration en BPA et indice de masse corporelle d'autre part: les taux sont significativement augmentés chez les femmes ayant un SOPK (obèses ou non) et les obèses sans trouble de l'ovulation. Commentaires: Les résultats restent difficilement interprétables en l'état, à cause de l'imprécision du plan d'échantillonnage, du manque d'information sur les critères d'inclusion et de l'absence de prise en compte dans les résultats des pathologies des témoins.	Études non retenues car présentant des limites méthodologiques majeures Cette étude a été écartée compte tenu des faiblesses méthodologiques suivantes: - faible effectifs, - analyse statistique peu détaillée, - la comparaison finale est faite par rapport aux femmes non obèses, sans trouble du cycle (considérées comme témoins) - absence d'ajustement à des facteurs confondants, - les dosages plasmatiques du BPA par la technique ELISA dans le plasma (limite mineure).	6.1 Apport des études épidémiologiques 6.3 Effets sur le système reproducteur femelle 6.5 Effets sur le métabolisme et le système cardio-vasculaire (

Effets sur le poids à la naissance								
Référence **Titre de l'article**	**Type d'étude**	**Population investiguée**	**Mesure de BPA**	**Méthode analytique**	**Ajustements**	**Résultats/discussion**	**Qualité de l'étude**	**Chapitre(s) concerné(s)**
(Padmanabhan *et al.*, 2008) Maternal bisphenol-A levels at delivery: a looming problem?	Étude transversale	Population investiguée: population générale: femme au moment de l'accouchement N = 40 femmes enceintes → Effectif faible	Plasmatique (chez les mères) (libre)	HPLC/ESI-MS/MS	Age: non Sexe: s/o Médicaments: non Tabac: non IMC: non Autres contaminants: non Autres: non	Résultats: Pas d'association entre les concentrations plasmatiques de BPA et la durée de gestation et le poids à la naissance Commentaires: - Un seul dosage de BPA réalisé à la naissance et non en début ou milieu de grossesse	Études non retenues car présentant des limites méthodologiques majeures Cette étude a été écartée en raison des faiblesses méthodologiques suivantes: - faible effectif, - pas d'ajustement sur les facteurs de confusion, - pas mesure de BPA conjugué.	6.1 Apport des études épidémiologiques 6.5 Effets sur le métabolisme et le système cardio-vasculaire
(Wolff *et al.*, 2008b) Prenatal Phenol and Phthalate Exposures and Birth Outcomes	Étude prospective	Population investiguée: population générale (femme) N = 367 → Effectif Ok	Urinaire	HPLC	Age: oui (âge gestationnel) Sexe: oui (des enfants) Médicaments: s/o Tabac: oui (pendant la grossesse) IMC: oui (pré-gestationnel) Autres contaminants: oui Autres: créatinine, race, éducation des mères	Résultats: aucune association significative entre le BPA et poids à la naissance, taille de bébé, tour de tête et l'âge gestationnel Commentaires: - Un seul dosage réalisé; - Faible taux plasmatique de BPA; - Pas d'association entre les concentrations plasmatiques de BPA et effets sur les nouveau-nés	Études de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.5 Effets sur le métabolisme et le système cardio-vasculaire

Effets sur les hormones thyroïdiennes								
Référence **Titre de l'article**	**Type d'étude**	**Population investiguée**	**Mesure de BPA**	**Méthode analytique**	**Ajustements**	**Résultats/discussion**	**Qualité de l'étude**	**Chapitre(s) concerné(s)**
(Meeker *et al.*, 2010a) Urinary Bisphenol A Concentration in Relation to Serum Thyroid and Reproductive Hormone Levels in Men from an Infertility Clinic	Étude transversale	Population investiguée: hommes consultant pour des problèmes de fertilité N = 167 cas vs 190 témoins	Urinaire (BPA libre et conjugué) (0,4 à 36,4 ng/mL)	HPLC/MS/MS	Age: oui Sexe: s/o Médicaments: s/o Tabac: oui IMC: oui Autres contaminants: Non Autres: saison, moment de prélèvement, gravité spécifique	Résultats: - Association positive entre concentrations urinaires en BPA et la FSH et le ratio FSH/inhibine B - Association négative entre concentrations urinaires en BPA et le ratio œstradiol/testostérone, TSH, inhibine B et index de testostérone libre et œstradiol. Commentaires: Au moins 2 prises urinaires voire 4 fois.	Études non retenues car présentant des limites méthodologiques majeures Cette étude a été écartée pour le raisons suivantes: - elle porte sur une population particulière d'hommes consultant pour des problèmes d'infertilité dans le couple. - les aspects thyroïdes apparaissent comme « opportunistes », l'étude ayant été faite par rapport à une problématique de fertilité et le protocole ne prend pas en compte des particularités propres à l'exploration de la fonction thyroïdienne. - le degré de signification de cette corrélation dépend du nombre de prélèvements urinaires inclus dans le calcul de la moyenne géométrique (de 1 à 3 pour chaque individu). Or, seulement 75 patients ont fait l'objet de prélèvements répétés. Si l'analyse porte sur la corrélation entre TSH et la seule mesure de BPA urinaire effectuée le jour du prélèvement sur l'ensemble des patients (n=167), ou entre TSH au premier prélèvement et la moyenne géométrique des concentrations en BPA sur les 3 prélèvements, limitée aux 75 patients ayant eu des prélèvements multiples, aucune corrélation significative ne peut être mise en évidence.	6.1 Apport des études épidémiologiques 6.2 Effets sur le système reproducteur mâle 6.6 Effets sur la thyroïde

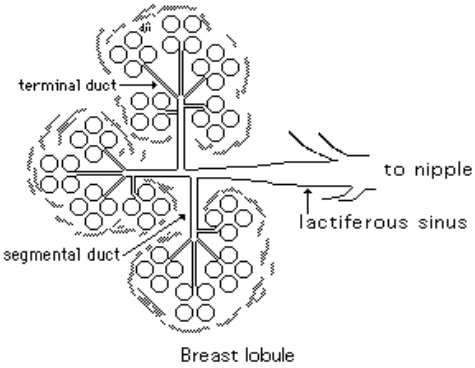
Effets sur le système immunitaire								
Référence **Titre de l'article**	**Type d'étude**	**Population investiguée**	**Mesure de BPA**	**Méthode analytique**	**Ajustements**	**Résultats/discussion**	**Qualité de l'étude**	**Chapitre(s) concerné(s)**
(Clayton *et al.*, 2011) The Impact of Bisphenol A and Triclosan on Immune Parameters in the US Population, NHANES 2003-2006	Étude nichée dans l'étude NHANES (2003-2006)	Population investiguée: population générale stratifiée avec une surreprésentation d'américains d'origine africaine, mexicaine et américaine et de plus de 60 ans N = 787 (Ac anti-CMV) N = 2133 (allergie)	Urinaire	HPLC/MS	Age: oui Sexe: oui Médicaments: non Tabac: non IMC: oui Autres contaminants: Non Autres: ethnie, niveau de créatinine, revenus, réussite scolaire	Résultats: Pour les > ou = 18 ans: concentrations les plus élevées en BPA sont associées avec une augmentation des Ac anti CMV, Pour les < 18 ans: relation négative entre concentration urinaires en BPA et les concentrations en Ac anti CM Commentaires: Une association positive avec les anticorps de cytomégalovirus a été observée, mais la portée et la causalité de cette relation reste incertaine. De plus, les auteurs ont travaillé sur une population avec « detectable BPA », ce qui laisse supposer que les individus avec les niveaux au dessous de la limite de détection ont été écartés, ce qui est vraiment discutable comme méthode de sélection.	Étude de bonne qualité ou ne présentant pas de limites méthodologiques majeures	6.1 Apport des études épidémiologiques 6.7 Effets sur le système immunitaire

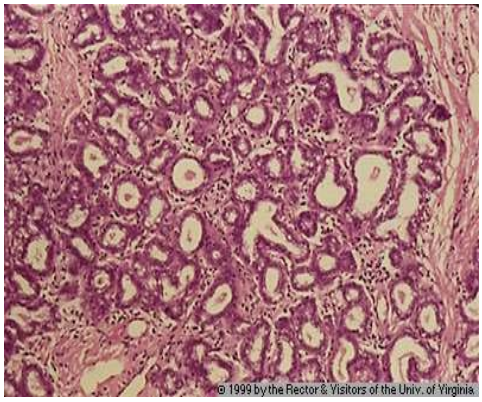
Cancer du sein								
Référence **Titre de l'article**	**Type d'étude**	**Population investiguée**	**Mesure de BPA**	**Méthode analytique**	**Ajustements**	**Résultats/discussion**	**Qualité de l'étude**	**Chapitre(s) concerné(s)**
(Yang *et al.*, 2009) Effects of bisphenol A on breast cancer and its risk factors	Étude transversale appariée sur l'âge	Population investiguée: population générale (femmes) N = 70 cas (femmes porteuses d'un cancer du sein) et 82 témoins → La taille de l'effectif est difficile à évaluer car la différence attendue est faible	Sang (BPA libre et conjugué) BPA conjugué utilisé comme biomarqueur (sang conservé plus de 10 ans dans des Ependorff)	HPLC/FD	Age: Oui (appariement et ajustement sur l'âge) Sexe: s/o Médicaments: non Tabac: oui MC: oui Autres contaminants: Non Autres: âge à la ménopause	Résultats: Pas de différence significative entre les concentrations sanguines de BPA chez les cas par rapport aux témoins.	Études non retenues car présentant des limites méthodologiques majeures Cette étude a été écartée pour le raisons suivantes: - La taille de l'effectif est difficile à évaluer car la différence attendue est faible, - prélèvement sanguin conservé dans des tubes Ependorff pendant plus de 10 ans, - recrutement fait en 1994-97, - BPA analysé uniquement dans le sang (sans préciser s'il s'agit du sang total ou du plasma) dans un seul échantillon - Absence de prélèvement urinaire.	6.1 Apport des études épidémiologiques 6.11 Effets sur le sein

Annexe 8: Etat des connaissances sur les lésions prénéoplasiques du sein - Eléments de langage

La fréquence avec laquelle les anatomopathologistes et les cliniciens sont confrontés aux lésions prénéoplasiques ou précancéreuses du sein a beaucoup augmenté avec la généralisation du dépistage mammographique et, à un moindre degré, avec l'amélioration de la prise en charge anatomopathologique des pièces opératoires.

Les lésions précancéreuses du sein correspondent à des proliférations épithéliales atypiques qui se développent à l'intérieur de l'arbre galactophorique et sont de deux types: canalaire et lobulaire. Ces deux types se différencient non pas par leur localisation mais par leur type cellulaire constitutif. En effet, en 1994, Wellings *et al* ont montré que la majorité des lésions préinvasives du sein prenaient naissance dans **l'unité terminale ductulolobulaire** ou TDLU très sensible aux facteurs hormonaux et située à la terminaison des canaux galactophoriques et à leur jonction avec les lobules (Wellings et Alpers, 1994).





TDLU: High power view showing the two-cell layer epithelium

Figure 1: anatomie du sein et unité terminale ductulolobulaire

Les deux types, canalaires et lobulaires, se distinguent au plan moléculaire par la présence au niveau des membranes cytoplasmiques d'une protéine du complexe de jonction intercellulaire: l'E-cathérine.

Le diagnostic histologique des lésions pré-cancéreuses est difficile et la **reproductibilité inter-pathologistes** est **médiocre** comme l'attestent un certain nombre d'études. Le diagnostic semble un peu amélioré grâce à l'apport de l'immunohistochimie dans les lésions qui présentent un profil spécifique (MacGrogan *et al.*, 2008).

Afin de faciliter le diagnostic et la prise en charge des lésions précancéreuses, une **nouvelle terminologie** a été proposée au début des années 2000 et adoptée par l'OMS en 2003. Elle se superpose à la terminologie traditionnelle et est **basée sur des critères morphologiques** et non moléculaires.

Classification OMS 2003 des lésions précancéreuses

Elle repose sur les termes de néoplasie intraépithéliale canalaire/ductal: DIN ou lobulaire/lobular: LIN. Les proliférations épithéliales canalaires se divisent en cinq catégories et les proliférations lobulaires en trois catégories.

Néoplasie intra-épithéliale de type canalaire ou ductal

1. Métaplasie cylindrique atypique (MCA): DIN-1A
2. Hyperplasie canalaire atypique (HCA): DIN-1B
3. Carcinome canalaire in situ (CCIS) de bas grade: DIN-1C
4. CCIS de grade intermédiaire: DIN-2
5. CCIS de haut grade: DIN-3

Les deux premières catégories correspondent à des lésions précancéreuses.

Le **carcinome canalaire in situ** (CCIS) n'est **pas une lésion précancéreuse** mais une lésion cancéreuse **préinvasive.** Aux Etats-Unis, les CCIS représentent près de 20% des cancers dépistés (1 cas de CCIS pour 1 300 mammographies de dépistage) (Ernster *et al.*, 2002).

Néoplasies mammaires intra-épithéliales de type lobulaire

1. Hyperplasie lobulaire atypique (HLA): LIN-1
2. HLA/CLIS: LIN-2
3. Carcinome lobulaire in situ (CLIS): LIN-3 qui inclue trois différents types

Cette nouvelle terminologie a permis une meilleure prise en charge des lésions précancéreuses. Par exemple, pour les lésions lobulaires, les recommandations de l'Institut National du Cancer (INCa) publiées en novembre 2009 préconisent une simple surveillance dans les LIN 1 et LIN 2 alors que lorsque le résultat histologique conclue à un LIN 3, le traitement initial repose sur une exérèse chirurgicale.

Risque de transformation en cancer invasif des lésions prénéoplasiques du sein

Lorsqu'elle est laissée en place, une lésion prénéoplasique ou précancéreuse peut se transformer en carcinome pré-invasif ou carcinome in-situ qui lui-même peut se transformer en carcinome invasif (cf. figure 2).

L'introduction du concept d'unité terminale ductulolobulaire a engendré la notion de transformation tumorale - non obligatoire - passant par différentes étapes, comme pour le cancer du colon. Des cellules normales situées dans l'unité terminale ductulolobulaire (TDLU) se transformeraient en hyperplasie atypique, puis en carcinome canalaire in situ jusqu'au cancer invasif (figure 2 ci-dessous)

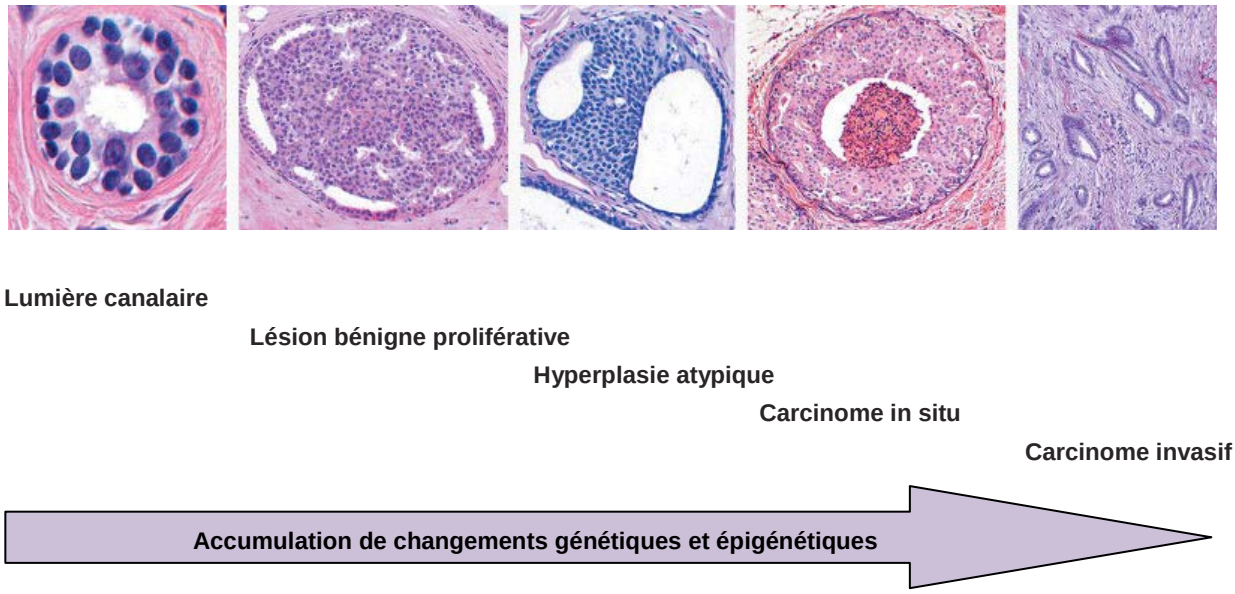


Figure 2: Coupes anatomopathologiques illustrant la transformation de la glande mammaire normale jusqu'au cancer invasif (figure tirée de (Burstein *et al.*, 2004))

L'hypothèse de l'existence d'un continuum entre la glande mammaire normale et le cancer invasif du sein, même si elle peut paraitre trop simpliste, est basée sur des arguments directs et indirects (Antoine *et al.*, 2010).

Des études historiques, depuis le début du 19ème siècle, ont permis d'observer que des lésions bénignes étaient plus fréquemment retrouvées chez les patientes présentant des cancers du sein. Des études épidémiologiques plus récentes ont montré que les femmes ayant des antécédents de lésions bénignes du sein avaient un risque augmenté de cancer du sein.

Jusqu'au début des années 1990, les anatomopathologistes et les cliniciens se sont référés à des travaux anciens, notamment ceux de Dupont et Page (Dupont et Page, 1985) qui ont calculé le risque de survenue ultérieur de cancer du sein chez des patientes présentant des lésions bénignes souvent découvertes sur des lésions palpables avant l'ère du dépistage par mammographie.

L'histoire naturelle des carcinomes canalaires in situ (CCIS) de bas grade a pu être déterminée par des études de suivi à long terme chez des femmes ayant eu une biopsie diagnostique sans autre thérapeutique, avant l'ère du dépistage de masse organisé. Après 10 ans de suivi, 14 à 60% de ces femmes ont eu un diagnostic de cancer invasif dans le même sein (Page *et al.*, 1995). La mise en évidence d'un tel risque d'invasion a d'ailleurs conduit à l'attitude actuelle de traiter activement ces lésions. L'histoire naturelle des CCIS de haut grade ou des CCIS palpable cliniquement n'est, en revanche, pas bien caractérisée car dans la plupart des cas, la tumeur est enlevée en totalité par chirurgie ce qui est également le cas des lésions d'hyperplasie canalaire atypique (HCA).

L'augmentation importante de la pratique de biopsies sur des images infra-cliniques et les données récentes apportées par l'étude moléculaire des lésions ont apporté un nouvel éclairage sur le risque de transformation de lésions hyperplasiques en cancer.

Lors de la transformation de lésions hyperplasiques en cancers in situ puis en cancer invasif, des déséquilibres sont observés à l'échelon chromosomique avec une perte de l'hétérozygotie dans plus de 70% des carcinomes in situ de haut grade comparé à près de 40% des cas d'hyperplasie atypique et 0% dans le tissu mammaire sain (Aubele *et al.*, 2000). Des marqueurs moléculaires de la transformation tumorale mammaire ont été identifiés. Le récepteur des estrogènes, exprimé par les cellules mammaires épithéliales normales est exprimé par plus de 70% des carcinomes canalaires *in situ* (CCIS). Le protooncogène HER2/neu est surexprimé dans la moitié des CCIS mais pas dans les hyperplasies atypiques (Allred *et al.*, 1992).

Des travaux publiés au début des années 2000 sur les voies moléculaires impliquées ont montré que le cancer du sein n'était pas une pathologie unique mais plutôt un ensemble de différentes maladies survenant dans les mêmes structures anatomiques (TDLU). Ces techniques de biologie moléculaire ont également montré que les **lésions précancéreuses et préinvasives** étaient aussi **hétérogènes** que les cancers invasifs.

Différents modèles d'évolution selon le **grade histologique** (bas grade ou haut grade) et la présence ou l'absence de **récepteurs aux estrogènes** ont été proposés (cf. figures ci-dessous tirées de la publication de (Lopez-Garcia *et al.*, 2010)).

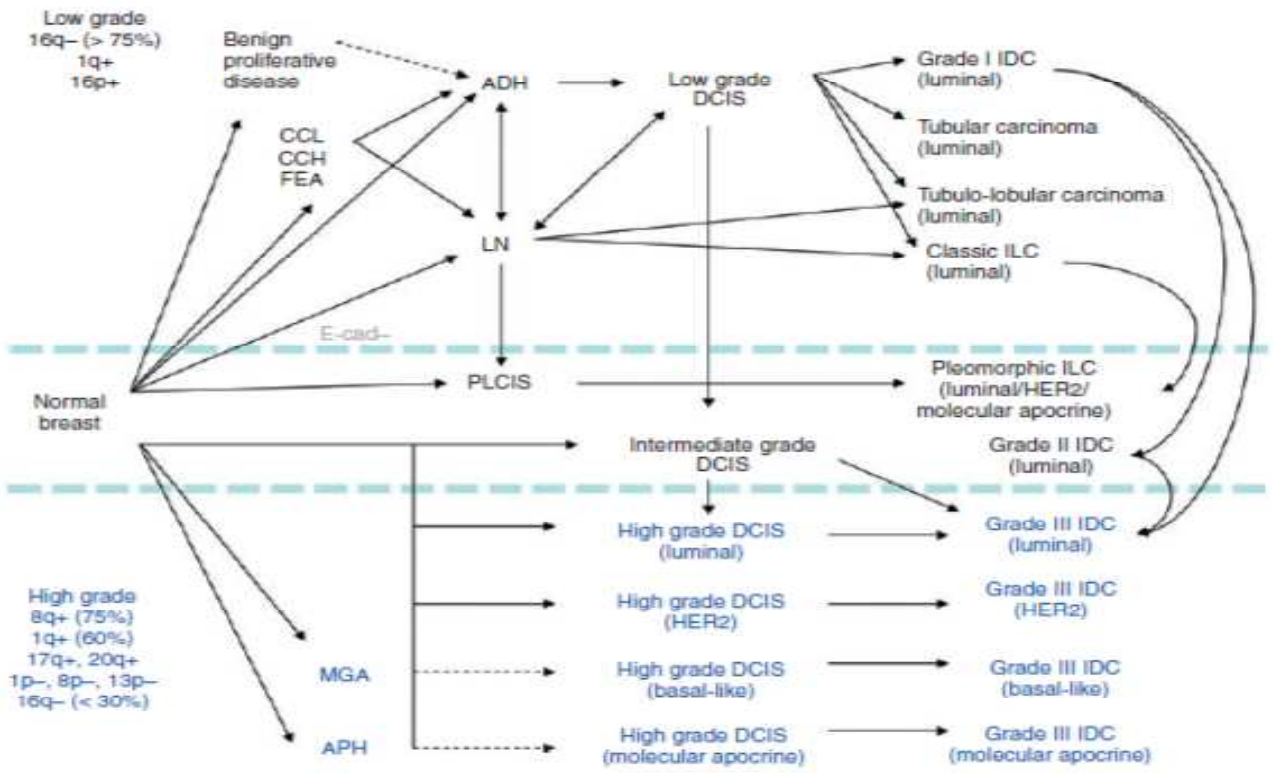


Figure 3. Low- and high-grade multistep model of breast cancer progression based on morphological, immunophenotypical and molecular features. Connectors drawn with continuous lines represent links between morphological entities which are demonstrated by morphological and/or molecular data. Connectors drawn with discontinuous lines represent hypothetical links yet to be demonstrated. ADH: atypical ductal hyperplasia; APH: atypical apocrine hyperplasia; CCH: columnar cell hyperplasia; CCL: columnar cell lesion; DCIS: ductal carcinoma *in situ*; E-cad: E-cadherin; FEA: flat epithelial atypia; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; LN: lobular neoplasia; MGA: microglandular adenosis; PLCIS: pleomorphic lobular carcinoma *in situ*.

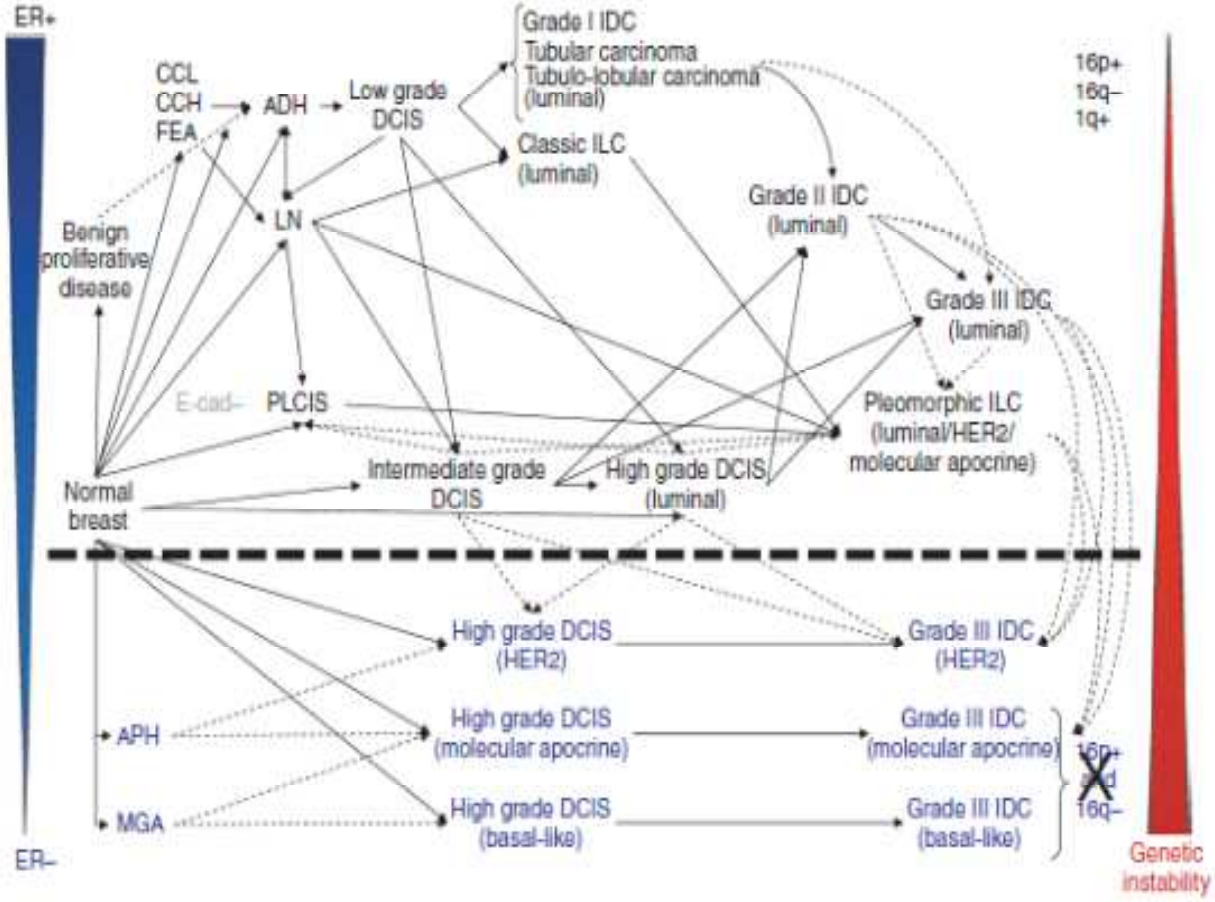


Figure 4. Revised multistep model of breast cancer evolution based on oestrogen receptor (ER)-status. Note that the two main pathways are defined by the expression of ER and ER-regulated genes. In this model, the ER-positive arm encompasses most of the precursor lesions and a range of invasive lesions which may progress from low to high grade due to the acquisition of genetic instability and accumulation of stochastic genetic events. The ER-negative arm includes ER-negative DCIS and invasive tumours; MGA and APH are proposed as non-obligate precursors of these lesions. ER and genetic instability bars on either side of the image represent the levels of ER expression and genetic instability, respectively. ADH: atypical ductal hyperplasia; APH: atypical apocrine hyperplasia; CCH: columnar cell hyperplasia; CCL: columnar cell lesion; DCIS: ductal carcinoma *in situ*; E-cad: E-cadherin; FEA: flat epithelial atypia; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; LN: lobular neoplasia; MGA: microglandular adenosis; PLCIS: pleomorphic lobular carcinoma *in situ*.

Par ailleurs, les données récentes montrent que les atypies épithéliales représentent non seulement un **facteur de risque de cancérisation secondaire** mais également un **marqueur de risque de cancer concomitant de voisinage** (de Mascarel *et al.*, 2007).

Conclusion

L'amélioration des connaissances apportées par l'immunohistochimie, la cytogénétique et la biologie moléculaire a permis d'améliorer la classification des lésions précancéreuses et la compréhension des liens de filiation entre atypies épithéliales et cancer du sein. Ces éléments ont permis d'affiner le diagnostic, de mieux déterminer le risque d'évolution en cancer invasif et donc d'optimiser la prise en charge des patientes.

Annexe 9: Note de synthèse sur les cancers mammaires chez les rongeurs

Les rongeurs, rat et souris, ont été largement utilisés pour l'étude de cancérogénèse mammaire, dans des modèles de tumeurs spontanés ou induits. Le principal avantage du modèle Rat est que le carcinome ressemble plus au cancer du sein de l'Homme, le cancer mammaire chez la souris étant souvent d'origine virale et hormono-indépendants (Cardiff *et al.*, 2000; Gould 1995). Chez la souris CD-1 , les lésions spontanées non néoplasiques et néoplasiques de la glande mammaire sont peu fréquentes (moins de 5%: (Gad 2007)).

Les différentes souches de rat utilisées classiquement ont montré des sensibilités diiférentes aux néoplasies induites chimiquement ou par radiations, Sprague-Dawley ou Wistar étant plus sensibles que le rat Fisher. Chez le rat Sprague-Dawley, l'incidence des tumeurs spontanées approche ou dépasse 50% dans les études chroniques (exemple, données historiques NTP, 2008). Il existe des souches présentant des sensibilités accrues à la cancérogénèse mammaire: c'est le cas de la souche Wistar-Furth très sensibles aux cancérigènes chimiques (Gould 1995).

Les facteurs de susceptibilitité de la glande mammaire incluent, outre les facteurs génétiques, le degré de différenciation du tissu mammaire au moment de l'exposition, le statut physiologique et hormonal, et l'alimentation. La sensibilité est accrue chez la femelle pré-pubère pendant la période du développement:les canaux se terminent par des bourgeons terminaux (TEB) qui vont progressivement se différencier en bourgeons alvéolaires et en lobules alvéolaires. Le plus grand nombre de tumeurs a été induit chez le rat femelle SD entre 40-46 jours, période de différenciation la plus active des TEB, considérés comme la cible des cancérogènes chimiques (Russo et Russo, 1996). Les carcinomes mammaires ont été induits chez les rats par des agents chimiques ou physiques. Les carcinogènes chimiques les plus utilisés comprennent l'hydrocarbure aromatique polycyclique DMBA (diméthylbenzanthracène) ou les agents alkylants ENU (N-éthyl-N-nitrosourée) et le NMU (N-méthyl-N-nitrosourée). Après une seule dose de DMBA ou de NMU, un adénocarcinome se développe en 20 jours chez les rats jeunes. Ces cancers envahissent parfois les tissus environnants mais métastasent rarement à des sites distants (Gould 1995). Un protocole de cancérogénèse à court terme par injection de NMU à 21 jours a permis de décrire la chronologie d'induction des lésions pré-néoplasiques et néoplasiques mammaires (Thompson *et al.*, 1998), et de comparer ces lésions à celles observées chez l'Homme (Singh *et al.*, 2000). Ainsi, certaines similitudes ont été décrites entre lésions observées chez l'Homme et celles induites chez les Rongeurs, mais des différences existent aussi.

- Les similitudes incluent:
  - Un développement selon un processus multi-étapes
  - La majorité des cancers induits par DMBA ou NMU sont hormonodépendants
  - Un schéma morphologique similaire: hyperplasies, hyperplasies intra-canalaires considérées comme prénéoplasiques, adénomes/ adénocarcinomes. Les carcinomes canalaires *in situ* (CCIS) sont considérés comme une progression morphologique vers le carcinome mammaire à partir de lésions prolifératives intracanalaires.

Tableau XVII: Comparaison des lésions histopathologiques pré-néoplasiques et néoplasiques de la glande mammaire, induites chez le rat pré-pubère, avec celles décrites chez l'Homme (Singh *et al.*, 2000)

	Homme	Rat
Lésions bénignes	Fibroadénomes pouvant présenter des carcinomes in situ (CIS) et des hyperplasies canalaires atypiques (HCA)	Pas d' HCA ni de CIS dans les fibroadénomes
Hyperplasies	Hyperplasies atypiques possibles	Pas d'hyperplasies atypiques
Carcinomes *in situ*	Carcinomes lobulaires *in situ* (CLIS) et carcinomes canalaires *in situ* (CCIS) peuvent être observés. Plusieurs sous-types histologiques. Microcalcifications possibles.	Moins de diversité histologique. Des CCIS) sont observés, notamment cribriformes et papillaires. Pas de microcalcifications des CCIS.
Carcinomes invasifs	Elastose et calcifications possibles. Plusieurs types. Ganglions impliqués.	Pas d'élastose ou de microcalcifications Absence de carcinomes lobulaires, etc. Beaucoup moins diversifiés sur le plan histologique Pas de métastase dans les ganglions

- Les différences incluent:
  - Morphologie de la plupart des tumeurs mammaires chez la souris ne ressemblent pas aux cancers du sein humains (Cardiff *et al.*, 2000);
  - Chez le rat, une similitude des lésions histologiques a été décrite, avec moins de diversités que chez l'Homme: par exemple pas d'hyperplasies atypiques, ni de microcalcifications, ni de forme lobulaire de CIS ou de carcinome invasif lobulaire n'ont été décrites dans le modèle de cancérogénèse à court terme induit par le carcinogène NMU (Singh *et al.*, 2000)

Les ganglions régionaux ne sont pas souvent envahis chez le rat par comparaison à l'Homme.

Annexe 10: Présentation des positions divergentes

Position exprimée par membre Mme Véronique Ezratty

Une proposition faite par un des membres du groupe de travail n'a pas été retenue par le groupe de travail. Cette proposition alternative consistait à établir une seule conclusion par effet intégrant à la fois les données humaines et animales en précisant respectivement pour chacune des deux catégories leur niveau de qualité ainsi que la plausibilité d'un mécanisme d'action, dans l'esprit des classifications des substances cancérogènes du CIRC et des agents cancérogènes, mutagènes et reprotoxiques CMR de l'Union Européenne. Cette proposition jugeait par ailleurs qu'il serait préférable d'éviter de parler d'effets avérés lorsqu'ils n'étaient observés que chez l'animal sans données humaines disponibles. En effet, séparer mais utiliser la même terminologie pour l'Homme et l'animal comme cela a été décidé par le GROUPE DE TRAVAIL pourrait induire un risque de mauvaise interprétation en cas d'extraction de certains éléments de la conclusion lors de la diffusion du rapport.

Annexe 11: Synthèse des déclarations publiques d'intérêts des experts par rapport au champ de la saisine

Cette partie présente une synthèse des liens déclarés par les experts, dans le domaine sur lequel se porte la saisine, et précise d'une part comment ces liens ont été analysés et d'autre part la manière dont ils ont été gérés, eu égard à un risque potentiel de conflit d'intérêts.

Les déclarations publiques d'intérêts sont mises à jour par les experts à chaque changement de situation.
Au cours des expertises, les liens d'intérêts sont réexaminés au vu de l'ordre du jour.

RAPPEL DES RUBRIQUES DE LA DÉCLARATION PUBLIQUE D'INTÉRÊTS

IP-A	Interventions ponctuelles: autres
IP-AC	Interventions ponctuelles: activités de conseil
IP-CC	Interventions ponctuelles: conférences, colloques, actions de formation
IP-RE	Interventions ponctuelles: rapports d'expertise
IP-SC	Interventions ponctuelles: travaux scientifiques, essais, etc.
LD	Liens durables ou permanents (Contrat de travail, rémunération régulière …)
PF	Participation financière dans le capital d'une entreprise
SR	Autres liens sans rémunération ponctuelle (Parents salariés dans des entreprises visées précédemment)
SR-A	Autres liens sans rémunération ponctuelle (Participation à conseils d'administration, scientifiques d'une firme, société ou organisme professionnel)
VB	Activités donnant lieu à un versement au budget d'un organisme

SYNTHÈSE DES LIENS DES MEMBRES DU CES, MENTIONNÉS DANS LA DECLARATION PUBLIC D'INTERET, EN RAPPORT AVEC LA THEMATIQUE DE LA SAISINE

NOM	**Prénom** ***Rubriques de la DPI*** Description de l'intérêt	**Date de déclaration des intérêts**

Analyse ANSES:	*en cas de lien déclaré*	
BELZUNCES	**Luc** ***LD*** Université d'Avignon: Enseignements (depuis 1998) (Vacations) Université d'Angers: Enseignements (depuis 2004) (Vacations) Université Aix-Marseille 3: Enseignements (depuis 2000) (Vacations) Centre Agronomique de Chania, Crête: Enseignements (01/11/2005 au 10/11/2010) (Salaire) ***IP*** ADAPi (Association pour le Développement de l'Apiculture Provençale): Conférences (2000-2006) (Rémunération personnelle) UNAF (Union nationale de l'apiculture française): Conférences (2000-2006) (Rémunération personnelle) et consultance (2011) (Rémunération ponctuelle personnelle) ADARA: Conférences (2000-2006) (Rémunération personnelle) GIE Apiculture Pays de Loire: Formation toxicologie abeille (02/02/2011) (Rémunération personnelle) Organisations apicoles: Conférences régulières (Permanent) (Rémunération personnelle) ***VB*** Bayer: Effets de l'imidaclopride chez l'abeille donnant lieu à versement à l'INRA (50 % du budget du laboratoire où l'expert est Directeur de recherche) (contrat clos en 2001)	10/02/2010 28/01/2011

Analyse ANSES:	Aventis-BASF: Action des sécrétions de tournesol chez l'abeille donnant lieu à versement à l'INRA (50 % du budget du laboratoire) (2002) Aventis: Mode d'action de l'acétamipride donnant lieu à versement à l'INRA (50 % du budget du laboratoire) (2001-2003) ***SR*** BASF: enfant stagiaire (2007) Pas de risque de conflit d'intérêts	
BONVALLOT **Analyse ANSES:**	**Nathalie** ***Démission le 31 mars 2011*** ***LD*** EHESP: CDD (oct. 2008-oct. 2011) ***IP*** PBE + (performance Bretagne environnement), association d'entreprises bretonnes de tous secteurs: Journées PBE +, Toxicologie (avril 2008) (Aucune rémunération) EDF GDF: Formation EQRS (juin chaque année) (Aucune rémunération) ***SR*** OBERTHUR: Conjoint responsable Hygiène, sécurité et environnement /	09/02/2010 25/01/2011
BOURGEOIS	**Damien** ***IF***	18/02/2010 24/01/2011

Analyse ANSES:	Rhodia: Plan d'épargne en entreprise, quelques actions ***SR*** Maintenance Service Environnement: Conjoint ingénieur (depuis 01/12/2010) Pas de risque de conflit d'intérêts	
CASSIER-CHAUVAT **Analyse ANSES:**	**Corinne** ***LD*** CNRS/CEA: chercheur CNRS dans unité mixte CEA-CNRS (depuis 1983) ***SR*** CEA: Conjoint Chercheur Chef de laboratoire (depuis 1985) Pas de risque de conflit d'intérêts	26/02/2010 01/02/2011
EMPEREUR-BISSONNET **Analyse ANSES:**	**Pascal** ***SR*** Institut de recherche international SERVIER: Épouse Directeur Support Opérationnel (1990-2011) Pas de risque de conflit d'intérêts	01/02/2010 26/01/2011
ENRIQUEZ **Analyse ANSES:**	**Brigitte** Aucun lien déclaré /	01/02/2010 02/02/2011
GUENOT	**Dominique** ***IP***	18/02/2010 02/02/2011

Analyse ANSES:	2eme édition de la manifestation « A votre santé ! » Le Mois de la santé et de la recherche médicale en Alsace: Intervention/débat suite à projection de film (1 journée en mars 2010) (Aucune rémunération) Pas de risque de conflit d'intérêts	
GUERBET **Analyse ANSES:**	**Michel** ***IP*** AFSSAPS: expert au sein du groupe réclinique (2006-2012) et au sein de la commission d'AMM des médicaments (2010-2013) (Aucune rémunération) Pas de risque de conflit d'intérêts	24/02/2010 02/02/2011
HUYNH **Analyse ANSES:**	**Cong Khan** Aucun lien déclaré /	19/01/2010 28/01/2011
KRISHNAN	**Kannan** ***IP*** Regulatory checkbook: Invited speaker and panelist, Naphthalene: State of the Science Symposium and Workshop (3 jours en 2006) (Vacation, frais de déplacement) ***VB*** ExxonMobil conjointement avec le conseil de recherches en sciences naturelles et en génie du Canada: Subvention de recherche pour « An integrated fugacity-pharmacokinetic model » donnant lieu à versement à l'University of Montréal, Trent University et Université du Québec à Montréal (< 10 % du budget) (2007-2010)	31/03/2009 02/03/2011

Analyse ANSES:	Pas de risque de conflit d'intérêts	
LAFON	**Dominique** ***LD*** Archives des maladies professionnelles: droits d'auteur (depuis 1995) Dassault Falcon service: Médecin du travail en CDI (depuis 1995) ***IP*** AFSSA: CES MCDA (2000-2006) (Vacations) AFSSAPS: Commission cosmétologie (2010)	15/02/2010 25/01/2011
Analyse ANSES:	Pas de risque de conflit d'intérêts	
LAGADIC-GOSSMANN	**Dominique** Aucun lien déclaré	25/02/2010 30/01/2011
Analyse ANSES:	/	
LAUDET	**Annie** Aucun lien déclaré	23/02/2010 17/03/2011
Analyse ANSES:	/	
MENETRIER	**Florence** ***IP*** ANR: Appel à projets Santé-environnement/santé-travail (juin 2006-août 2006) (Aucune rémunération)	23/02/2010 26/01/2011
Analyse ANSES:	Pas de risque de conflit d'intérêts	
PRAT	**Odette** Aucun lien déclaré	27/01/2010 30/01/2011

Analyse ANSES:	/	
SCHROEDER **Analyse ANSES:**	**Henri** ***IF*** Air France, Rexel, BNP: actions Fonds de placement LCL Protection: Produits financiers ***VB*** Industries agro-alimentaires: Contrat sur aliments fonctionnels donnant lieu à versement à l'université Henri Poincaré (50 % du laboratoire où l'expert est Enseignant-chercheur, responsable des études réalisées) (2001-2006) ANSES: Veille bibliographique (auteur des notes de veille) donnant lieu à versement à l'université Henri Poincaré (15 %du budget du laboratoire) (2009-2011) Pas de risque de conflit d'intérêts	27/02/2010 28/01/2011
SECRETAN-LAUBY	**Béatrice** ***LD*** CIRC: Salariée (depuis 2001) ***IP*** Encyclopedia of Cancer (Springer Verlag): Article sur "UV radiation (déc. 2007 à fév. 2008) (Aucune rémunération) AFTIM (Association française des techniciens et ingénieurs de sécurité et des médecins du travail): Journée AFTIM-ADHYS, Paris (2004) (Aucune rémunération) ADHYS (Association pour le développement de	02/03/2010 03/02/2011

	l'hygiène et de la sécurité dans les établissements de recherche ou d'enseignement supérieur): 22ème journées de l'ADHYS (2005) (Aucune rémunération) BTP: 28èmes journées Nationales du BTP (2005) (Aucune rémunération) DRASS: Journée d'échange de pratiques professionnelles sur les CMR (2005) (Aucune rémunération) TSR (Télévision Suisse romande): Emission "A bon entendeur" sur le benzène dans les boissons de table (2006) (Aucune rémunération) AFSSET: Substitution: un enjeu pour les CMR (2007) (Aucune rémunération) University of Grenoble: Journée FMC Quoi de neuf en Cancérologie (2008) (Aucune rémunération) Université Claude Bernard Lyon 1er: Journée de formation de la Société de SMST Lyon: Les cancers professionnels (2010) (Aucune rémunération) University of Grenoble: Enseignement (1 journée en 2006, 2007, 2010) (Aucune rémunération)	
Analyse ANSES:	Pas de risque de conflit d'intérêts	
TISSOT	**Sylvie** ***Démission le 14 janvier 2011*** Aucun lien déclaré	05/02/2010 27/01/2011
Analyse ANSES:	/	

SYNTHÈSE DES DÉCLARATIONS PUBLIQUES D'INTÉRÊTS DES MEMBRES DU GT PAR RAPPORT AU CHAMP DE LA SAISINE

NOM **Analyse ANSES:**	**Prénom** ***Rubriques de la DPI*** Description de l'intérêt *en cas de lien déclaré*	**Date de déclaration des intérêts**
ANTIGNAC **Analyse ANSES:**	**Jean-Philippe** ***IP*** Rapport d'expertise INSERM sur les substances reprotoxiques (depuis 2009) (Rémunération personnelle) Pas de risque de conflit d'intérêts	22/02/2010 10/03/2011
BELZUNCES	**Luc** (Membre du CES « Evaluation des risques liés aux substances chimiques) ***LD*** Université d'Avignon: Enseignements (depuis 1998) (Vacations) Université d'Angers: Enseignements (depuis 2004) (Vacations) Université Aix-Marseille 3: Enseignements (depuis 2000) (Vacations) Centre Agronomique de Chania, Crête: Enseignements (01/11/2005 au 10/11/2010) (Salaire) ***IP*** ADAPi (Association pour le Développement de l'Apiculture Provençale): Conférences (2000-2006) (Rémunération personnelle) UNAF (Union nationale de l'apiculture française):	10/02/2010 28/01/2011

Analyse ANSES:	Conférences (2000-2006) (Rémunération personnelle) et consultance (2011) (Rémunération ponctuelle personnelle) ADARA: Conférences (2000-2006) (Rémunération personnelle) GIE Apiculture Pays de Loire: Formation toxicologie abeille (02/02/2011) (Rémunération personnelle) Organisations apicoles: Conférences régulières (Permanent) (Rémunération personnelle) ***VB*** Bayer: Effets de l'imidaclopride chez l'abeille donnant lieu à versement à l'INRA (50 % du budget du laboratoire où l'expert est Directeur de recherche) (contrat clos en 2001) Aventis-BASF: Action des sécrétions de tournesol chez l'abeille donnant lieu à versement à l'INRA (50 % du budget du laboratoire) (2002) Aventis: Mode d'action de l'acétamipride donnant lieu à versement à l'INRA (50 % du budget du laboratoire) (2001-2003) ***SR*** BASF: enfant stagiaire (2007) Pas de risque de conflit d'intérêts	
BENAHMED	**Mohamed** ***IP*** Galderma: Travaux d'expertise (décembre 2009) BASF-Galderma: Rapport d'expertise (janvier 2010) BASF-Galderma: Formation sur l'utilité des	04/03/2010

Analyse ANSES:	nouveaux marqueurs (En cours) (Rémunération personnelle) ECETOC: Workshop (Rémunération personnelle) ***VB*** BAYER, GALDERMA: Contrats de collaboration, conventions sur perturbateurs endocriniens donnant lieu à versement à l'INSERM (5 % du budget) BASF, BAYER: Contrats CIFRE sur perturbateurs hormonaux, reprotoxicité donnant lieu à versement à l'INSERM (5 % du budget) Pas de risque de conflit d'intérêts	
BERTRAND **Analyse ANSES:**	**Nicolas** ***LD*** Salarié de l'INRS (2007-2011) Pas de risque de conflit d'intérêts	17/03/2010 09/03/2011
BLANCHARD **Analyse ANSES:**	**Olivier** ***LD*** Salarié de l'INERIS (jusqu'en 2009) Pas de risque de conflit d'intérêts	22/02/2010 28/01/2011
CLAUW	**Martine** ***LD*** Sanofi-Aventis: Membre extérieur du comité d'éthique pour la protection des animaux de laboratoire, Toulouse (depuis 2009) (Aucune	16/03/2010 02/02/2011

Analyse ANSES:	rémunération) ***IP*** Nutreco (The Netherlands): Convention de recherches sur l'impact digestif du stress thermique (depuis 2010) Royal Canin, SAS (Aymargues, France): Convention de recherches sur Gériatrie canine et stress (depuis 2010) ***VB*** Taxe d'apprentissage de Pierre Fabre médicaments et Sanofi-Aventis au bénéfice de l'École vétérinaire de Toulouse (0,01 % du budget) Pas de risque de conflit d'intérêts	
CRAVEDI	**Jean-Pierre** ***IF*** Total, Arcelor Mittal: Actions ***LD*** EFSA: Expertise (panel Contaminants) (depuis 2003) (Rémunération personnelle) ***IP*** DANONE: Analyse d'échantillons d'eau (depuis octobre 2010) donnant lieu à rémunération à l'organisme d'appartenance (INRA) L'OREAL: Bioaccumulation chez le poisson (2008-2009) (Vacations) DANONE: Détection des perturbateurs endocriniens dans l'eau (2009-201) (Vacations) ***VB***	19/11/2009 18/04/2011

Analyse ANSES:	Pierre FABRE: Bourse de thèse donnant lieu à versement à l'INRA (5 % du budget) DANONE: Analyse de xénobiotiques dans l'eau donnant lieu à versement à l'INRA (1 % du budget) Pas de risque de conflit d'intérêts	
DUPUPET **Analyse ANSES:**	**Jean-Luc** ***Démission le 22 octobre 2010*** ***LD*** CCMSA: Médecin conseiller technique national /	31/03/2010
ELEFANT **Analyse ANSES:**	**Élisabeth** Aucun lien déclaré ***/***	11/03/2010
EMOND **Analyse ANSES:**	**Claude** ***IP*** Health Canada: Revue expert (2006-2008) (Rémunération personnelle) US EPA USA: Développement d'un modèle PBPK sur les PBDE (2007) et travaux de consultation pour la réévaluation des niveaux d'exposition au dioxine (2009) (rémunération personnelle) Pas de risque de conflit d'intérêts	24/02/2010 14/07/2011
EUSTACHE	**Florence** Aucun lien déclaré	08/03/2010

Analyse ANSES:	***/***	
EZRATTY **Analyse ANSES**:	**Véronique** ***IF*** EDF, Gaz de France-Suez: Actions ***LD*** EDF: Salarié (depuis 1998) ***IP*** RSEIN: Analyse d'articles scientifiques (depuis 2007) (Rémunération personnelle) OMS: Rédaction de trois chapitres pour les guidelines QAI (Septembre 2008) (Rémunération personnelle) Pas de risque de conflit d'intérêts	10/02/2010 29/03/2011
LABADIE **Analyse ANSES:**	**Pierre** ***Démission le 12 novembre 2010*** ***IP*** ONEMA: Comité de suivi scientifique du plan national d'actions sur les PCB (Aucune rémunération) (Décembre 2009) /	22/02/2010
LE MAGUERESSE-BATTISTONI **Analyse ANSES:**	**Brigitte** Aucun lien déclaré ***/***	26/02/2010 09/03/2011
LEMARCHAND	**Frédérick**	01/02/2010

Analyse ANSES:	Aucun lien déclaré ***/***	15/03/2011
MANDIN	**Corinne** ***LD*** Salariée du CSTB ***IP*** Vacations à l'Institut Supérieur de la Santé et des Bioproduits d'Angers (1 fois par an) (Rémunération personnelle) Analyse d'articles scientifiques pour le bulletin de veille du réseau RSEIN de l'INERIS (1 à 2 fois par an) (Rémunération personnelle) ***SR*** Conjoint Directeur des ventes chez Taiki Europe Pas de risque de conflit d'intérêts	15/06/2009 11/03/2010 08/03/2011
MINIER **Analyse ANSES:**	**Christophe** Aucun lien déclaré /	10/03/2010 09/05 /2011
MULTIGNER **Analyse ANSES:**	**Luc** Aucun lien déclaré ***/***	03/03/2010
PASCUSSI	**Jean-Marc** ***Démission le 11 janvier 2011***	22/01/2010

Analyse ANSES:	***IP*** SERVIER: Cours sur les xénorecepteurs CAR et PXR (Rémunération personnelle) (Mai 2009) ***VB*** SERVIER: Bourse CIFRE pour une thèse (2006-2009) donnant lieu à versement à l'organisme d'appartenance (ONSERM) (3 % du budget) /	
SANCHEZ **Analyse ANSES:**	**Wilfried** ***LD*** Salarié de l'INERIS Pas de risque de conflit d'intérêts	04/03/2010
STEENHOUT	**Anne** ***LD*** ULB (Université Libre de Bruxelles): chef de service et directeur de recherche (depuis 1990) ***IP*** US Environmental Protection Agency: travaux scientifiques donnant lieu à versement à l'organisme d'appartenance (ULB) Conseil Supérieur d'Hygiène de Belgique: Rapport d'expertise sur le dichlorométhane donnant lieu à versement à l'organisme d'appartenance (2000) Commission européenne: Participation, intervenant à des réunions, GT, comités donnant lieu à versement à l'organisme d'appartenance (depuis 2002) CSTC (Centre Scientifique et Technique de la	25/02/2010 07/03/2011

Analyse ANSES:	Construction)/BATIBOUW (Salon international du bâtiment, de la rénovation et de l'aménagement intérieur): Intervenant GT et conférences (Aucune rémunération) (2003, 2004-2005) ECETOC (the European Centre for Ecotoxicology and Toxicology of Chemicals): Evaluation de projets donnant lieu à versement à l'organisme d'appartenance (2002) Écoles d'architecture: conférencier, formations donnant lieu à versement à l'organisme d'appartenance (2001-2005) Ministère Communauté française de Belgique: conférencier, formations donnant lieu à versement à l'organisme d'appartenance (2006) Sociétés scientifiques: Conférencier (Aucune rémunération) ***VB*** Divers ministères/ région de Bruxelles, Communauté française: recherche scientifique en environnement et Santé donnant lieu à versement à l'organisme d'appartenance LRI (Long-range research initiative)-CEFIC (European Chemical Industry Council): recherche scientifique, exposition donnant lieu à versement à l'organisme d'appartenance Pas de risque de conflit d'intérêts	
TAKSER **Analyse ANSES:**	**Larissa** Aucun lien déclaré ***/***	02/02/2010 06/05/2011
THONNEAU	Patrick Aucun lien déclaré	02/03/2010 28/05/2011

Analyse ANSES:	/	
VIGUIE **Analyse ANSES:**	Catherine Aucun lien déclaré /	18/02/2010 24/06/2011

Notes

French agency strongly disagrees with EFSA on BPA

EU Food Policy today
2014

The French Food Safety agency has responded to EFSA's latest assessment that Bisphenol A is safe by saying it stands by its own findings on the food contact material which led France to pass a law banning the substance.

In its formal response, published on Monday, ANSES says EFSA's draft conclusions suffer from limitations in the way they deal with "uncertainties" and challenges the European Authority's analysis of key scientific studies.

ANSES says a particular "point of disagreement" with EFSA concerns the possible risk of breast cancer in girls born to women exposed to BPA.

It insists an increased cancer risk could arise in the children of women who have a high level of endogenous oestrogens or xeno-oestrogens during pregnancy and are then exposed to tumour-initiating agents.

"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."

EFSA's experts only included analysis of the direct carcinogenic effects of BPA on the development of neoplastic lesions, it says.

"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 (eg DMBA or NMU) during puberty."

ANSES says its own conclusions of March 2013 remain valid. It calls for an "active watch" to update data on BPA and for a more standardised EU-wide approach to assessing toxicological studies on the substance.

The French agency questions EFSA's refusal to consider some biochemical and histological signs as potentially significant indicators of health risk, such as on the central nervous system and mammary gland.

Uncertainty factor

"EFSA often uses uncertainty as an argument to consider that an effect is not likely or to even exclude an effect that is considered likely from the risk assessment," says ANSES.

The French agency concedes that some of the 450 studies assessed by EFSA were more up to date than those available by the June 2012 deadline for its own report, particularly relating to "critical" effects on the metabolism.

"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."

The French agency recognizes that the new EFSA assessment takes into account studies on oral exposure as well as on subcutaneous exposure, "which was not the case in previous EFSA opinions".

ANSES's response was submitted four weeks ago as part of the consultaton on EFSA's draft Opinion.

While stressing high scientific uncertainty, EFSA concludes that BPA does not pose risks to consumers taking account of exposure from both food and skin contact with BPA such as in thermal cash receipts.

However, partly reflecting lower than expected exposure data, it recommends cutting the temporary tolerable daily intake to one tenth of its current level to avoid even the low risks it identifies, notably to the kidney and liver.

Key studies

ANSES says many of its divergences with EFSA's assessment relate to different methodologies for classifying the relevance and reliability of studies.

For instance, EFSA found a major weakness in a study by *Rubin et al* which was considered by ANSES to be "a key study for the identification of hazards to the female reproductive system".

ANSES said it was "surprised" EFSA dismissed as unconvincing another study it considered to be key - by *Xu et al* on BPA's effect on memory and learning in mice.

The divergence is also explained by EFSA's tendency to separate effects rather than take an overview, says ANSES, such as in the evaluation of BPA's effect on metabolism.

"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."

ANSES also disputes EFSA's calculation of exposure to BPA, mentioning that it takes

no account of exposure in the workplace, and questioning its evaluation of exposure through skin and breathing.

EFSA has consulted separately on its exposure estimate and ANSES says it is not yet known to what extent this had been revised in light of the comments received.

EFSA is holding a public meeting later this month to discuss the conclusions it reached.

Under EFSA's founding regulation, it is required to present to the European Commission a joint document with ANSES investigating the differences between the two.

ANSES attacks EFSA's "weight of evidence" approach

The French food safety agency ANSES this week called on EFSA and other national food safety agencies to agree "objective criteria" for assessing studies on potential endocrine disrupting substances.

In its criticism of EFSA's work on Bisphenol A, ANSES also called into question the "weight of evidence" approach to risk assessment which the Authority used for the food contact material and is using for risk assessments on other substances.

ANSES said that there are "differences in interpretation" with regard to methodological limitations of BPA toxicity studies.

These concern 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," it said.

ANSES issued pretty strong criticism on EFSA's "weight of evidence" approach, saying it causes the body of data to become 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," said ANSES.

There is fragmentation which "can cause confusion for the reader."

ANSES also argues that the criteria for "likely" and "likely as not" in the weight of evidence approach are not clearly justified.

It accuses EFSA of granting "a low level of reliability to the data whether the lines of

evidence are strong or limited."

And it asks how divergences of working group members were taken into account.

Overall, ANSES considers the weight of evidence approach to be too subjective and to use ill defined terms.

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

The BPA draft Opinion is one of the first times EFSA has used the weight of evidence approach. However, the Authority has stated that it intends to use this approach for other assessments.

One of the key issues is whether "likely" and "likely as not" are meaningful terms which can be clearly defined.

CHEM Trust
Protecting humans and wildlife from harmful chemicals

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EFSA corrects its risk assessment of bisphenol A to acknowledge that experts didn't say 'no health concern'

by Michael Warhurst on April 2, 2015

CHEM Trust have discovered that the European Food Safety Authority (EFSA) have admitted that the abstract of January's risk assessment of the commonly used hormone disrupting chemical bisphenol A incorrectly stated that there was 'no health concern'.

CHEM Trust wrote to the EU Health Commissioner on 25th February complaining about EFSA's misrepresentation of the risk assessment and had a reply from DG Health dated 24th March. EFSA changed the text of the abstract on 25th March 2015, the next day. It seems highly likely that CHEM Trust's letter triggered this change by EFSA.

The new abstract notes (our emphasis):

> *"This Executive Summary of the Scientific Opinion, published on 25 March 2015,* ***replaces the earlier version published on 21 January 2015.****"*
>
> *"***** An editorial amendment was made to the abstract* ***to align the wording with that of the summary and body of the opinion****. This does not affect the contents or conclusions of the opinion. The original opinion is available on request, as is a version showing all the changes made."*

The original abstract stated (our emphasis):

> *"By comparing this t-TDI with the exposure estimates, the CEF Panel concluded that there* ***is no health concern for any age group from dietary exposure or from aggregated exposure****."*

We pointed out the error in this abstract in our blog on the day the opinion was published, 21st January 2015. The assessment actually said that there was 'low health concern from aggregated exposure', which is not the same as 'no health concern'.

The only change in the revised abstract is to correct the sentence we had highlighted as incorrect (our emphasis):

> "*By comparing this t-TDI with the exposure estimates, the CEF Panel concluded that there is no health concern for any age group from dietary exposure* ***and low health concern from aggregated exposure****."*

Michael Warhurst, Executive Director of CHEM Trust, said:

> *"CHEM Trust welcomes EFSA's belated admission that they misrepresented the results of the bisphenol A risk assessment in the abstract of the assessment.*
>
> *However, as we set out in our letter to EFSA's Director General, the incorrect press release remains on their web site, along with an incorrect briefing.*
>
> *They need to correct all their materials relating to this risk assessment, and announce that they are doing this, otherwise people will be confused as to the real outcome.*
>
> *EFSA must also establish – and reveal – how the abstract and communications materials ended up misrepresenting such an important issue"*

CHEM Trust sent a letter to EFSA's Executive Director on 31st March 2015 – details here – before we found that the abstract had been changed. We haven't yet received a reply.

For the latest information on bisphenol A in our blog, click here.

This story has been covered by Chemical Watch and Food Packaging Forum.

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@EFSA_EU correct their abstract

But their press release is still wrong!

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WP Admin

Conflicts of interets at EFSA – season 10*

Stéphane Horel
11 December 2012

(*EFSA celebrates its 10th anniversary this autumn)

On October 1st 2012, the European Food Safety Agency (EFSA) announced the creation of a working group on endocrine disrupters – a question that has become highly political. Ten of its eighteen members have conflicts of interests. Josef Schlatter, a Swiss toxicologist, received fundings from the tobacco industry during twelve years. The scientific work of only four of these experts is directly related to the field of endocrine disrupters.

A first preliminary meeting took place in Brussels on 18th-19th October in order to determine the composition of this working group. EFSA published the list of its members on December 3rd. Still, the proceedings of the discussions are still not available online. A spectacular gesture of transparency. Especially as the very existence of this group is subject to controversy.
To understand the context of the group creation, see at the bottom of the article « The context »*

The « Scientific Committee working group on endocrine active substances » comprises 18 experts.

A // Four members of EFSA's Scientific Committee
– Jan Alexander (chair)
– Anthony Hardy
– Robert Luttik
– Josef Schlatter

B // Three members of EFSA's Panel on « Plant Protection Products and their Residues » (with decoder : the Pesticides panel)
– Karen Hirsch-Ernst
– Susanne Hougaard-Benekou
– Daniel Pickford

C // Ten experts
–Jacques Auger – France
Reproductive biologist. Assistance Publique – Hôpitaux de Paris.
– Diane Benford – UK
Civil servant. Head of Chemical risk assessment unit at the Food standards agency.
– Susy Brescia – UK
Regulatory toxicologist, Health & Safety Executive.
– Gisela Degen – Germany
Toxicologist. Leibniz research Centre for working environment and human factors at Dortmund technical University (IfADo).
– Peter Hoet – Belgium
Researcher. Public health department Catholic university Leuven.
– Peter Matthiessen – UK
Ecotoxicologist. Consultant. Retired.
– Wim Mennes – Netherlands
Toxicologist. Dutch National Institute for Public Health and the Environment (RIVM).
– Thomas Platzek – Germany
Toxicologist. Head of toxicology of consumer products, German Federal Institute for Risk Assessment (BfR).
– Peter Pärt – Denmark
Doctor. Seconded expert. European Environment Agency (EEA).
– Emanuela Testai – Italy
Researcher at the Instituto Superiore di Sanità in Roma.
– Theo Vermeire – Netherlands
Doctor. Dutch National Institute for Public Health and the Environment (RIVM).

Conflicts of interests in the working group

All EFSA's experts have to fill in a declaration of interests (DoI) – an administrative document where they record their collaborations – remunerated or not – with the private sector during the previous five years. The aim is to allow EFSA to prevent any conflict of interest. « Omissions » can lead to sanctions. (Decision implementing EFSA's Policy on Independence and Scientific Decision Making Processes regarding Declarations of interests 21.02.2012)

1 – Jan Alexander (Chair) – Norway

Medical doctor. Toxicologist. Deputy Director-General of the Norwegian Institute of public health.
Member of several working groups at EFSA since 2006.
Vice-chair of EFSA's Scientific Committee (2012-2015).
No conflicts of interests in his 5.04.2012 declaration of interests.

2 – Jacques Auger – France

Reproductive biologist. Assistance Publique – Hôpitaux de Paris.
No conflicts of interests in his 4.12.2012 declaration of interests.

3 – Diane Benford – UK

Civil servant. Head of Chemical risk assessment unit at the Food standards agency.
Member of several working groups at EFSA since 2006.
In her 5.11.2012 declaration of interests, she declared :

= Member of expert group of the International Life Sciences Institute (ILSI) – Europe on « Data Selection for BMD Modelling » on behalf of the Food Standards Agency (09/2009 - 04/2012).
>> ILSI is an industry pressure group (see box 1 on ILSI below).

= Member of ILSI Europe expert group on the « Margin of Exposure approach to substances that are genotoxic and carcinogenic » on behalf of the Food Standards Agency (07/2006 - 01/2010).

>> A certain number of « omissions » in Diane Benford's DoI :
= Co-author of an ILSI publication : Benford D, Bolger PM, Carthew P, Coulet M, DiNovi M, Leblanc JC, Renwick AG, Setzer W, Schlatter J, Smith B, Slob W, Williams G, Wildemann T. *Application of the Margin of Exposure (MOE) approach to substances in food that are genotoxic and carcinogenic. Food Chem Toxicol*. 2010 Jan;48 Suppl 1:S2-24. Review. One of the co-authors, Josef Schlatter, is also a member of EFSA's working group (see below : Josef Schlatter).

= Member of the editorial board of the scientific journal « Regulatory Toxicology and Pharmacology ».
>> *Regulatory Toxicology and Pharmacology* is an industry-captured journal (see box 2 below).

= Speaker on behalf of ILSI at EUROTOX 2008 congress. « Overview of the work of the export group on MoE and report back on the workshop ». (Eurotox 2008 Report)

= Chair of the expert group on the « Application of the Margin of Exposure (MOE) Approach to Compounds in Food which are both Genotoxic and and Carcinogenic » at an ILSI workshop (1-3/10/2008, Rhodes, Greece).

>> Before 2007 :
= Co-chair at the FOSIE third plenary meeting. FOSIE is an ILSI research project co-funded by the European Commission. Lisbon, Portugal, 23-25/10/2002 (ILSI Europe-Newsletter49. Février 2003).

= Author of an ILSI monography « Principles of risk assessment of food and drinking water related to human health ». ILSI Europe concise monograph series, 2001.

= Author of an ILSI monography « The acceptable daily intake : a tool for ensuring food safety ». ILSI Europe concise monograph series, 2000.

Box 1 / International Life Sciences Institute (ILSI)

ILSI was created in 1978 by American giants of the food industry like Heinz, Procter & Gamble, General Foods and Kraft Foods. It was largely controled by Coca-Cola at its beginnings. Until 1991, besides, ILSI was presided by Alex Malaspina, one of the Atlanta's multinational vice-presidents.
ILSI's sources of financing have since diversified : major corporations selling food (Coca-Cola, Danone, Kraft Foods, Ajinomoto – world leader for food additives), chemistry (BASF, Dow Chemicals, DuPont...), pesticides and GMOs (Monsanto, Bayer CropScience, Syngenta...), detergents (Procter & Gamble, Unilever), pharmaceuticals (Pfizer, Merck...), and even oil (Exxon Mobil) are ILSI members. (Source : ILSI annual report 2011).
With the notable exception of Antarctica, all continents host an ILSI branch. The headquarter is established in Washington DC. The European offices opened in Brussels in 1986.
ILSI describes itself as a « nonprofit, worldwide foundation » which « bring[s] together scientists from academia, government, industry, and the public sector » and « seeks a balanced approach to solving problems of common concern for the well being of the general public ».
ILSI is in fact a lobby pressure group and has a sophisticated, efficient strategy. Many EFSA experts work for ILSI. Even the chair of EFSA's management board, Diana Banati. In a press conference in September 2010, European Green MEP José Bové revealed that she also was member of ILSI's Board of directors. Diana Banati resigned from EFSA in May 2012 after she was appointed ILSI executive and scientific director.
>> For a detailed account of the EFSA-ILSI connections, see the report by Corporate Europe Observatory and EarthOpenSource – *Conflicts on the menu*.)

Box 2 / The International Society of Regulatory Toxicology & Pharmacology & *Regulatory Toxicology and Pharmacology*

« Regulatory Toxicology and Pharmacology » is an industry-captured journal. Its editor Gio Gori has worked many years for the tobacco industry (Source : David Michaels. *Doubt is their product*. Oxford University Press, 2008).
The journal's owner, the International Society of Regulatory Toxicology & Pharmacology (ISRTP) is an association dominated by industry scientists and consultants of regulatory firms who work for the industry. ISRTP has organised several workshops on endocrine disrupters where presentations were made by representatives of firms like Monsanto and Dow Chemicals or regulatory consultancy firms like Exponent, Gradient Corp and The Weinberg group.

4 – Susy Brescia – UK

Regulatory toxicologist, Health & Safety Executive.
No conflicts of interests in her 14.11.2012 declaration of interests.

>> She left out :
= Speaker and session moderator at an ECETOC workshop on endocrine disrupters, « Risk assessment of endocrine disrupting chemicals » (Florence 9-10/05/2011 – Workshop report). The European Centre for ecotoxicology and toxicology of chemicals (ECETOC) is a lobby structure for the chemical industry.
>> Daniel Pickford also took part in the worskhop but only as an attendant. He nonetheless recorded that in his declaration of interests (see below : Daniel Pickford).

>> As a Health & Safety Executive employee, Susy Brescia represents the position of her country. Now, the UK took an official stand on the endocrine disrupters criteria issue in May 2011 (>> to understand what this is all about, see below « The Context »). In a joint position paper with the German Federal Institute for Risk Assessment (BfR), the UK favoured the approach of potency threshold cut-off criteria. Because it would filter out certain chemicals from a stricter regulation, the industry have a preference for it. Potency-based cut-off criteria are severely criticised by NGOs and by a leading endocrine disrupter expert, Pr Andreas Kortenkamp, who considers that « such values are largely arbitrary and not scientifically justifiable ».
Susy Brescia is also member of the AdHoc group on the definition and criteria of endocrine disrupters at DG Environment – European Commission (idem : to understand what this is all about, see below « The Context »). Because she represents her country, one can hardly imagine she would adopt an independent position. Besides, she presented the UK proposition in person at a Food Standards Agency workshop on endocrine disrupters on October 19th 2010.

5 – Gisela Degen – Germany

Toxicologist. Leibniz research Centre for working environment and human factors at Dortmund technical University (IfADo).
Member of the Scientific Committee on Consumer safety – SCCS (European Commission).

In her 23.10.2012 declaration of interests, Gisela Degen declared :
= Research project on phytoestrogen exposure in children at and before puberty funded by CEFIC / Long-range research initiative (LRI) (07/2007 - 12/2009).
>> The European Chemical Industry Council (CEFIC) is the European organisation for the chemical industry. It employs 72 people for its lobbying activities in Brussels. CEFIC devotes 6 millions euros to these activities, according to the information it recorded on a voluntary basis on the transparency registry of the European Parliament.

6 – Anthony Hardy – UK

Honorary Professor. Worked at the UK Department for Environment, Health and Rural Affairs (DEFRA).
Member of several working groups at EFSA since 2006.
Chair of EFSA's Scientific Committee (2012-2015)
In his 9.08.2012 declaration of interests, he declared :

= Member of ILSI « Task Force on Evaluation of Agronomic Practices for Mitigation of Natural Toxins » (09/2008 – 10/2010). (Evaluation of Agronomic Practices for Mitigation of Natural Toxins)

= Director of the Centre for Low Carbon Futures, a public structure at the University of York (04/2009 – 01/2010).
>> In his declaration of interests, Anthony Hardy does not mention the fact that the Centre is supported by about one hundred private partners. Note : among the members of the Centre's International advisory board is Ian Johnson, operations director at Coca-Cola.

= Unpaid chairman of Venturefest Yorkshire, a non-profit making organisation (since September 2009).
>> In his declaration of interests, Anthony Hardy explains that Venturefest Yorkshire organises an annual « one-day exhibition and expo of entrepreneurial Technical, Digital and Creative businesses from around Yorkshire ». The 2013 sponsors are in majority investment societies and commercial organisations.
What's more, Venturefest Yorkshire is organised by York Professionals, whose goal is, according to its website, to « promot[e] business opportunities ». Anthony Hardy is one of the directors, which he omits to mention in is declaration of interests.

7 – Karen Hirsch-Ernst – Germany

Toxicologist. Federal Institute for Risk Assessment (BfR).
Member of EFSA's Panel on Plant Protection Products and their Residues – PPR / Pesticides
Member of several EFSA working groups at EFSA since 2009.
No conflicts of interests in her 16.10.2012 declaration of interests.

Intellectual conflict of interests, though, like Susy Brescia (see hereabove : Susy Brescia) : Mrs Hirsch-Ernest is employed by the Federal Institute for Risk Assessment (BfR) which took an official stand on the endocrine disrupters criteria in May 2011 (>> to understand what this is all about, see below « The Context »). In a joint position paper with the German Federal Institute for Risk Assessment (BfR), the UK favoured the approach of a potency threshold cut-off criteria. Because it would filter out certain chemicals from a stricter regulation, the industry have a preference for it. Potency-based cut-off criteria are severely criticised by NGOs and by a leading endocrine disrupter expert, Pr Andreas Kortenkamp, who considers that « such values are largely arbitrary and not scientifically justifiable ».

Mrs Hirsch-Ernst is also co-author of an article in the scientific literature exposing the cut-off criteria favoured by BfR. She was thus personally involved in the decision-process of the institute.

Besides, she is a member of the AdHoc group on the defintion and criteria of endocrine disrupters at DG Environment – European Commission as a BfR representative (idem : to understand what this is all about, see below « The Context »). One can hardly imagine she would stand for a position that would different from her employer's.

8 – Peter Hoet – Belgium

Researcher. Public health department Catholic university Leuven.
Member of the Scientific Committee on Emerging and Newly Identified Health Risks – SCENIHR (European Commission).
In his 28.11.2012 declaration of interests, Peter Hoet declares :

= Board member of BelTox, the Belgian societey of toxicology and Ecotoxicology.
>> BelTox sponsors are the following firms : Janssen Pharmaceutica, Solvay (platina sponsors), Eusapharma,

UCB, GlaxoSmithKline Vaccines (gold sponsors), Umicore, Total Petrochemicals, ECETOC (silver sponsors). See the « bronze sponsors » on BelTox website.

9 – Susanne Hougaard-Benekou – Denmark

Toxicologist. Danish Environment Agency.
Member of several EFSA working group at EFSA since 2009.
Member of EFSA's Panel on Plant Protection Products and their Residues – PPR / Pesticides
No conflicts of interests in her 8.10.2012 declaration of interests.

10 – Robert Luttik – Netherlands

Ecotoxicologist. Dutch National Institute for Public health and the Environment .
Member of several working groups at EFSA since 2009.
No conflicts of interests in his 15.11.2012 declaration of interests.

11 – Peter Matthiessen – UK

Ecotoxicologist. Consultant. Retired.
In his 22.10.2012 declaration of interests, he mentioned :

= Consultant for Regulatory Science associates (RSA), a private consultancy firm (chemicals, pharmaceuticals) (05/2012 – 09/2012).

12 – Wim Mennes – Netherlands

Toxicologist. Dutch National Institute for Public Health and the Environment (RIVM).
Member of several working groups at EFSA since 2008.
No conflicts of interests in his 30.10.2012 declaration of interests.

13 – Daniel Pickford – UK

Ecotoxicologist. University lecturer at Brunel University – Institute for the Environment.
Member of EFSA's Panel on Plant Protection Products and their Residues – PPR / Pesticides
In his 16.10.2012 declaration of interests, Daniel Pickford declared :

= Research supervision financed by Syngenta. £35,000 over three years paid directly to Brunel University (05/2006 – 05/2009).

= Invited attendance at ECETOC Workshop on endocrine disrupters « Risk assessment of endocrine disrupting chemicals » (Florence 9-10/05/2011 – Workshop report). The European Centre for ecotoxicology and toxicology of chemicals (ECETOC) is a lobby structure for the chemical industry.

>> Some of the collaborations he mentioned in his 2.05.2012 declaration of interests no longer appear in his 16.10.2012 declaration. (NB : According to EFSA rules, only collaborations during the previous five years have to be recorded).

= Consultant for Syngenta (09/2003 – 05/2005).

= Research scientist employed by AstraZeneca (02/1999 – 06/2003).

>> During his time at AstraZeneca, Daniel Pickford carried out two studies on private funding :
= Assessing chronic toxicity of Bisphenol A on frog for CEFIC (the European organisation for the chemical industry), the Society of the Plastics Industry Bisphenol A Environmental Working Group (£89,000 in 2000). (Source : tab « research »).
= Conducting baseline studies on a marine snail for the Bisphenol A Environmental Research Task Group of the American Plastics Council (£174,000 in 2002). (Source : tab « research »).

14 – Thomas Platzek – Germany

Toxicologist. Head of toxicology of consumer products, German Federal Institute for Risk Assessment (BfR).
No conflicts of interests in his 16.10.2012 declaration of interests.

But Thomas Platzek, like Karen Hirsch-Ernst, is employed by the German Federal Institute for Risk Assessment (BfR) which took position for potency-based cut-off criteria for endocrine disrupters (>> see hereabove : Karen Hirsch-Ernst).

15 – Peter Pärt – Denmark

Doctor. Seconded expert. European Environment Agency (EEA).
No conflicts of interests in his 12.10.2012 declaration of interests.

16 – Josef Schlatter – Switzerland

Toxicologist. Swiss federal office of public health.
Member of several working groups at EFSA since 2009.
In his 15.11.2012 declaration of interests, he listed numerous conflicts of interests and a tight, decade-long collaboration with ILSI :

= Member of ILSI EUROPE expert group on the « Application of the Thresholds of toxicological concern (or TTC) Approach to Cosmetic Ingredients » (11/2011 – 04/2012). This work was done within the frame of COSMOS, a research project jointly funded by the European Commission and Cosmetics Europe (European federation of the cosmetics industry).
>> Josef Schlatter omits to mention that the project has the firms Henkel and Merck among its « partners » (Source : Partners of the COSMOS project).
>> More important, ILSI is the inventor – in the mid 1990s – of the Thresholds of Toxicological Concern (TTC) concept and has been its main promoter since then. TTC aim to determine a threshold beneath which no toxicological test is required before marketing a chemical. This approach *de facto* eliminates the possibility of a substance to have effects at low doses.
In 2008, curiously enough, EFSA deemed necessary to create a working group on TTC. A detailed investigation by NGO PAN-Europe revealed major conflicts of interests inside this group in 2011. Several members had been actively promoting TTC on the behalf of ILSI. Among them : Josef Schlatter, who was a co-author for several articles on TTC (Kroes 2004, ILSI 2000, Dybing 2002 also co-authored by Syngenta employee John Doe). See also the Le Monde article (in French) where journalist Stéphane Foucart relates that the chair of the group, Susan Barlow, had been working for Philip Morris until the end of the 1990s.

= Non-remunerated member of the Board of Trustees and member of the program strategy and stewardship committee of the Health and Environmental Sciences Institute (HESI), an ILSI structure. Scientific research on a « range of public health and environmental issues, for the most part on generic issues ». (01/ 2008 – 04/2012).
(List of HESI Board of trustees members in 2011)

= Member of ILSI Europe expert group « Application of the Scientific Criteria to Food Allergens of Public Health Importance » (07/2007 – 04/2012) and of expert committee « Eliciting Dose of Allergens in Food (07/2003 – 7/2007).

= Member of ILSI Europe « Risk Assessment of Genotoxic Carcinogens Task Force » and some of its experts groups : « Margin of Exposure approach to substances that are genotoxic and carcinogenic », « Data Selection for BMD Modelling » (07/2002 – 04/2012). Diane Benford also participated in this work which lead to an ILSI publication : Benford D, Bolger PM, Carthew P, Coulet M, DiNovi M, Leblanc JC, Renwick AG, Setzer W, Schlatter J, Smith B, Slob W, Williams G, Wildemann T. *Application of the Margin of Exposure (MOE) approach to substances in food that are genotoxic and carcinogenic. Food Chem Toxicol.* 2010 Jan;48 Suppl 1:S2-24. Review. (See hereabove : Diane Benford).

= Participant in workshops, annual meetings and congresses (co-)organized by ILSI and/or HESI (01/1999 – 06/2011).

= Member of ILSI Europe « Process-related Compounds and Natural Toxins Task force » (01/1999 – 04/2012).

= Member of ILSI Europe Scientific advisory committee « Nutrition, food safety, natural toxins in food » (01/1999 – 12/2010).

= Member of the European food information council (EUFIC) scientific advisory board since March 2009.
>> In his declaration of interests, Josef Schlatter presents EUFIC as a « non-profit organisation ». EUFIC is in fact a lobbying structure for the food industry financed by AB Sugar, Ajinomoto Sweeteners Europe, Bunge, Cargill, Cereal Partners, Coca-Cola, Danone, DSM Nutritional Products Europe Ltd., Ferrero, Kraft Foods, Mars, McDonald's, Nestlé, PepsiCo, Pfizer Animal Health, Südzucker and Unilever (List of members 2012).

= Ad-Hoc Consultant to the Flavor and Extract Manufacturers Association of the U.S. (FEMA) expert panel (10/2009-09/2011).

= Member of the International Society of Regulatory Toxicology & Pharmacology (ISRTP) since January 1998.
>> Josef Schlatter presents it as a « scientific society » but ISRTP is an industry-captured journal (see hereabove box 2)

= Finally, Josef Schlatter has worked a long time with the tobacco industry. All but madly elegant in a scientist's resume. As this collaboration took place at the beginning of his career, he had no obligation whatsoever to record

it in his EFSA declaration of interests. Nonetheless, here are a few details gathered during this investigation. Josef Schlatter benefited from fundings from the tobacco industry between 1972 and 1984. His thesis, in 1973, was about the effects of nicotine (Ref. A). Led under the direction of Pr Bättig at the Swiss Federal Institute of technology Zurich (ETH), his research was financed up to 237.500 US dollars between 1972 and 1974 (Ref. B). During the following years, Josef Schlatter obtained several research grants from the scientific commission of the Swiss association of cigarettes manufacturers in Friburg (Ref. C). In 1983, he wrote an article on second-hand smoke on the demand of the industry (Ref. D). A document dated 1984 explicitely says that the purpose of a study on second-hand smoke led by Josef Schlatter is to be « used as a souce of arguments for popularized press articles ». « The industry has to "get wet", because the general public is unilateraly informed at present. Mr Schlatter's work demonstrates once again that tobacco smoke can be a nuisance but that it is not harmful », says the document (Ref. E-F). The attendance of Josef Schlatter at the « Cigarettes working group » meetings of the Swiss industry is documented until November 1985 (Ref. G). In 1990, Mr Schlatter occupies a position at the Swiss federal office of public health... where he is an interlocutor for the tobacco industry regarding regulatory issues for additives in cigarettes (Ref. H).
(Download the documents in .zip)

17 – Emanuela Testai – Italy

Researcher at the Instituto Superiore di Sanità in Roma.
Expert for EFSA since 2010.
No conflicts of interests in her 13.11.2012 declaration of interests.

18 – Theo Vermeire – Netherlands

Doctor. Dutch National Institute for Public Health and the Environment (RIVM).
Member of the Scientific Committee on Emerging and Newly Identified Health Risks – SCENIHR (European Commission).
No conflicts of interests in his 16.10.2012 declaration of interests.

Conclusion in figures

On eighteen members, eight declared collaborations with the industry / the private sector :
– Diane Benford
– Susy Brescia
– Gisela Degen
– Anthony Hardy
– Peter Hoet
– Peter Matthiessen
– Daniel Pickford
– Josef Schlatter
Including three with ILSI.

Three experts have an intellectual conflict of interest because of the official position taken by their countries and/or their employer organisms :
– Susy Brescia, Health & Safety Executive, UK
– Karen Hirsch-Ernst, German Federal Institute for Risk Assessment (BfR), Germany
– Thomas Platzek, German Federal Institute for Risk Assessment (BfR), Germany

Apparently, EFSA did not see fit to include an expert in human endocrinology. A nothing less but surprising choice.
However, EFSA enroled three ecotoxicologists – who study the impact of chemicals on the environment (Robert Luttik, Peter Matthiessen, Daniel Pickford).

The scientific work of only four of these experts is directly related to the field of endocrine disrupters (Peter Matthiessen, Daniel Pickford, Emanuela Testai).

Finally, one can wonder whether a countries count reflects a politically-oriented choice from EFSA.
The group is dominated by the UK (5) and Germany (3) – which positions on the criteria for endocrine disrupters are crystal clear.
Then, we have : Netherlands (3), Denmark (2), Norway (1), Belgium (1), Switzerland (1), Italy (1). In the first version of the list (3rd December), France was not represented in the group. The name of French scientist Jacques Auger – a specialist in male fertility issues – was added on Thursday 6th December. Why would the Dutch National Institute for Public Health and the Environment (RIVM) have three representatives, the German BfR – two, and the French National Agency for Food, Environmental and Occupational Health & Safety (ANSES) not a single one ?
Note that ANSES acknowledges the low-dose effects of bisphenol-A since September 2011 (Collective expert report on health effects of bisphenol A). Which is not the case for EFSA.

In 2007, EFSA even increased the tolerable daily intake of bisphenol A from 10 to 50µg/d and reaffirmed its safety despite numerous studies showing its effects at low-doses (Scientific references on low-dose effects of bisphenol A gathered by Fred vom Saal in October 2006. For April 2012 update, see Fred Vom Saal's website).

The conclusions of EFSA's « Scientific Committee working group on endocrine active substances » will be published in March 2013.

The context

The European Commision has one year left to attribute a definition and precise criteria to endocrine disrupters. You wouldn't think, but it is an arduous task. All the more that this decision will impact the regulation of all chemicals in the European Union.

Using simple words, let's say that endocrine disrupters are chemicals that have the ability to hijack the hormonal system and influence the levels of hormones secreted by the body. Hormones are estrogens, testosteron, insulin, steroids, thyroid hormones etc. There are about 50 of them. No need to have a triple Ph. D. to understand that non-programmed variations in hormone levels can have an impact. And especially during pregnancy : hormones play a crucial role during the fetus development. Endocrine disrupters are linked to numerous « modern » diseases like declining male fertility, abnormalities of the genital tract of baby boys, cancers (testis, breast, prostate, thyroid), obesity, diabetes, brain development. To date, scientists have identified around 870 endocrine disrupters.
(For further explanations on endocrine disrupters, see Theo Colborn's TEDX or my documentary *The Great Invasion* – part 1, 2, 3, 4).

Using more scholarly terms, the Endocrine Society says : « An endocrine-disrupting chemical (EDC) is an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action ».
(Endocrine-Disrupting Chemicals and Public Health Protection: A Statement of Principles from The Endocrine Society)

But not everyone agrees on the choice of words. A scientific definition is one thing. A definition meant to be used within a regulatory frame is another one.

From that choice will stem the regulation of hundreds of chemicals that we use every day. Phthalates and bisphenol A are well known by the general public. But the decision also implies pesticides and biocides – this sweet name covers pesticides intended to kill cockroaches, fleas and other little bugs inside our homes.

To wich extent, in this time of crisis, economic stakes will weigh in Brussels's choice of words ? That is the question.

Direction (or DG) Environment started the decision process in 2011 by creating an AdHoc working group. DG Environment can rely on a report written by Pr Andreas Kortenkamp (State of the Art of the Assessment of Endocrine Disruptors). In June 2012, it organised an important conference. The whole work was supposed to end at the beginning of 2013.

Everything was on the right track until October 1st 2012. Then, to everyone's surprise, the European Food Safety Agency (EFSA) anounced that it would, too, set up a working group on endocrine disrupters. As its supervision authority, DG SANCO (Health and consumers) mandated EFSA.

This is not just about a bureaucratic squabble – DG SANCO stepping on DG Environment's toes. This is above all a proper by-pass of the process led by DG Environment that shocked all European NGOs (NGOS Letter to Commissioners Dalli and Potočnik).

This autumn 2012, EFSA celebrates its 10th anniversary in total denial of its more than questionable management of conflicts of interets. The European Parliament has expressed its concern about that precisely and refused to vote EFSA's budget discharge in April 2012. So has the European Court of auditors. On October 11th, it published a special report on the « management of conflicts of interests in selected EU agencies », including EFSA, and judged that « selected EU agencies did not adequately manage conflict of interest situations ». NGOs, for their part, have continuously documented the conflicts of interests at EFSA and their potential consequences for the health of the European population (See the detailed report by Corporate Europe Observatory and EarthOpenSource – *Conflicts on the menu*). Around twenty NGOs have troubled the Agency's anniversary festivity last November in Parma and have called for radical changes in EFSA's organisation.

For the record, EFSA is in charge of the authorisation of thousands of chemicals and products that end up in our food : GMOs, pesticides, additives, nanotechnologies. EFSA is also in charge of the bisphenol A case file.

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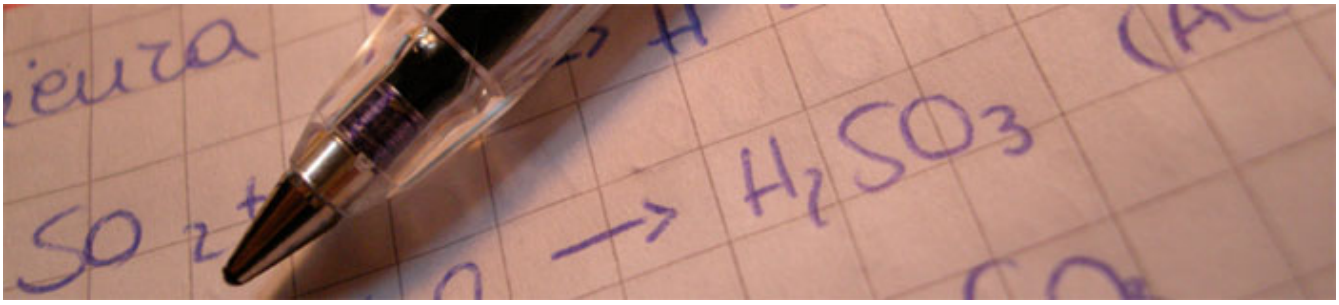
Link to the article : www.stephanehorel.fr/efsa

Scientist with extensive industry ties quits EU advisory panel

A German scientist who is critical of the European Union's plan to regulate chemicals and has extensive financial ties to regulated companies has resigned from a key scientific committee of the European Commission. Wolfgang Dekant was one of 18 scientists who authored a controversial editorial that criticized Europe's plans to regulate hormone-disrupting chemicals. A September investigation by Environmental Health News revealed that Dekant and 16 others had collaborations with companies and industry trade groups. A professor at the University of Würzburg, Dekant resigned last month from the European Commission's Scientific Committee on Emerging and Newly Identified Health Risks, which provides scientific opinions to EU decisionmakers on various topics. Dekant, who has served as an expert for the European Commission since 2000, said he resigned on his own initiative.



Valentina Pasquale/flickr

By Stéphane Horel
Environmental Health News

Oct. 15, 2013

A German scientist who is critical of the European Union's plan to regulate chemicals and has extensive financial ties to regulated companies has resigned from a key scientific committee of the European Commission.

Wolfgang Dekant was one of 18 scientists who authored a controversial editorial that criticized Europe's plans to regulate hormone-disrupting chemicals. A September investigation by Environmental Health News revealed that Dekant and 16 others had collaborations with the chemical, pharmaceutical, cosmetic, tobacco, pesticide and biotechnology industries.

A professor of toxicology at the University of Würzburg, Dekant resigned last month from the European Commission's Scientific Committee on Emerging and Newly Identified Health Risks. The committee provides scientific opinions to EU decisionmakers on various topics, such as antimicrobial resistance, fertility reduction and new technologies, such as nanomaterials.

Europe would be the first region in the world to adopt a specific policy that could lead to bans of some of these chemicals, which are used in everyday consumer products.

Dekant, who has served as an expert for the European Commission since 2000, said he resigned on his own initiative. His resignation letter was dated Sept. 6, after he signed the editorial that sparked a firestorm among scientists.

Toxicology Forum
Wolfgang Dekant

"Due to a number of recent developments which have severely restricted my time availability, I am not able to devote sufficient time to contribute to the work," he wrote to the Commission. "I therefore resign from the committee effective immediately."

Dekant said in an email that he had been considering resigning since "March 2013 onwards" because serving on the panel required traveling to Luxembourg and that the "very long opinions/large number of meetings require more time." Also, he said, the "topics seem to move away from my expertise."

"Members (and Commission) wanted me to stay," he said.

Frédéric Vincent, a spokesperson for the European Commission, declined to comment other than to confirm that Dekant resigned last month. Dekant's name, however, was not removed from the Commission's list of advisors until a few days after the EHN article was published.

"I don't know why Mr. Dekant resigned," said Michèle Rivasi, a French member of the European Parliament and vice president of the Green/EFA group. "But I know that the criteria of excellence that are required for members of the scientific committees imply that scientists are as independent as possible and respect the most basic rules of science ethics, like disclosing their interests."

EHN's investigation shows that Dekant has a long history of ties to companies that would be regulated by a policy that Europe is developing for endocrine disruptors – chemicals that can interfere with hormones such as estrogen, testosterone and thyroid hormones.

Included are bisphenol A, a controversial compound in hard plastics and food can liners; phthalates found in vinyl and fragrances; furniture flame retardants and pesticides. Lab animal studies and some human studies have linked these chemicals to reproductive problems, cancers, neurological effects and other diseases.

Stéphane Horel

The European Commission is headquartered in Brussels.

Europe would be the first region in the world to adopt a specific policy that could lead to bans of some of these chemicals, which are used in everyday consumer products. The stakes are high since companies worldwide that sell products in Europe would have to comply.

Earlier this year, Dekant and the 17 other scientists, all editors of scientific journals, signed an editorial published in 14 journals. They criticized a precautionary approach to regulating the chemicals outlined in a leaked draft proposal by Europe's Environment Directorate-General. They called the proposal "scientifically unfounded" and "defying common sense, well-established science and risk assessment principles."

The editorial sparked outrage from other scientists with no declared industry ties, including 41 who wrote in a rebuttal that it "appears to be intended as an intervention designed to impact imminent decisions by the European Commission." Another 104 scientists and journal editors wrote a second rebuttal calling the editorial "a profound disservice" to public health.

This debate focuses largely on two issues for the Commission: determining whether there are safe concentrations for endocrine-disrupting compounds and defining criteria to identify pesticides that would be removed from the market.

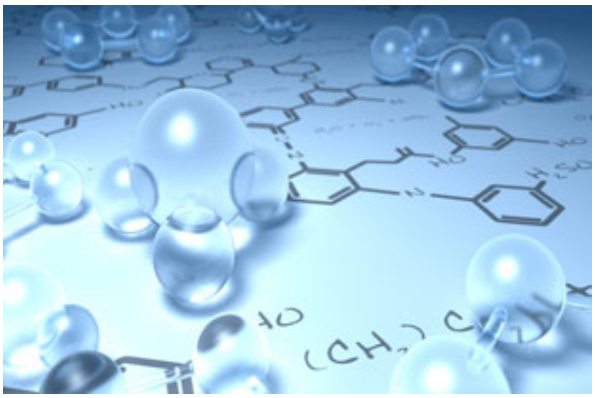
Of the scientists who signed the editorial, Dekant appears to have the most ties to industries that would be regulated by the new strategy.

Dekant, editor in chief of the journal *Toxicology Letters*, reported in an April 2013 online declaration of interest to the Commission's scientific committee that he had 18 consultancy contracts with companies. He did not disclose the company names, however.

Another document shows that Dekant received money from the Polycarbonate/BPA Global Group – part of the American Chemistry Council, which represents manufacturers of bisphenol A. He was paid for a 2008 review that concluded that "exposure to bisphenol A does not result in a health risk to the general population." During that same period, from 2006 through 2008, he served on the European Food Safety Authority's scientific panel, which reported that the EU's acceptable daily intake for BPA was safe.

Dekant also wrote a review on phthalates for a European group representing phthalates manufacturers, ECPI, in 2012 and a review on TBBPA, a flame retardant, for the global association of the brominated flame retardant industry, EBFRIP, in 2010.

He also co-authored a 2010 article on degradation products of pesticides with an employee from chemical and pesticide manufacturer BASF and a private consultant; and two studies on 3-(4-methylbenzylidene)camphor, a UV filter, with employees from this chemical's manufacturer – Merck, in 2006.



European Commission

Getting money from a mix of sources – including industry – is "the normal way" to do research. "You can't do research anymore if you don't go for money from all sources." –*Wolfgang Dekant*

BPA, phthalates, TBBPA, 3-(4-methylbenzylidene)camphor, and some pesticides are endocrine disruptors that could be regulated under the new EU policy.

Since 2011, Dekant also has been a member of the scientific advisory panel of the Research Institute for Fragrance Material, an organization for the fragrance, detergents and cosmetics industry, whose members include BASF, L'Oréal and Unilever. In 2007, he was an advisor for the German Association of the Automotive Industry.

Some of Dekant's research activities are supported by private benefactors, such as Honeywell, since 2006. For a 2013 study, he received funding from the Tetrahydrofuran Task Force, a consortium of U.S. manufacturers of tetrahydrofuran, European Chemical Industry Council (CEFIC) and The Toxicology Forum.

Dekant collaborated with an industry lobby group, International Life Sciences Institute (ILSI Europe), in 2005.

When asked about his industry ties, Dekant replied, "I don't consider conflict of interest as measure tool to judge scientific debate."

Getting money from a mix of sources – including industry – is "the normal way" to do research nowadays, Dekant said. "You can't do research anymore if you don't go for money from all sources."

Read EHN's investigation here.

Stéphane Horel, based in Paris, France, is a freelance journalist and documentarian who investigates conflicts of interest and influence on public health issues. She is working on a documentary about the regulation of endocrine disruptors in Europe.

Send questions or comments to EHN Editor-in-Chief Marla Cone at mcone@ehn.org

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Environmental Health News

18 October **In the public eye: Mascara exempt from UN mercury treaty.** Under a new global treaty that limits the use of mercury, some light bulbs will be banned. Some batteries, thermometers and medical devices will be banned

too. But mascara is exempt. Environmental Health News.

15 October **Scientist with extensive industry ties quits EU advisory panel.** A German scientist who is critical of the European Union's plan to regulate chemicals and has extensive financial ties to regulated companies has resigned from a key scientific committee of the European Commission. Wolfgang Dekant was one of 18 scientists who authored a controversial editorial that criticized Europe's plans to regulate hormone-disrupting chemicals. Environmental Health News.

10 October **Tropics first region on globe to hit a new climate era, research finds.** Forget, for the moment, melting Arctic ice and polar bears. The tropics – and the 5.5 billion people living there – will be the first region on the globe to experience a "radically different" climate. Daily Climate.

10 October **Opinion: A call for action in Minamata.** The U.N. mercury treaty now bears Minamata's name. This creates a special obligation to meet the demands of mercury-poisoned victims in the Japanese seaside city and transform a human tragedy into an opportunity for change. During negotiations of the treaty, the victims opposed naming the treaty after their decades-old, unresolved disaster. But they hope the pact will mean that no one ever again will suffer as they have. Environmental Health News.

10 October **The Minamata Convention: 12 things it does (or doesn't) do.** The Minamata Convention, a United Nations pact launched Thursday, is designed to limit mercury use and emissions internationally. Finalized after three years of negotiations and expected to be adopted by about 130 nations, it includes many exemptions. Here is what the treaty does – and doesn't do. Environmental Health News.

7 October **Mine may open next year as last-chance appeal languishes.** As a tribe awaits resolution of a last-chance appeal to stop mining in Michigan's Upper Peninsula, an international company is moving closer to production. The Keweenaw Bay Indian Community has challenged a state permit that allows sulfide mining to extract copper and nickel on public lands in the Upper Peninsula. Environmental Health News.

4 October **Mercury increasing in birds downstream of Canada's oil sands.** Mercury levels are increasing in the eggs of water birds that nest downstream of Canada's oil sands region, according to a new study. Eggs of Ring-billed Gulls collected from northern Alberta's Mamawi Lake in 2012 had 139 percent more mercury than in 2009. Environmental Health News.

3 October **Warming Lake Superior prompts a tribe to try a new fish.** On the shore of Lake Superior, the Keweenaw Bay Indians are raising walleye in addition to the traditional trout at their hatchery. They need to keep pace with their changing lake. A 'Climate at your Doorstep' story. Daily Climate.

2 October **Opinion: Tracking global warming trends? Think like a stockbroker.** In climate science, as with the stock market, the long-term trend completely overwhelms short-term fluctuations. Don't let the variability confuse you. Daily Climate.

1 October **The man who made sea-level rise go away.** Armed with a 168-slide PowerPoint and a message that resonates with North Carolina's conservative Legislature, John Droz has notched a remarkable record fighting sea-rise science, coastal development limits and renewable energy plans. Daily Climate.

1 October **From the Mac Wars, a political warrior emerges.** The man who helped persuade North Carolina lawmakers to ignore sea-rise predictions got a start in state politics fighting his school district over Apple computers. Daily Climate.

30 September **Coal fields to coast, a totem pole rallies tribes against exports.** Washington state's Lummi Tribe trucked a 22-foot tall totem pole across the West, following the coal trains in a bid to unit tribes against fossil fuel development and export. Daily Climate.

27 September **What we're seeing now: Climate scientists get Swift-boated.** Six years after the IPCC's massive Fourth Assessment Report was excoriated for a handful of errors, four years after the uproar over leaked emails put scientists on the defensive, the climate denial camp still controls the message. Daily Climate.

25 September **Flame retardants banned years ago finally declining in women, study suggests.** Scientists

have documented for the first time that banned flame retardants have declined in people in the United States, where levels of the chemicals had been growing exponentially. The small study, published today, reported that levels in pregnant California women were 65 percent lower than in a similar group of women tested three years earlier. Environmental Health News.

23 September **Special report: Scientists critical of EU chemical policy have industry ties.** An investigation by Environmental Health News reveals that of 18 scientists who signed a controversial published editorial, 17 have collaborated with the chemical, pharmaceutical, cosmetic, tobacco, pesticide or biotechnology industries. The stakes are high in the controversy because it involves the European Union's strategy to regulate hormone-altering chemicals – the first attempt in the world to do so. Environmental Health News.

Recent Environmental Health News coverage

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LETTER TO EDITOR

Report of Very Low Real-World Exposure to Bisphenol A is Unwarranted Based on a Lack of Data and Flawed Assumptions

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Key Words: **metabolism; biotransformation and toxicokinetics; pharmacokinetics; endocrine disruptors; endocrine toxicology.**

The article by Teeguarden *et al.* (2011) contains numerous flawed assumptions, omissions, and misrepresentations of the published literature on bisphenol A (BPA) that demand our rebuttal. We herein raise serious issues regarding the validity of the conclusions reached by the authors. The title itself, "24-h Human Urine and Serum Profiles of Bisphenol A During High Dietary Exposure," clearly states that study participants were exposed to high amounts of BPA. A fatal flaw of this study is the complete absence of the critical and necessary data concerning actual BPA levels in the meals fed to the small number (20) of adult participants isolated in a BPA-free clinical facility during the study. In fact, the results suggest that BPA exposure from many of the different meals was very low, the opposite of what the title states.

Teeguarden *et al.* state: "Very little $_{TOT}$BPA was eliminated after ingestion of all study breakfasts and lunches 1 and 2 evidence that these meals contained very little $_{TOT}$BPA relative to the other meal." This directly contradicts the misleading title and acknowledges that, in fact, an unknown but clearly low exposure to BPA occurred during much of the study. How this translates into "High Dietary Exposure" as used in the title is entirely unclear. The consequence was that all serum samples had no detectable unconjugated (biologically active) BPA, 86% of serum samples had no detectable total BPA, and 51% of urine samples taken between breakfast and lunch were below the level of detection for total BPA. The urine data vary sharply from detection of total BPA in urine from 93% of the U.S. general population, based on the National Health and Nutrition Examination Survey (NHANES) conducted by the U.S. Centers for Disease Control and Prevention (CDC) (Calafat *et al.*, 2008; Stahlhut *et al.*, 2009).

A serious omission in the "Discussion" section by Teeguarden *et al.* is a study showing that removal of known sources of BPA from the diet of five families resulted in a 66% reduction in total urine BPA levels from 3.7 ng/ml (16.2nM) preintervention to 1.2 ng/ml (5.3nM); a return to the diet being consumed before the experiment resulted in a rapid rebound to preintervention urine BPA levels, with 100% of samples containing measurable BPA (Rudel *et al.*, 2011). This finding clearly documents that removal of BPA from the diet reduces measurable BPA, whereas return to using BPA-containing products increases BPA levels—the classic scientific experimental design. This publication directly contradicts the conclusion by Teeguarden *et al.* that in the real world, there is little BPA exposure. This is further supported by over 20 publications reporting measurable serum unconjugated BPA in the general human population (Vandenberg *et al.*, 2010a) at median levels well above those that result in adverse effects in experimental animals (Prins *et al.*, 2011; vom Saal *et al.*, 2007). It is notable that none of the publications showing substantial BPA in human serum and other tissues from multiple experiments using a variety of methods are cited in the Teeguarden *et al.* study.

A likely explanation for the discrepancy between the CDC's NHANES and Teeguarden *et al.* data, and that also negates the exposure estimates cited by Teeguarden *et al.*, is that there are multiple unknown sources and routes of exposure to BPA. Thus, it is highly unlikely that all BPA exposure is due solely to leaching from cans and polycarbonate food and beverage containers. For example, it is known that thermal paper is coated with milligrams of free BPA (Mendum *et al.*, 2011), which could be just one of many nondietary sources of BPA, and cashiers have higher levels of BPA than people in other jobs (Braun *et al.*, 2011). In the Rudel *et al.* (2011) study in the real world, even after limiting BPA exposure via the diet, there remained detectable levels of

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BPA in all parents and children. The assumption that all BPA exposure is via the diet, and that BPA is rapidly and completely cleared from the body within 24 h, is also not supported by published data for total urine BPA in the CDC's NHANES study (Stahlhut *et al.*, 2009). Food and Drug Administration (FDA) administrators have stated that under the current laws, they do not have the authority to require reporting of products that contain BPA (FDA, 2010); this makes it impossible to account for the sources of exposure contributing to BPA levels that are detected in people after the few known food packaging sources are eliminated from the diet (Rudel *et al.*, 2011).

It is important to note that the authors of this study do not provide brand identification of the canned foods that were fed to the study participants. This is significant because published data and publicly available information have shown that within a given food type, high BPA levels are found in certain brands but much lower levels are measured in the same canned food from an alternate company. Furthermore, it is known that most cans used for canned fruit—a major portion of the diet in the Teeguarden study—do not use BPA-based epoxy lining on the main wall of the can. Consistent with this, Braun *et al.* (2011) report that consumption of canned fruit is not a predictor of higher BPA exposure. Consequently, in the absence of quantifying the BPA in food given to the study participants, there is no basis for the claim that they had High Dietary Exposure.

Since the investigators failed to directly measure BPA levels in the participants' meals and due to limited data from their study, they were forced to make exposure estimates based on other reports. The Teeguarden *et al.* exposure estimates are flawed in that they are all based on single oral bolus studies, although they examined BPA levels after ingestion in food. This matters for BPA because the assumptions concerning pharmacokinetic parameters from single oral bolus studies (Volkel *et al.*, 2002) have been shown to not be applicable to BPA consumed in the diet, which anyone familiar with differences in pharmacokinetics due to method of drug delivery would predict (Sieli *et al.*, 2011).

Instead of actually measuring BPA in the diet, Teeguarden *et al.* state that they used data from an oral bolus BPA administration study with a small number of people (Volkel *et al.*, 2002) to estimate exposure of participants in the present study. However, the Volkel *et al.* study itself has been criticized due to assay insensitivity and the inability to detect unconjugated BPA in serum owing to high background contamination relative to any other published study that measured BPA in serum or urine (Taylor *et al.*, 2011; Vandenberg *et al.*, 2010a, 2010b). The Volkel *et al.* (2002) study assay insensitivity was actually acknowledged by Teeguarden *et al.*: "The current results obtained using analytical methodology 10–45 times more sensitive than the previous human study by Volkel *et al.* . . ." However, in the process of developing a high-throughput assay for BPA, the sensitivity of the BPA assay reported in the Teeguarden *et al.* study has decreased fourfold from the detection limit reported by the CDC in 2005 (Calafat *et al.*, 2005), and other studies dismissed as flawed were more sensitive than the assay used by Teeguarden *et al.* (Vandenberg *et al.*, 2010a).

Teeguarden *et al.* draw the conclusion that "reported BPA concentrations in human blood of 1.4–19.2nM (Vandenberg *et al.*, 2010a) are highly unlikely in the general population." The idea that estimates of BPA exposure, given without actually measuring BPA intake, would be used to assure the public that there should be little concern about exposure to BPA, even though extensive other data contradict that conclusion, is very disturbing. Teeguarden *et al.* seek to simply dismiss all other studies contradicting their conclusions as flawed and state: "Such BPA blood concentrations would require 'oral exposure' to BPA 2–3 orders of magnitude greater than aggregate exposure levels derived from urinary BPA concentrations." To justify the above statement, Teeguarden *et al.* cite three articles (Dekant and Volkel, 2008; Lakind and Naiman, 2008, 2011) that were funded by The Polycarbonate/BPA Global Group of the American Chemistry Council, although these estimates have been deemed to be flawed by others (Vandenberg *et al.*, 2010a,b; vom Saal *et al.*, 2007).

The statements above by Teeguarden *et al.* indicate that they believe that assumptions based on models that lead to estimates (guesses) about exposure can be used to dismiss data from about 20 peer-reviewed published studies that used a wide variety of techniques and showed serum levels of unconjugated BPA in the 1–19nM range; this is similar to conclusions reached in all industry-funded studies (Vandenberg *et al.*, 2010a,b). This conclusion has been rejected as violating basic principles of science in a consensus report from a meeting on BPA held by the German EPA (Gies *et al.*, 2009). The consensus report identified that when data contradict a model, it is unacceptable to reject the data (based on unsupported allegations of contamination), but instead, scientists are required to change the model to account for the data.

Most disturbing is that Teeguarden *et al.* assure the public that BPA is not a concern for babies: "Taken together, our findings are evidence that on the basis of internal exposures to BPA, the active form of the compound, and the Prins *et al.* (2011) rat model of histological changes from neonatal exposure to BPA, that BPA promotion of PIN (early-stage prostate cancer) would not be expected in the general human population . . ." The Teeguarden *et al.* study did not measure BPA levels in babies, nor did it measure BPA in the general human population. Rather, they measured BPA in adult subjects isolated from the real world in a clinical research facility with controlled diets containing unknown amounts of BPA, although as the authors identify, in the majority of the diets, BPA levels were likely very low. That the public can be assured that babies are safe based on data presented in this study is preposterous, given that both drug and chemical (including BPA) metabolism in fetuses and newborns is known to be limited relative to adults (Taylor *et al.*, 2008, 2011).

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There is currently a plethora of data in experimental animal models that low-dose exposures to BPA during development that leads to blood levels of unconjugated BPA found in human fetuses (Vandenberg *et al.*, 2010a) increases adult risk for prostate and breast cancer and causes reproductive, immune, neurobehavioral, and metabolic abnormalities throughout life (Richter *et al.*, 2007; Vandenberg *et al.*, 2009). There are also published human data relating neurobehavioral problems in children to maternal levels of BPA during pregnancy (Braun *et al.*, 2009). Furthermore, there is ample evidence in animals and humans that adult low-dose BPA exposures have negative health consequences (Richter *et al.*, 2007). This study by Teeguarden *et al.* had the potential to add to our understanding of the contribution of dietary exposure to BPA on human serum and urine levels in a highly controlled environment without other sources of BPA exposure encountered in the real world. However, due to the flaws described above, this study actually has minimal value.

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Review

Evidence that bisphenol A (BPA) can be accurately measured without contamination in human serum and urine, and that BPA causes numerous hazards from multiple routes of exposure

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ABSTRACT

There is extensive evidence that bisphenol A (BPA) is related to a wide range of adverse health effects based on both human and experimental animal studies. However, a number of regulatory agencies have ignored all hazard findings. Reports of high levels of unconjugated (bioactive) serum BPA in dozens of human biomonitoring studies have also been rejected based on the prediction that the findings are due to assay contamination and that virtually all ingested BPA is rapidly converted to inactive metabolites. NIH and industry-sponsored round robin studies have demonstrated that serum BPA can be accurately assayed without contamination, while the FDA lab has acknowledged uncontrolled assay contamination. In reviewing the published BPA biomonitoring data, we find that assay contamination is, in fact, well controlled in most labs, and cannot be used as the basis for discounting evidence that significant and virtually continuous exposure to BPA must be occurring from multiple sources.

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Abbreviations: ACC, American Chemistry Council; AUC, area under the concentration time curve; BPA, bisphenol A; CERHR, Center for the Evaluation of Risks to Human Reproduction; CLARITY-BPA, Consortium Linking Academic and Regulatory Insights on BPA Toxicity; EDSP, US-EPAs Endocrine Disruptor Screen and Testing Program; EFSA, European Food Safety Authority; EPA, Environmental Protection Agency; FDA, US Food and Drug Administration; GLP, Good Laboratory Practices; LC–MS/MS, HPLC followed by tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantitation; NCTR, National Center for Toxicological Research; NHANES, National Health and Nutrition Examination Survey; NIEHS, National Institute of Environmental Health Sciences.

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Highlights

- The basis for the FDA declaring BPA safe is shown to be false by findings reported in a large number of studies.
- The FDA claims that BPA is a ubiquitous environmental contaminant that is so pervasive that their BPA assay is contaminated.
- Numerous studies with uncontaminated assays show human blood BPA levels are high and associated with common human diseases.
- The FDA's assay contamination hypothesis is a manufactured controversy to reject findings relating BPA to human diseases.
- Statements in the abstracts of articles by authors concluding that BPA is safe are often discordant with their own data.

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1. Introduction

Bisphenol A (BPA) is a high volume production chemical, with 15-billion pounds reported being produced in 2013 (GrandViewResearch, 2014). BPA is used in a wide variety of consumer products, including polycarbonate and other forms of plastics, resins used to line food and beverage containers, thermal print papers, and composites used in dentistry. Based on data from the National Health and Nutrition Examination Survey (NHANES), virtually all people in the USA are exposed to measurable levels of BPA (Calafat et al., 2008). BPA contaminates our air, water, and soil (EnvironmentCanada, 2008), and thus the pervasiveness of human exposure is not disputed (Calafat et al., 2008; Vandenberg et al., 2010a). BPA exposure appears to be from multiple routes on a near continuous basis, since only a portion the urine total BPA drops as a function of fasting time (Stahlhut et al., 2009).

Beginning in 1999, studies were published of the results of methods to measure BPA in human serum (Table 1). These initial studies reported determinations solely of the unconjugated (also referred to as aglycone or parent) BPA that is the biologically active endocrine disrupting molecule; BPA has estrogenic and anti-estrogenic activity and also disrupts other aspects of endocrine function (Reif et al., 2010). Endogenous hormones are evaluated clinically by the parent, hormonally active compound, not by less active or inactive metabolites, and BPA conjugates were reported to be devoid of estrogenic activity by a number of groups (Welshons et al., 2006). However, the possibility of *in vivo* deconjugation has not been examined, and recent work suggests other BPA conjugates have biological activities; specifically, BPA conjugates disrupt non-genomic, rapid estrogen-response systems associated with the cell membrane (Viñas et al., 2013).

The approaches required in method development for hormonally active chemicals such as BPA include controls, typically required for publication by endocrine journals, for specificity, accuracy, precision and sensitivity. Sensitivity is typically defined in assays of hormones as two or three standard deviations above background; therefore, to achieve high sensitivity, endocrine assays minimize variance and minimize or preferably eliminate background, i.e., contamination.

The reason that this issue is so important to endocrinologists is that the circulating levels of hormones, with their research and clinical implications, are very low, often below levels of detection using the most sensitive approaches in analytical chemistry. For example, free estradiol in fetal mouse and rat serum (measured by highly sensitive and specific radioimmunoassay and ultrafiltration dialysis) is below 1 pg/ml (Montano et al., 1995; vom Saal et al., 1997). Therefore, contamination, with what are by chemical analysis invisible levels, is a very substantial and serious problem in endocrine assays and taken very seriously by laboratories involved in endocrine assay development and use (vom Saal et al., 1990). For this reason, investigators studying hormones and endocrine active chemicals assume a high level of awareness and management of contamination, which may be missing or not assumed to be necessary in non-endocrine laboratory investigations. We will address these issues in this review.

2. Routes and sources of BPA exposure: Is assay contamination a significant problem?

2.1. Background

As will be discussed in more detail below, over the last 17 years there has been a huge amount of research conducted on the hazards of BPA in a wide variety of animal models, and there have also been a large number of studies conducted relating BPA to numerous diseases in humans. The published literature showing adverse effects of BPA is thus vast, and only a very small number of studies report being unable to detect any effects of BPA within the "low dose" range (Myers et al., 2009; Richter et al., 2007; vom Saal and Welshons, 2006; Vandenberg et al., 2013a); "low dose" refers to administered doses that are below the lowest levels typically examined in guideline studies for regulatory purposes (NTP, 2001).

In 2006 the National Institute of Environmental Health Sciences (NIEHS) sponsored a workshop that resulted in a consensus statement (The Chapel Hill Consensus Statement) signed by 38 experts from the USA, Europe and Japan that concluded the following about human exposure and blood levels of BPA: "Based on existing data we are confident of the following. 1. Human exposure to BPA is widespread. 2. Human exposure to BPA is variable, and exposure levels cover a broad range [central tendency for unconjugated BPA: 0.3–4.4 ng/ml (ppb)] in tissues and fluids in fetuses, children and adults." (vom Saal et al., 2007). Together with the extensive hazard data (Richter et al., 2007), which was supported by extensive evidence concerning the underlying mechanisms based on *in vitro* studies (Welshons et al., 2006; Wetherill et al., 2007), it seemed as if the argument about the safety of BPA and need to regulate it was over. However, the reasons that this did not happen will be discussed later.

2.2. Exposure models are used to reject BPA biomonitoring data

Estimates of sources and amounts of exposure to BPA differ markedly (Dekant and Volkel, 2008; Taylor et al., 2011; Vandenberg et al., 2010a). Of importance is that the different estimates of exposure to BPA are based on the exposure models that are used (Gies et al., 2009), with one set of pharmacokinetic models being based entirely on single intra-gastric gavage exposure (LaKind et al., 2008; Volkel et al., 2002). In contrast, other exposure models assume that gavage exposure alone is inadequate to explain human serum levels of bioactive BPA (Vandenberg et al., 2010a, 2010b, 2013b, 2014b).

Central to our review is an examination of data in studies (Table 1) reporting significant (ng/ml or parts-per-billion) concentrations of unconjugated, bioactive BPA in human serum (also see tables listing studies in Vandenberg et al., 2007, 2010a). These data have been rejected by industry-funded studies (Dekant and Volkel, 2008; LaKind et al., 2008) and subsequently in studies supported by the FDA (Patterson et al., 2013). The position that the FDA took in its 2008 draft risk assessment (FDA, 2008a) has not changed in spite of a dramatic increase in data over the last 6 years (Rochester, 2013; Vandenberg et al., 2013a). The FDA draft risk assessment did not adequately explain the basis for ignoring all of the published biomonitoring and hazard data by academic investigators, and the draft was rejected in the October 31, 2008 report by the FDA Science Board Subcommittee Report on Bisphenol A (FDA, 2008b). The Board stated in their review of the FDA's risk assessment that: "The draft FDA report does not articulate reasonable and appropriate scientific support for the criteria applied to select data for use in the assessment." The rejection of the published biomonitoring data because the data were not consistent with exposure models was also criticized by other scientists (Gies et al., 2009; Vandenberg et al., 2010b). Nevertheless, the current position of the FDA remains that rejection of published biomonitoring data reporting measurable

Table 1
Controls for contamination in human biomonitoring papers 1999–2013 (Y = yes; N = no).

Authors	Year	Detection method	Sensitivity (ng/ml)	Sample volumes	Std curve range (ng/ml)	Human Endpoint(s)	Levels found [ng/ml (ppb), mean ± SD or SEM]	Other chemicals measured	Contam eval before data collection?
Sajiki et al., 1999	1999	HPLC/ Electrochemical detection	0.2	0.2–0.5 ml serum or plasma	0–500	Healthy human serum:	0–1.6		Y
		HPLC/MS/ESI	0.1			12 women 9 men	0.33 ± ***0.16*** (0–1.6) 0.59 ± ***0.07*** (0.38–1.0)		
		MS/ESI	0.1						
Inoue et al., 2000	2000	HPLC/electro-chemical detection, or with - Coulometric array	LOQ 0.01 in solvent, 0.05 in serum 19844	3 ml/1.5 ml eq	0.1–100	Healthy human serum *n* = 5	0.32 ± 5%		
Schonfelder et al., 2002	2002	Derivatization- GC/MS	0.01 in serum			Fetal (cord) serum	2.9 ± 0.411		Y
						Maternal serum Placenta	4.4 ± 0.641 11.2 ± 1.5 12 ng/g tissue		
Yoshimura et al., 2002	2002	Derivitization-GC/MS GC/ECD	0.005 "0.15 pg/ml"	1 ml	0.01–10 pg/ml in serum	Pooled 20 human serum samples of ≥5 individuals/ serum pool	0.54 ± 0.037; range 0.39–0.92		Y
Kuroda et al., 2003	2003	HPLC fluorescence derivitization, column switching clean-up	0.04 (at signal/ noise = 3)	0.1 ml	0.1–7.0	Maternal serum from 9 healthy women at delivery	0.46 ± 0.067 range 0.21–0.79		
						Fetal cord serum	0.62 ± 0.043 range 0.45–0.76		
						21 Sterility female serum	0.46 ± 0.044 range 0.22–0.87		
						Ascitic (peritoneal) fluid	0.56 ± 0.041 range 0.15–0.88		
Tan and Mohd, 2003	2003	GC-MS	0.05	1 ml	0 - 50	Fetal cord plasma	ND – 4.05 (88% with positive detection)	Nonylphenol pesticides, other alkylphenols; 7 chemicals total	
Padmanabhan et al., 2008	2008	HPLC-ESI-MS/MS	0.5	0.8–1 ml	0.2–100	Maternal blood from 40 women at delivery	5.9 ± 0.94; range, ND-22.3		Y
Sprague et al., 2013a, 2013b	2013	SPE, HPLC tandem MS	0.24	4.5 ml		Human serum 264 postmenopausal women	Median 0.55;(ND – 8.77); two "outlier values" of 10.7 and 14.5 excluded from analyses; 69% pos for BPA	Mono-alkyl phthalates, propyl and butyl parabens, octylphenol, nonylphenol; estradiol	Y
Gerona et al., 2013	2013	SPE, HPLC-tandem MS, with direct simultaneous measures of BPA, BPA glucuronide and BPA sulfate	0.05 (LOQ 0.1)	0.25 ml	0.1–80	Human fetal cord serum, mid-21013 pregnancies	2.18 ± 8.10 GM 0.16 GSD 7.22 range ND – 52.26 (47% positive) total BPA 0.08–62.77, GM 0.79	BPA GlucuronideBPA SulfateTotal BPA as sum of direct measures	Y, Extensive

(*continued on next page*)

Table 1 (*continued*)

Authors	Contamination and other evaluated endpoints	Contam detected… (ng/ml)	…that was removed before assay?	Samples collected into vessels pre-tested <LOD or <LOQ?	Contam Control Blanks in Each Assay?	Blanks < LOD or <LOQ?	Pre-tested sample assay equipment to < LOQ?	Lab Bkgd BPA (ng/ml)	Assay "contamination-free"?
Sajiki et al., 1999	Commercial fetal bovine serum Commercial sheep plasma Bovine serum albumin Fresh sheep plasma Polycarbonate tubes (incubated with fresh sheep plasma)	Y Y Y N Y	Y	Y	Y	Y	Y (glass syringes, glass tubes, SPE cartridges)	<LOD	Y "In the experiments, no contamination was observed during clean-up and analysis of BPA. …" (Sajiki et al., 1999), p. 257
Inoue et al., 2000	Tap water Milli-Q DI water Disc-purified water SPE catridges	Y (0.01) Y (0.02) N Y	Y Y; MeOH pre-rinse	Y				<LOD	Y
Schonfelder et al., 2002		Y	Y	Y	Y	Y	Y	<LOQ	Y
Yoshimura et al., 2002	BPA-free water prepared by SPE filtration; SPE columns MeOH-washed		Y					<LOD	Y
Kuroda et al., 2003	Background noise = sensitivity defined 0.04/3 = 0.013 ng/ml							<LOD	Noise background = sensitivity 0.04/3 = 0.013 ng/ml
Tan and Mohd, 2003	Blank sample extractions parallel to serum sample extractions				Y, Blank extractions < LOD	Y	Y	<LOD	Y for BPA, and Y for all six other chemicals measured
Padmanabhan et al., 2008	Procedural blank; sheep serum; glass vs. plastic pipetting	<0.1 ng/ml	subtracted	Y	Y	Y	Y	<0.1 ng/ml (<LOD)	Y
Sprague et al., 2013a, 2013b	Solvent and method blanks < LOD; use of glass labware, prep-arative steps and 450 °C to remove potential con-taminants from lab-ware, handling of lab-ware and specimens in biosafety cabinets, and assessment of method blanks; serum collected into glass	all < LOD	Y	Y	Y	Y	Y	<LOD	Y, "Assessment of method blanks showed that iatrogenic contamination was lower than the limits of detection for BPA and phthalates.", published letter (Sprague et al., 2013b), p. 403
Gerona et al., 2013	Glass or polypropylene sample collection, hand-ling; all sample contact-ing or extracting materials and assay equip-ment; field control blanks with synthetic human serum	All < LOD	Y	Y	Y, Procedural quality controls and blanks in each assay	Y	Y	<LOD	Y, Extensive contamination evaluation and controls published with the report

unconjugated BPA in humans serum is justified based on the hypothesis that any study that reports finding unconjugated BPA in human blood must have experienced contamination (Churchwell et al., 2014).

The FDA's position regarding contamination may reflect the fact that they recently acknowledged that they have not been able to eliminate sources of contamination from their BPA LC–MS/MS assay: "Mean BPA aglycone levels in vehicle and naive control rat serum (0.02–0.5 ng/ml) indicated sample processing artifact, consistent with literature reports of a propensity for post-exposure blood contamination by BPA." (Churchwell et al., 2014). Other recent FDA-sponsored publications also report that their assays for BPA in the circulation of lab animals were contaminated by levels of BPA in a variable range of 2 ng/ml and even higher in adult and neonatal rats (Doerge et al., 2010a) and rhesus monkeys (Patterson et al., 2013). These authors insist that because they were unable to eliminate sources of contamination from their BPA assay, all other laboratories that measure BPA in blood must also be experiencing similarly uncontrolled contamination. The FDA scientists also indicated that contamination was present even after taking all precautions, despite the fact that other laboratories nationally and internationally achieve contamination-free BPA assays as a matter of course (Table 1).

In sharp contrast to the position of the FDA, a NIH-sponsored round robin study to assess whether laboratories could accurately measure BPA in human serum showed that, in fact, laboratories could accurately measure BPA without contamination (Vandenberg et al., 2014a). Similarly, the issue of assay performance was also examined using a round robin validation process in Europe for a number of chemicals, including BPA, which identified that some laboratories were able to accurately assay BPA and other chemicals without contamination, while other laboratories were unable to assay BPA or other chemicals accurately (Vanderford et al., 2014).

2.3. The NIH-sponsored round robin analysis of BPA in human serum

The recently published NIH round robin study addressed this issue of the ability to accurately assay BPA without contamination in human serum. Four participating laboratories screened materials to identify BPA contamination in collection and analysis materials. Serum was spiked with concentrations of unconjugated BPA and/or BPA glucuronide ranging from 0.09 to 19.5 ng/ml (unconjugated BPA) and 0.5 to 32 ng/ml (glucuronidated BPA). Samples were coded and provided by NIEHS blind to laboratories for LC–MS/MS analysis. To determine whether inadvertent hydrolysis of BPA metabolites occurred, samples spiked with only BPA glucuronide were analyzed for the presence of unconjugated BPA.

The results of the round robin clearly contradicted the FDA's universal contamination hypothesis (Vandenberg et al., 2014a). The round robin authors reported that: "BPA contamination can be controlled during sample collection and inadvertent hydrolysis of BPA conjugates can be avoided during sample handling". The study reported that (1) extensive sampling of collection materials indicated that contaminating BPA was not ubiquitous in collection materials or analytical methods, (2) precautions of screening and selection of materials and reagents, if taken before sampling, could manage BPA contamination to below the limit of detection (LOD), and (3) three labs of the four total were able to achieve contamination-free measures of unconjugated BPA in human serum. All laboratories were able to distinguish low, moderate and high concentrations of unconjugated BPA and glucuronidated BPA. Linear relationships between the amount spiked and the amount measured by the four participating labs ranged from 0.920 to 0.999 for unconjugated BPA and from 0.976 to 1.0 for BPA glucuronide (Vandenberg et al., 2014a).

In the NIH-sponsored round robin study, the limit of detection (LOD) was defined as three times (3 SD), and limit of quantitation (LOQ) was defined as 10 times (10 SD) of the standard deviations (SD) of three replicate analyses using the lowest calibration standard of BPA. The LOQ ranged from 0.01 to 0.13 among the laboratories participating in the round robin. The round robin authors stated that: "Each laboratory independently analyzed their sample extraction protocol, processing materials (including pipet tips, test tubes, cartridges, conical tubes, sample vials, water, methanol) and their liquid chromatography (injection needle, injection port, capillaries, column, mobile phase solvents, mobile phase reservoir) and mass spectrometry procedures (injection valve, ion source, collision cell, quadrupole detector) to ensure that their materials and reagents did not introduce BPA contaminations in the laboratory. In subsequent testing of the collection materials selected for the remainder of the Round Robin experiments, BPA was not observed in either water or stripped human serum, with the exception of a low concentration (0.17 ng/ml) measured in the water sample in one laboratory" (Vandenberg et al., 2014a).

The authors of the NIH-sponsored round robin made it clear that it was essential that equipment used in the sample collection process should be determined prior to sample collection to not contain potentially contaminating BPA. This should be examined for BPA using a solvent appropriate for extraction of a lipophilic compound. Thus, assays of stored samples from previously conducted experiments should only be assumed to be valid if potential sources of contamination had been examined and eliminated.

2.4. Claims that assay contamination is common are not supported by the published literature and data

Given the successful measurement of serum BPA in this round robin, an important question is why the FDA lab cannot do the same. The FDA chemists were initially participants in the NIH-sponsored BPA round robin analysis but withdrew prior to completion of the study. A question is why do experienced FDA scientists publish studies in which BPA contamination above 1 ng/ml is tolerated, accompanied by repeated claims that contamination is unavoidable? The contamination with BPA should be addressed and controlled to below the LOD before any further studies begin, and significant contamination with the chemical of study should not be tolerated in any further publications. The NIH-sponsored round robin and descriptions of assay control procedures in the prior biomonitoring literature demonstrate that this is feasible.

Importantly, it is clear that there are potential sources of BPA contamination that need to be determined and eliminated prior to assaying samples for BPA, which is also the case for other ubiquitous environmental pollutants. For example, in developing and validating our LC–MS/MS BPA assay, we identified and found clean substitutes for sources of water that were found to contain detectable background levels of BPA. Sources of BPA contamination have been identified and eliminated by other investigators, which demonstrates the ever-present need to examine equipment and utilize field blanks in experiments that measure BPA or other ubiquitous contaminants.

There are publications devoted to accomplishing this set of required preliminary studies prior to conducting analyses of samples (Salgueiro-Gonzalez et al., 2012). As correctly noted in a chemical-industry study, adequate control of contamination is required in order to publish results if analysis of human serum and urine levels are to be believed (Markham et al., 2010). Supporting the contention that BPA contamination can be controlled in human biomonitoring studies is a report from the CDC in which sources of contamination were identified and systematically eliminated during the successful development of LC–MS/MS assays for BPA and three other chemicals (Ye et al., 2013).

The potential for assay contamination is thus not unique to BPA, and simply requires the use of standard assay procedures and appropriate controls that should be routinely employed, which has also been the conclusion reached by others (Calafat and Needham, 2009; Markham et al., 2010; Vandenberg et al., 2014a). It is important to note that the two groups of scientists that have promoted the belief that BPA cannot be assayed in human serum without contamination, the FDA laboratory (Doerge et al., 2010a; Churchwell et al., 2014), and a plastic-industry funded laboratory (Dekant and Volkel, 2008; Volkel et al., 2002), are the two laboratories that in their publications have reported uncontrolled contamination and high background BPA in their assays.

FDA scientists (Patterson et al., 2013) stated in a recent publication that: "a significant body of evidence has shown that contamination from ubiquitous environmental sources of BPA during sample collection, storage, and analysis has a propensity to introduce artifactual aglycone [unconjugated] BPA in extracts from blood and tissues". Given this statement as a pillar for their argument that biologically active serum levels of unconjugated BPA reported in numerous studies can be ignored, along with the fact that it is contradicted by data we have presented earlier and inconsistent with standard analytic laboratory procedures, it is crucial to identify what exactly was stated in the eight studies Patterson et al. (2013) cited as support for their statement (the articles cited were: Doerge et al., 2012; Koch et al., 2012; Markham et al., 2010; Salgueiro-Gonzalez et al., 2012; Teeguarden et al., 2011; Twaddle et al., 2010; Vandentorren et al., 2011; Volkel et al., 2002). In fact, examination of these publications reveals that the Patterson et al. (2013) statement is not consistent with the conclusions drawn by most of the authors that they cite.

First, Koch et al. (2012) stated for the assay of BPA in urine: "… we were able to keep the laboratory blank value of BPA (caused by contamination with omnipresent BPA) below the LOD of 0.05 μg/L", and they also identify that this was true for plasma. Second, the objective of the Markham et al. (2010) study was "to account for and/or eliminate background contamination from all sources." Markham et al. reported on a comparison of BPA serum and urine assay performance in two laboratories, and they reported very good accuracy and precision, although this was reduced at the very lowest dose examined compared to higher doses, consistent with typical assay performance. Third, Salgueiro-Gonzalez et al. (2012) conducted an analysis of sources of blank contamination in BPA, as well as nonylphenol and octylphenol, assays and concluded that: "the main contamination sources in DLLME–LC–MS/MS were considered and >90% of the contamination could be removed by following the guidelines described here". These were standard procedures for identifying and eliminating sources of contamination in assays.

Another study cited by Patterson et al. was by Teeguarden et al. (2011), which is particularly interesting. Their study reports results of assays conducted by the CDC laboratory that subsequently reported on development of the BPA assay and procedures used to successfully eliminate sources of contamination for BPA as well as three other chemicals (Ye et al., 2013). In Teeguarden et al. (2011) study some serum samples that had been assayed by the CDC lab were re-assayed at the FDA lab, and again problems with contamination were identified.

The Vandentorren et al. (2011) study that was cited by Patterson et al. regarding ubiquitous contamination of samples in biomonitoring studies concerned contamination from urinary catheters and is thus irrelevant to the general issue of analysis of urine by methods other than via indwelling catheters. The Doerge et al. (2012) and Twaddle et al. (2010) studies involved the administration of deuterated BPA to mice and rats and were thus also not relevant to the issue of contamination of assays by authentic BPA from environmental sources (although information about contamination in another study from this lab using a BPA isotope is to be discussed later). In summary, eliminating contamination is required if the objective is a sensitive assay, and if the contamination is variable and a low constant background value cannot be subtracted from all assayed values, then accuracy and precision will be impacted and the assay will not produce valid results (Salgueiro-Gonzalez et al., 2012).

As discussed earlier, for a number of the studies cited by Patterson et al. (2013), assay contamination was not identified as interfering with measurement of unconjugated BPA. However the two laboratories that experienced contamination and a high background level of BPA included Volkel et al. (2002) and the FDA lab (Doerge et al., 2010a). For example, assay sensitivity was compromised by assay contamination in a study designed to measure BPA in human blood and urine after placing BPA contained in a capsule into the stomach of adult men and women (Volkel et al., 2002). Volkel et al. stated that: "When this method was used to quantify bisphenol A content from "unexposed" individuals, a small background of bisphenol A (up to 50 nM [11.4 ng/ml]) was detected in all urine and blood samples analyzed. Identical background concentrations as seen in the human blood and urine samples were also observed when water samples purified by a Millipore Water purification system were subjected to the workup procedures." In the Doerge et al. (2010a) study, they reported that: "All dosing was done using stable isotope labeled BPA to avoid contamination with unlabeled BPA from laboratory materials or other sources, which were found to be significant (buffer blanks contained approximately 2 ng/ml, data not shown)."

Even though a number of the authors of studies described earlier reported that contamination was identified and controlled, some of these authors then went on to state that contamination was, in fact, a problem. For example, in their abstract Markham et al. (2010) stated that: "Trace contamination of BPA from exogenous sources or hydrolysis of BPA-G to free BPA, either during or after biomonitoring specimen collection, may have contributed to the reported concentrations of free BPA." However, these authors actually reported that hydrolysis of conjugated BPA, which would lead to overestimation of unconjugated BPA, was not observed in their study, similar to findings reported in the NIH-sponsored round robin study (Vandenberg et al., 2014a). While Koch et al. (2012) agreed that: "circulating unconjugated BPA in blood (or serum) is of special interest for toxicological and mechanistic valuations, because only unconjugated BPA is regarded as hormonally active", they went on to accept that because gavage exposures lead to such a low percent of unconjugated BPA in serum (<1%), any findings of serum unconjugated BPA cannot be valid based on accepting that assays must be experiencing BPA contamination, even though they did not have a contaminated assay.

In the Ye et al. (2013) study from the CDC, despite the successful control of contamination, they strangely contradict themselves in the paper's title: "Potential external contamination with bisphenol A and other ubiquitous organic environmental chemicals during biomonitoring analysis: an elusive laboratory challenge". The casual reader would not expect that the conclusion drawn from their data was that eliminating contamination by BPA or other chemicals was, in reality, not "an elusive laboratory challenge". Thus, even though Ye et al. systematically identified and eliminated potential sources of BPA contamination, they emphasized in their conclusion that: "Unfortunately, until all of the environmental sources of these chemicals are known, totally eliminating external contamination is practically impossible. However, judicious application of the measures below will allow the identification of contamination scenarios, thus facilitating the implementation of measures to isolate and track external contamination and minimize as much as possible its recurrence and impact." A similar argument by the senior (corresponding) author on the Ye et al. study that serum BPA assays must always be contaminated recently led to a criticism that is was

"misuse of blood serum" to study the relationship between serum unconjugated BPA and mammographic breast density in postmenopausal women (Calafat et al., 2013). The authors of the original study had reported that there was a significant relationship between serum BPA and increased breast density (Sprague et al., 2013a), which is a marker of increased risk for breast cancer that has been shown to be a consequence of very low dose BPA exposure during development in a number of studies with laboratory mice, rats and monkeys (Soto et al., 2013; Tharp et al., 2012; Vandenberg et al., 2013a). In responding to this criticism the authors of the breast density study pointed out that numerous steps had been taken to ensure the absence of contamination (also identified in the initial published article), that method blanks were below the limit of detection, and that random contamination would tend to decrease, not increase, the likelihood of a statistically significant association (Sprague et al., 2013b). The conclusion by Ye et al. (2013) that eliminating contamination by environmental BPA "is practically impossible" directly contradicts that they did successfully eliminate BPA contamination, as did laboratories that participated in the Markham et al. (2010) study, the NIH round robin study, and many other laboratories for both biological (Gerona et al., 2013; Schonfelder et al., 2002) and environmental (Watabe et al., 2004) samples.

3. Unknown sources of contamination of control animals is unacceptable in experimental research

The FDA scientists promoting the idea that BPA contamination was to be expected and was the basis for finding unconjugated BPA in serum have acknowledged problems with BPA contamination not only in their assay procedures (Churchwell et al., 2014), but in addition, the animal research facility at the FDA's toxicology center (National Center for Toxicological Research, NCTR) also has an unidentified source of contamination (Delclos et al., 2014). Churchwell et al. acknowledged that their negative controls in an experiment with rats had been contaminated with BPA in the preliminary study conducted as part of an ongoing collaborative research program (identified by the acronym CLARITY). The contamination was from a source that they were unable to identify, and that serum levels of unconjugated BPA in the negative controls were not different from serum BPA levels detected in all six low dose groups in animals at postnatal day 80, and total serum BPA in the negative controls overlapped with total serum BPA levels in the lowest two BPA dose groups (Churchwell et al., 2014). The FDA scientists drew the conclusion that there were no adverse effects at low doses and stated: "Clear adverse effects of BPA …were observed only at the two high doses of BPA" (Delclos et al., 2014). This conclusion was harshly criticized as violating the basic principle of experimental research that a valid negative (reference) control is required to draw any conclusion of safety from an experiment and that the results of their contaminated animal experiment cannot be interpreted (Hunt et al., 2014).

The use of data from fundamentally flawed studies that are intended to be used to assure the public that BPA does not pose a risk to the public health has been ongoing under the supervision of product protection firms funded by chemical industry lobbying organizations, such as the American Chemistry Council (ACC), since the publication of "low dose" BPA findings beginning in 1997 (Nagel et al., 1997; Steinmetz et al., 1997; vom Saal et al., 1998). The results of the Nagel et al. study were disputed by two industry-funded studies (Ashby et al., 1999; Cagen et al., 1999). However, both of these industry-funded studies were declared flawed and rejected for inclusion by the National Toxicology Program CERHR panel's review of published BPA findings. In its final 2007 draft report to the NTP, the CERHR panel stated with regard to both the Ashby et al. and Cagen et al. studies that with regard to: "Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to absence of response of the positive control group" (CERHR, 2007).

Importantly, the initial findings by Nagel et al. (1997) were replicated and extended by Gupta (2000a, 2000b). This replication was acknowledged by a senior FDA scientist as resolving the dispute over the validity and reliability of our original findings (Sheehan, 2000). The issue of the need for appropriate controls (both negative and positive controls) in experimental research on endocrine disrupting chemicals such as BPA has been previously reviewed (vom Saal and Welshons, 2006; Welshons et al., 2003).

Since the study with contaminated negative controls was published by FDA scientists (Delclos et al., 2014), the concern is that the conclusions drawn by the authors will be uncritically accepted as valid, even though they have been harshly criticized, as were the editorial policies of the journal for allowing a flawed study to be published (Hunt et al., 2014). Previously, a group of 24 scientists, including us, identified the need for the editors of this same journal to understand, and incorporate into journal editorial policy, the importance of appropriate controls in experimental research (vom Saal et al., 2010). The second issue relating to publication of the Delclos et al. study by the FDA is that it was conducted using Good Laboratory Practices (GLP). A large group of scientists pointed out that GLP does not guarantee good science, just good record keeping, since the use of GLP was instituted as a result of fraud at commercial laboratories (Myers et al., 2009; vom Saal and Myers, 2010). The logical question here is: can a study with contaminated negative controls even be considered GLP-compliant?

An issue that should be considered in the future is whether regulatory agencies that are charged with making decisions regarding the safety of chemicals in commerce should be tasked with conducting scientific research (such as the FDA's CLARITY collaboration) that could reveal that the agency's prior assessments of safety were incorrect. This can create a potential conflict of interest.

4. The impact of age on pharmacokinetics of BPA

The most concern with exposure to endocrine disrupting chemicals is during fetal development and during early postnatal life through adolescence. During these critical periods in organ development permanent adverse effects can occur, including effects that can be transmitted across generations due to changes in the germ line (see Skinner review in this theme issue). The maxim in pediatric medicine is that babies are not little adults, and it is well understood that fetuses and babies are more susceptible to toxic exposures than adults (Bearer, 1995a, 1995b). A major factor in a criticism of the inadequacy of the US-EPAs Endocrine Disruptor Screen and Testing Program (EDSP) in a position paper by the Endocrine Society (Zoeller et al., 2012) was the absence of developmental assays that took the issue of age-related changes in susceptibility into account.

The increased susceptibility of fetuses and infants to exposure to BPA and other chemicals is, in part, due to the age-related change that occurs in the ability to metabolize BPA in all species that have been examined. The primary phase 2 BPA metabolizing enzyme, UDP-glucuronosyltransferase, is not expressed in the human fetal liver until after birth (de Wildt et al., 1999), and infant (5-day-old) rhesus monkeys have 3.8-fold higher unconjugated serum BPA values relative to adult monkeys after oral administration of the same single oral dose, based on the area under the concentration time curve or AUC (Doerge et al., 2010b). We have published that there is a virtually identical age-related increase in phase II metabolism of BPA in mice, with infant (3-day-old) mice (Taylor et al., 2008) having a 4.0-fold higher serum unconjugated BPA (based on the AUC) relative to adults (Taylor et al., 2011) after oral administration of the same dose of BPA. Matsumoto et al. (2002) reported that the BPA-conjugating enzyme (UDP-glucuronosyltransferase) was not detected

in the liver of fetal rats, but showed a linear 4.5-fold increase between postnatal days 3 and 21, at which age adult levels of BPA-glucuronide were reached.

In adult rats there is approximately 10-fold higher serum unconjugated BPA after IV administration relative to gavage administration (Pottenger et al., 2000); the lower unconjugated BPA in serum after gavage administration is a result of direct transport via the mesenteric vessels of BPA from the GI tract to the liver. However, due to the limited BPA conjugating activity of the liver in infant rats and mice, the effect of route of administration on the levels of serum unconjugated BPA that are achieved is greatly reduced (Prins et al., 2011; Taylor et al., 2008). Although, similar to other species, there was a steady increase in phase II metabolism of BPA between infancy and adulthood in rhesus monkeys (Doerge et al., 2010b), consistent with an age related change in BPA pharmacokinetics reported in other species, the authors of this study concluded in the abstract that: "No age-related changes were seen in internal exposure metrics for aglycone [unconjugated] BPA in monkeys." Thus, there was discordance between this statement in the abstract and actual findings concerning the fact that oral exposure to the same amount of BPA resulted in higher serum unconjugated BPA in infants relative to adults.

5. BPA pharmacokinetics in humans is similar to other species

The only previously published attempt at a human pharmacokinetic study involved placing BPA directly into the stomach by administering it in a capsule (gavage administration) (Volkel et al., 2002). This experiment was of limited value because the assay was about 10-fold less sensitive than current LC–MS/MS BPA serum assays (Vandenberg et al., 2014a). In addition, the study by Volkel et al. did not allow levels of serum unconjugated BPA to be determined because of the lack of control of background contamination (Volkel et al., 2002). While the study by Volkel et al. has been used to justify the conclusion that there should be no unconjugated BPA detected after exposure to BPA in humans, the insensitivity of the assay used precludes drawing this conclusion. We have thus had to rely on rodent, primate and other species, such as sheep (Corbel et al., 2013) to estimate human pharmacokinetics of BPA. The available evidence is that both rodent and primate data are relevant to human pharmacokinetics without allometric adjustment of pharmacokinetic parameters for body size, which is unexpected. The conclusion that there was a lack of need for allometric scaling across species was based on comparison of conjugated BPA levels in serum after oral administration in mice, monkeys and humans, where it was found that there was no significant difference in pharmacokinetic parameters over a 24-h period (Taylor et al., 2011).

Pregnancy involves complex physiological changes and there are species differences in the interaction between the mother, placenta and fetus (Corbel et al., 2013; Gerona et al., 2013; Inoue et al., 2005; vom Saal et al., 2014). However, unconjugated BPA, but not conjugated BPA, readily passes across the placenta from the maternal circulation into the fetus in all species examined (Gerona et al., 2013; Miyakoda et al., 1999; Padmanabhan et al., 2008; vom Saal et al., 2014; Zalko et al., 2003).

6. Important routes of exposure to BPA that are not modeled by intra-gastric gavage administration: Relevance of the serum conjugated/unconjugated BPA ratio

6.1. Sublingual exposure to BPA bypasses first-pass metabolism in the liver

Gayrard et al. (2013) reported that BPA is rapidly absorbed in the mouth, which is a known method for rapid (and virtually complete) uptake of drugs such as nitroglycerine. The Gayrard et al. study was conducted in dogs, which are an accepted model for human oral exposures. Gayrard et al. reported high sublingual absorption and bioavailability of BPA (about 70%), close to that from IV administration, but much higher than the <1% absorption and bioavailability values following gavage administration in side-by-side comparisons (Gayrard et al., 2013). This finding by Gayrard et al. thus contradicts the prediction by others that virtually all BPA exposure can be modeled by pharmacokinetics based on a single gavage administration, which bypasses sublingual absorption and leads to very low serum unconjugated BPA (Patterson et al., 2013).

The disagreement about the safety of BPA has been over the substantial difference between the estimated and measured human blood levels of unconjugated BPA. The estimate of very low, virtually undetectable, serum unconjugated BPA is based on exposure estimates from urine total BPA data coupled with the prediction that all human exposure to BPA can be modeled by an acute gavage administration that results in significant first-pass metabolism in the liver and less than 1% bioavailability of the administered dose. In contrast, there are numerous biomonitoring studies that report what would clearly be high enough unconjugated BPA to cause adverse effects (Vandenberg et al., 2007, 2010a). In fact, given the typical LOQ of 0.1 ng/ml for serum unconjugated BPA in LC–MS/MS assays (Vandenberg et al., 2014a), any detection of unconjugated BPA in human serum would be biologically active, based on a very large *in vitro* and experimental animal *in vivo* literature (reviewed in Vandenberg et al., 2013a; Welshons et al., 2003, 2006; Wetherill et al. 2007): as well as a large epidemiology literature [reviewed in Rochester, 2013). For example, Prins et al. (2011) reported that a 10 μg/kg oral dose of BPA (fivefold below the EPA's reference or safe daily dose 0f 50 μgkgday) resulted in a maximum concentration (Cmax) of 0.26 ng/ml unconjugated BPA in neonatal rat pups, with the consequence of an increased incidence of prostate intraepithelial neoplasia in adulthood. Angle et al. (2013) reported that a 20 μg/kg oral dose to pregnant mice resulted in a Cmax of 14 pg/ml unconjugated BPA in fetal blood and an average serum level over 24 h (based on the area under the concentration time curve or AUC) of 7 pg/ml (these data are based on administering tritiated BPA to the pregnant females as these levels would be undetectable by LC–MS/MS). Serum BPA within this range resulted in a wide range of hormonal and metabolic abnormalities, including glucose intolerance, in male offspring when examined during postnatal life (Angle et al., 2013). These data also emphasize that estimates of pharmacokinetics based on studies in adults are not valid for predicting pharmacokinetics in fetuses or infants, nor are they predictive of the types of adverse outcomes that can occur at very low serum concentrations of unconjugated BPA; clearly the results identified earlier would be labeled as adverse and were caused by concentrations of serum unconjugated BPA in the pg/ml (parts-per-trillion) range.

The importance of the data in Gayrard et al. is that they are the first experimental evidence that provides an answer regarding how relatively low exposures can lead to high blood concentrations of unconjugated BPA that have escaped first pass metabolism in the liver and that are clearly in the bioactive range. Regarding the model Gayrard et al. challenge, a paper funded by the trade organization Polycarbonate/BPA Global Group presents estimates concerning amounts and routes of human exposure to BPA (Lakind and Naiman, 2008). These estimates have become the basis for predicting the levels of unconjugated BPA in human blood from urine BPA data. This "leap of faith", as anyone knowledgeable about pharmacokinetics knows, is impossible unless one is confident that all routes and amounts of exposure are known and have been accounted for in the exposure model. Gayrard et al. reported that sublingual exposure can lead to very high absorption and bioavailability of BPA, resulting in high concentrations of unconjugated BPA in arterial blood, which is the blood circulating directly to tissues and cells,

while bioavailable BPA in blood is less than 1% after an acute gavage administration in dogs. This should not be surprising, since sublingual administration is the route used to rapidly deliver nitroglycerine into the blood (Narang and Sharma, 2011).

The current FDA model that all BPA exposure can be modeled by a single bolus gavage administration (Patterson et al., 2013) not only has to now take into account the high absorption and bioavailability of BPA associated with sublingual exposure reported by Gayrard et al., but also underestimates BPA exposure via other routes that can bypass first-pass metabolism, such as dermal exposure from thermal receipt paper coated with milligram levels of free BPA per gram paper (Hormann et al., 2014; Mendum et al., 2011). If one accepts the NHANES data that fasting time does not show the predicted inverse relationship to urine BPA (Stahlhut et al., 2009), then there has to be concern that exposure estimates based on an acute gavage exposure are significantly underestimating human exposure to BPA.

6.2. Thermal receipt paper as a source of transdermal exposure to BPA that bypasses first-pass metabolism in the liver

Free, unpolymerized BPA is present in the print surface of thermal paper, which is used for airline tickets, gas, ATM, cash register and other types of receipts. The print surface of thermal paper is coated with milligrams of free BPA per gram paper as a heat-activated print developer (Hormann et al., 2014; Mendum et al., 2011), and free BPA appears to be readily transferred to anything that the thermal paper contacts (Liao and Kannan, 2011), although the characteristics of the material contacted by thermal paper impact the amount of BPA transferred (Hormann et al., 2014). We have recently completed a study of the consequence of adult men and women holding a thermal receipt coated with BPA after using a commonly used skin-care product containing dermal penetration enhancing chemicals (these are chemicals used to enhance transdermal drug delivery but are commonly found in skin-care products). The volunteers also ate French fries that were picked up with the BPA-contaminated hand that had held the receipt paper (Hormann et al., 2014). We found rapid transfer (due to holding a thermal receipt for 2 s) of hundreds of micrograms of free BPA from the surface of thermal receipt paper to the hand. Maximum levels of free BPA were swiped off of the surface of the hand after holding a receipt for only 45 s (over 500 μg of BPA was swiped from the surface of the hand). Importantly, many people touch thermal receipts multiple times per day and may hold the receipts for variable periods of time.

There are many factors that impact the ability of compounds to pass through skin, including differences due the location of skin on the body, gender, age, molecular weight and lipophilicity (Singh and Morris, 2011), in addition to the use of personal care products that contain chemicals that impact the integrity of the dermal barrier (Funke et al., 2002; Karande and Mitragotri, 2009). While lipophilic compounds such as BPA ($\log P = 3.4$) can pass through skin (Zalko et al., 2011), regulatory agencies have assumed that this route of human BPA exposure should be limited in spite of the lack of data and acknowledged "significant uncertainties" around the issue of human exposure to BPA from thermal paper (EFSA, 2013). However, a factor that has not been considered in estimating transdermal exposure to BPA from thermal paper is that many skin-care products, including hand sanitizers, lotions, soaps and sunscreens, contain mixtures of chemicals that are also used as dermal penetration enhancers to increase the transdermal delivery of drugs. The dermal penetration enhancing chemicals present in personal care products as well as hand sanitizers cause a breakdown of the dermal barrier that normally functions to reduce transdermal absorption (Funke et al., 2002; Karande and Mitragotri, 2009).

Our data provide the first evidence that the use of very large amounts of free BPA as a developer on the print surface of thermal paper (~20 mg BPA/g paper) could be an important factor in accounting for the high levels of bioactive serum unconjugated BPA reported previously in human biomonitoring studies (Vandenberg et al., 2010a). We conducted this study to mimic aspects of the behavior of people in a fast-food restaurant where we observed people handling a thermal receipt prior to picking up and eating food with their hands after using hand sanitizer. In both men and women there was a dramatic increase in serum unconjugated BPA after holding thermal receipt paper and then eating French fries with the BPA-contaminated hand. While we only examined five men and five women, the data suggest that absorption through the skin is more rapid in females relative to males, consistent with men having a thicker stratum corneum (the outermost layer of the epidermis) relative to women (Fitzmaurice and Maibach, 2010; Polak et al., 2012). Thus, the skin of females may allow greater transdermal transport of BPA relative to males due to sex differences in skin permeability (Singh and Morris, 2011). One possible contributor to the sex differences we observed would be a greater use of skin cream in females than in males, which could impact both the transfer of BPA to the hand from the surface of thermal paper as well as transdermal penetration of BPA.

Our finding that thermal receipt paper is a potential source of high exposure to BPA are supported by data showing that environmental contamination caused by the use of unpolymerized (free) BPA in thermal paper is widespread (Liao and Kannan, 2011). While BPA was reported to be absorbed through pig and human skin *in vitro* (Zalko et al., 2011), our data show that use of hand sanitizer containing dermal penetration enhancing chemicals significantly enhanced by over 100-fold extraction of free BPA from the surface of thermal receipt paper, thus providing a much greater amount of BPA to be absorbed through the skin.

6.3. The significance of high vs. low ratio of conjugated BPA/unconjugated BPA in serum

When examining all of our data for serum unconjugated and conjugated BPA, a critical finding is that the ratio of conjugated BPA/unconjugated BPA was similar to the ratio observed by Gayrard et al. (2013) after sublingual administration in dogs; the ratio we observed and Gayrard et al. observed was close to 1:1 rather than >100:1 observed after gavage administration in dogs (Gayrard et al., 2013) and rhesus monkeys (Patterson et al., 2013). These new findings have to be considered in relation to prior findings that thermal paper also poses an occupational risk with regard to increased BPA exposure (Braun et al., 2011; Ehrlich et al., 2014). Together with the findings by Gayrard et al. regarding high serum levels of unconjugated BPA after sublingual absorption of BPA (Gayrard et al., 2013), these findings further challenge predictions that high levels of biologically active unconjugated BPA in blood are not possible.

Rhesus monkey data showed that continuous exposure to BPA via Silastic capsules produced a profile of conjugated BPA/unconjugated BPA in maternal serum that ranged from 0.99:1 to 3.87:1 during pregnancy (vom Saal et al., 2014). Because the ratios obtained from continuously exposed animals are more similar to the profiles observed in cross-sectional studies in people, where the ratio of conjugated BPA to unconjugated BPA is less than 10:1 (Gerona et al., 2013; Liao and Kannan, 2012a), our results suggest that continuous exposure (via subcutaneously implanted capsule) may better model human exposures than oral bolus exposure one time per day. This is important because in the prior NTP-CERHR panel analysis of the BPA literature up to 2007, all studies that used non-oral sources of BPA administration were eliminated from consideration in the assessment of potential hazards caused by BPA, which dramatically reduced their level of concern in assessing the hazards posed by BPA (CERHR, 2007).

6.4. Incompatibility between observations in human exposure data and predictions based on pharmacokinetic models based on gavage

There are four kinds of paradoxes between the observations in the current human exposure data and the predictions of the current pharmacokinetic model for BPA of sole-oral exposure and acute-rapid metabolism. First, the published biomonitoring data require at least some kind of non-oral exposures to be accepted as valid (as discussed in the prior sections) because circulating levels of unconjugated BPA in human serum are higher than are predicted from acute oral intake models; reviewed median serum unconjugated BPA values are reported to be 1 or 2 ng/ml, extending from high sub ng/ml to over 10 ng/ml (Vandenberg et al., 2007, 2010a). As shown in Table 1, the prediction of contamination for many of these studies is not credible given the method development and explicit contamination controls, including field blanks, in many of these studies.

Second, as reviewed in Vandenberg et al. (2010a), human urinary BPA and human serum BPA (nonpregnant and pregnant) concentrations are reported in similar overlapping ranges of low ng/ml, which vary substantially from the acute studies where ratios of up to 250-fold higher in urine than in serum are reported (Teeguarden et al., 2011). Rapidly cleared chemicals are found with a high ratio in urine relative to serum, but bioaccumulated chemicals, such as PCBs, are found at similar concentrations in both urine and serum. The discordant urinary-serum ratio from acute studies further suggests that acute pharmacokinetics do not model the reality of human BPA exposure.

Third, the decline in urinary BPA with time of fasting, which would be predicted to be rapid based on data from acute pharmacokinetic studies, was in sharp contrast, only partial with a decrease of approximately one-third between 4 and 8.5 h fasting time, and with no decrease in urinary BPA being observed after 8.5 h fasting time in an evaluation of the NHANES BPA data set (Stahlhut et al., 2009). This evaluation of the NHANES BPA data set could be explained by continuous BPA exposure, or by reduced clearance rates after prolonged exposures or both, but the data are not consistent with current models of rapid clearance within a few hours of ingestion and that all human exposure is from food and beverage packaging and modeled by gavage exposure.

Fourth, there are a small number of current reports and some unpublished data of the ratio of circulating BPA conjugates to unconjugated BPA, ratios which are much smaller than predicted by solely oral route of exposure. For example, Schonfelder et al. (2002) published the first data on unconjugated BPA in maternal serum with a median of 3.1 ng/ml (mean 4.4 ng/ml), while Lee et al. (2008) published the first total BPA (after enzymatic hydrolysis) in maternal serum with a median value of 2.73 ng/ml (mean 9.04 ng/ml). Additional unpublished human serum data sets have been reported at meetings with ratios of conjugated BPA/unconjugated BPA of 6 or 10, discordant with acute oral pharmacokinetic predictions of ratios over 100:1. These new data will have to be published for this issue to be resolved. While this issue is being resolved, an unbiased approach needs to be applied in analyzing serum BPA data. In the Teeguarden et al. (2011) study, some serum samples were eliminated from consideration because the ratio of conjugated BPA to unconjugated BPA did not match the profile that would be predicted after gavage administration. Discarding data that do not fit your model is clearly unacceptable (Gies et al., 2009).

7. Extensive BPA hazard data dispute the assumption that bioactive levels of BPA cannot occur in humans due to rapid first-pass metabolism

There is a complete lack of knowledge of exposure information and hazards for the great majority of even high volume chemicals; the lack of information was pointed out in the 1997 report "Toxic Ignorance" (EDF, 1997), and the situation has not changed since then. Thus, the huge amount of information based on hundreds of BPA publications reporting data on human and wildlife exposure, *in vitro* mechanisms, developmental and adult health effects in laboratory animal studies, as well as a large number of epidemiological studies, is highly unusual. One fascinating aspect of why this is the case is the controversy created by the aggressive response of the chemical industry to the initial findings that a BPA dose 25,000-times lower than had been previously tested in animals disrupted development of the male reproductive system in mice, associated with an unexpectedly high free concentration of BPA in serum relative to estradiol due to limited binding to plasma estrogen binding proteins (Nagel et al., 1997; vom Saal et al., 1998). The elevated free serum BPA due to reduced binding to plasma estrogen-binding proteins is similar to another estrogenic drug with which BPA shares many characteristics, diethylstilbestrol or DES (Nagel et al., 1999; Sheehan and Young, 1979; Welshons et al., 2003, 2006).

The idea that the high dose testing paradigm used in chemical risk assessments, followed by linear extrapolation using safety factors, was an approach that could not be applied to any endocrine active compound challenged the core assumptions of chemical risk assessments (vom Saal and Sheehan, 1998; Vandenberg et al., 2012; Welshons et al., 2003; Zoeller et al., 2012). If these challenges to the assumptions used in chemical risk assessments were to be accepted as valid, it would mean that the FDA in the USA and European Food Safety Authority (EFSA) in Europe, as well as other regulatory agencies around the world, would have to acknowledge that their assessments of "safe" exposure levels for endocrine disrupting chemicals were no longer valid. The aggressive reaction to the possibility that very low doses of endocrine disrupting chemicals could have effects that were unpredicted by high dose studies (vom Saal and Hughes, 2005), which everyone knowledgeable about hormonally active compounds knows to be possible (Vandenberg et al., 2012), led scientists from a wide range of disciplines outside of toxicology to test for effects of BPA. BPA turned out to impact a wide range of systems other than just nuclear estrogen receptors based on both *in vitro* and *in vivo* experiments. For example, in the EPA's ToxCast program, Reif et al. (2010) developed a weight-of-evidence Toxicological Priority Index (ToxPi) score based positive findings using a battery of 467 *in vitro*, high-throughput screening assays, and BPA had the third highest ToxPi score out of 309 environmental chemicals that were tested. This finding is consistent with the now enormous published literature reporting adverse effects of BPA at low exposure levels (Peretz et al., 2014; Richter et al., 2007; Rochester, 2013; vom Saal et al., 2007; Vandenberg et al., 2013a). When combined with massive amount of hazard information, biomonitoring data identifying high levels of unconjugated BPA in human serum are clearly of concern (Vandenberg et al., 2007, 2010a, 2010b).

The problem from a public health perspective of the blanket rejection of human biomonitoring data is that if there are sources of BPA exposure that are not modeled by gavage exposure, then there should be great concern with the health effects of BPA that have been identified. Our concern with this issue is based on published findings from hundreds of experimental animal studies for "low dose" effects of BPA; there was a NIH-sponsored review of published studies up to 2007 (Richter et al., 2007; vom Saal et al., 2007). Follow-up reviews of additional hundreds of studies published between 2007 and 2013 were recently published (Peretz et al., 2014; Vandenberg et al., 2013a). In addition, there have been over 90 epidemiological studies, both cross-sectional and prospective, reporting relationships between total BPA in urine and a wide array of adverse health outcomes, including a significant increase in the likelihood of developing cardiovascular disease and type 2 diabetes, obesity, impaired liver function, impaired immune and kidney function,

inflammation, reproductive effects in women (polycystic ovary syndrome, altered ovarian response to hormones, reduced fertilization success, implantation failure, endometrial disorders, reduced embryo quality, miscarriage, premature delivery and breast cancer), reproductive effects in men (reduced libido, sperm quality, altered sex hormone concentrations and embryo quality), altered thyroid hormone concentrations, and neurobehavioral deficits such as aggressiveness, hyperactivity and impaired learning (Rochester, 2013; Vandenberg et al., 2013a). The estimate of the costs per year of additional cases of just cardiovascular disease in the USA attributable to BPA is $1.5 billion (Trasande, 2014).

For government public health agencies to reject such a massive amount of information about the hazards of BPA, that are remarkably consistent across hundreds of *in vitro* mechanistic studies and experimental animal studies as well as dozens of human studies, one would imagine that there would be a very high level of certainty regarding routes of BPA exposure rather than this decision being based on estimates derived from models (Lakind and Naiman, 2008) that are contradicted by other findings (Gayrard et al., 2013; Hormann et al., 2014; Stahlhut et al., 2009; Vandenberg et al., 2010b).

The majority of experimental studies of the hazards due to exposure to BPA have been conducted with rodents, which are often criticized as not being predictive of effects in primates. However, unconjugated BPA levels in pregnant rhesus monkeys (vom Saal et al., 2014) that were within the range reported in numerous human biomonitoring studies (Vandenberg et al., 2007, 2010a) were reported to have adverse effects in a series of studies examining fetal tissues (ovary, mammary gland, brain, lung, uterus and heart) from the female fetuses carried by pregnant rhesus monkeys (Calhoun et al., 2014; Chapalamadugu et al., 2014; Elsworth et al., 2013; Hunt et al., 2012; Tharp et al., 2012; Van Winkle et al., 2013). The monkey fetus findings for ovary, mammary gland and brain recapitulate previously reported effects from numerous studies in rodents (reviewed in Vandenberg et al., 2013a). This underscores the importance of pharmacokinetic data based on measuring unconjugated BPA in serum rather than estimating serum unconjugated BPA based on levels of total BPA in urine. Data from this cohort of monkeys demonstrate that low, human-relevant concentrations of unconjugated BPA in maternal and fetal serum disrupt normal fetal development in a primate model and produce effects similar to those observed in rodents.

8. Conclusions

The universal contamination hypothesis appears to be a manufactured controversy (Michaels and Monforton, 2005) without a basis in the literature (Table 1). The data concerning the validity of contamination-free assays demonstrating that unconjugated BPA can be accurately measured in human serum obtained with contamination-free sample collection procedures have now been confirmed (Vandenberg et al., 2014a). The prediction that any assay that detected unconjugated BPA in human serum must have had an unknown source of BPA contaminate the sample is thus not supported by data and ignores that numerous published biomonitoring studies were careful to examine potential sources of contamination.

Regulatory agencies in the USA and Europe are using models and estimates of human exposure based on a single gavage route of administration to reject published data (Gies et al., 2009) by holding on to the assumption that all detected unconjugated BPA in human serum is due to contamination (Churchwell et al., 2014). Here we reviewed evidence demonstrating that this assumption has been falsified by controlled studies from a number of laboratories (Markham et al., 2010; Vandenberg et al., 2014a). Since there remain some laboratories that report being unable to control assay contamination (Churchwell et al., 2014), there is legitimate concern about the usefulness of data from studies in which there is inadequate information provided regarding assay performance or high background contamination is evident.

A significant problem encountered in reviewing publications related to these issues is that conclusions stated in abstracts are often discordant with data in the results section of articles, and those interested in these issues thus must carefully review the actual data before drawing conclusions. Similar problems were faced as debate occurred over the safety of the use of lead in products such as paint (Markowitz and Rosner, 2000) and the safety of second-hand tobacco smoke (Ong and Glantz, 2001).

In summary, contamination of assays from environmental sources should not be tolerated in studies of endocrine disrupting chemicals such as BPA. Statements that elimination of contaminating BPA cannot be achieved in biomonitoring studies are disputed by laboratories in round robin blinded studies reporting the ability to eliminate contamination. Assay development before sample analysis to accepted standards for clinical laboratories in which environmental sources of contamination are identified and eliminated needs to be a requirement for publication of data.

Competing financial interest declaration

FVS and WVW have no conflicts to declare.

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Why Public Health Agencies Cannot Depend on Good Laboratory Practices as a Criterion for Selecting Data: The Case of Bisphenol A

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Background: In their safety evaluations of bisphenol A (BPA), the U.S. Food and Drug Administration (FDA) and a counterpart in Europe, the European Food Safety Authority (EFSA), have given special prominence to two industry-funded studies that adhered to standards defined by Good Laboratory Practices (GLP). These same agencies have given much less weight in risk assessments to a large number of independently replicated non-GLP studies conducted with government funding by the leading experts in various fields of science from around the world.

Objectives: We reviewed differences between industry-funded GLP studies of BPA conducted by commercial laboratories for regulatory purposes and non-GLP studies conducted in academic and government laboratories to identify hazards and molecular mechanisms mediating adverse effects. We examined the methods and results in the GLP studies that were pivotal in the draft decision of the U.S. FDA declaring BPA safe in relation to findings from studies that were competitive for U.S. National Institutes of Health (NIH) funding, peer-reviewed for publication in leading journals, subject to independent replication, but rejected by the U.S. FDA for regulatory purposes.

Discussion: Although the U.S. FDA and EFSA have deemed two industry-funded GLP studies of BPA to be superior to hundreds of studies funded by the U.S. NIH and NIH counterparts in other countries, the GLP studies on which the agencies based their decisions have serious conceptual and methodologic flaws. In addition, the U.S. FDA and EFSA have mistakenly assumed that GLP yields valid and reliable scientific findings (i.e., "good science"). Their rationale for favoring GLP studies over hundreds of publically funded studies ignores the central factor in determining the reliability and validity of scientific findings, namely, independent replication, and use of the most appropriate and sensitive state-of-the-art assays, neither of which is an expectation of industry-funded GLP research.

Conclusions: Public health decisions should be based on studies using appropriate protocols with appropriate controls and the most sensitive assays, not GLP. Relevant NIH-funded research using state-of-the-art techniques should play a prominent role in safety evaluations of chemicals.

Key words: bisphenol A, endocrine disruptors, FDA, Food and Drug Administration, GLP, good laboratory practices, low-dose, nonmonotonic, positive control. *Environ Health Perspect* 117:309–315 (2009). doi:10.1289/ehp.0800173 available via *http://dx.doi.org/* [Online 22 October 2008]

Regulatory agencies in the United States and the European Union (EU) have justified the decision to declare the estrogenic chemical bisphenol A (BPA) safe at current levels of human exposure based on a few studies conducted using Good Laboratory Practices (GLP). In contrast, these agencies have rejected for consideration in their risk assessment of BPA hundreds of laboratory animal and mechanistic cell culture studies conducted by academic and government scientists reporting harm at very low doses of BPA. These studies were rejected primarily because they were not conducted using GLP. We suggest that decisions based on this logic are misguided and will result in continued risk to public health from exposure to BPA, as well as other manmade chemicals.

GLP is a federal rule for conducting research on the health effects or safety testing of drugs or chemicals submitted by private research companies for regulatory purposes. The GLP outlines basic guidelines for conducting scientific research, including the care and feeding of laboratory animals, standards for facility maintenance, calibration and care of equipment, personnel requirements, inspections, study protocols, and collection and storage of raw data (Goldman 1988). These regulations were developed in response to widespread misconduct by private research companies; this misconduct was possible because their data usually do not go through the rigorous, multistage scientific review that is normal for academic data funded by federal agencies and published in the peer-reviewed literature. The lack of these safeguards from academic science had enabled fraud. The U.S. Food and Drug

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Administration (U.S. FDA) first issued rules for GLP in 1978 after a 2-year federal investigation into sloppy laboratory practices of a number of private research companies (Lublin 1978; Markowitz and Rosner 2002). What began as serious concerns about poor quality research expanded into a criminal investigation of Industrial Bio-Test (IBT), one of the largest private laboratories at the time and a subsidiary of Nalco Chemical Company. In response to the federal investigation, the U.S. Environmental Protection Agency (EPA) demanded that 235 chemical companies reexamine the > 4,000 tests conducted by the laboratory. In 1983, three men from IBT were found guilty of deliberating doctoring data and were sentenced to prison (Lublin 1978; Markowitz and Rosner 2002). The fraudulent practices of IBT brought into question 15% of the pesticides approved for use in the United States. That same year, the U.S. EPA issued similar GLP rules for regulatory testing.

Both the U.S. FDA (2008a) and European Food Safety Authority (ESFA 2006) have recently published documents demonstrating that their decision to continue to declare BPA safe at current exposure levels was based primarily on the results of a few industry-funded studies that followed GLP guidelines. These decisions stand in stark contrast to the decisions concerning the potential risks to human health reached by a panel of 38 experts at a U.S. National Institutes of Health (NIH)-sponsored conference, who published The Chapel Hill Consensus Statement (vom Saal et al. 2007), as well as five review articles (Crain et al. 2007; Keri et al. 2007; Richter et al. 2007a; Vandenberg et al. 2007a; Wetherill et al. 2007). These peer-reviewed articles covered approximately 700 articles concerning BPA and represented a comprehensive review of the literature as of the end of 2006. In addition, the U.S. FDA draft decision contradicted the conclusions reached by the National Toxicology Program (NTP), which had spent 2 years investigating this question (NTP 2008). An important role of the NTP is to advise the U.S. FDA about the science relating to toxic chemicals in food, but in an unusual move, the U.S. FDA chose to release its draft report before the release of the final report on BPA by the NTP and without indicating who at the U.S. FDA was involved in preparing the draft report (U.S. FDA 2008b). At a hearing on 16 September 2008 regarding the draft report on BPA, the U.S. FDA announced that their goal was to have a subcommittee of the U.S. FDA Science Board complete a review of the draft decision by the end of October 2008. This would presumably also involve review by the subcommittee members of the approximately 1,000 articles relating to BPA.

We believe that the methods employed in chemical industry–sponsored GLP studies are incapable of detecting low-dose endocrine-disrupting effects of BPA and other hormonally active chemicals. Detecting endocrine-disrupting effects at low doses of chemicals such as BPA requires sophisticated and modern assays and analyses that have been developed in advanced, usually federally funded laboratories over the past decade. This is especially apparent when one examines what is now known about functional effects of BPA on a wide range of end points (Richter et al. 2007a; Welshons et al. 2006; Wetherill et al. 2007). These end points include those mediated by recently discovered estrogen response pathways initiated in human and animal cell membranes (nonclassical or alternative estrogen response mechanisms), which multiple laboratories have shown to be equally sensitive to BPA and estradiol in terms of activating effects in human and animal cells at low picomolar through low nanomolar concentrations (Alonso-Magdalena et al. 2008; Wetherill et al. 2007; Wozniak et al. 2005; Zsarnovszky et al. 2005).

The effects of BPA documented in these studies include a diverse array for which there are no data from GLP studies because the end points have not been examined: altered metabolism related to metabolic syndrome (Alonso-Magdalena et al. 2005, 2006, 2008; Ropero et al. 2008); altered adiponectin secretion (Hugo et al. 2008), which is a condition predicting heart disease and type 2 diabetes (Lang et al. 2008); altered epigenetic programming leading to precancerous lesions of the prostate (Ho et al. 2006); differential growth patterns in the developing prostate (Timms et al. 2005); abnormal growth, gene expression, and precancerous lesions of the mammary glands (Soto et al. 2008); and adverse effects on the female reproductive system, including uterine fibroids, paraovarian cysts, and chromosomal abnormalities in oocytes (Newbold et al. 2007; Susiarjo et al. 2007). There is also a large literature on neuroanatomic, neurochemical, and behavioral abnormalities caused by low doses of BPA (Leranth et al. 2008; Richter et al. 2007a), which also are not capable of being detected by current GLP studies conducted for regulatory purposes because of their out-of-date assays.

The approaches used by academic and government scientists to study the potential health hazards of BPA contrast sharply with those still used by the chemical industry that are relied on by regulatory agencies in the United States and Europe, including the two studies identified by both the U.S. FDA and European Food Safety Authority (EFSA) as central to the decision to declare BPA safe at current human exposure levels (Tyl et al. 2002, 2008a). By using outdated and insensitive assays that were supposed to have been replaced by a new battery of screens and tests by 2000 [as mandated by the U.S. Congress in 1996 in the Food Quality Protection Act (1996), but which has, as yet, still not occurred], these studies conducted using GLP fail to find any adverse effects.

Reliability and Validity

Reliability and validity are separate issues, although in the experimental research described here, validity and reliability basically refer to research that is credible. Golafshani (2003) noted that "reliability" refers to the extent to which results are consistent over time and are an accurate representation of the total population under study. Of central importance is that the results of a study must be reproduced under a similar methodology to be considered to be reliable. "Validity" refers to whether the research measures what it was intended to measure, and valid findings are considered to be true. In other words, reliability is determined by whether the results are replicable, whereas validity is assessed by whether the methods used result in finding the truth as a result of the investigator actually measuring what the study intended to measure.

Use of GLP in Regulatory Decision Making

Despite strong evidence of aberrations caused by low doses of BPA in animals exposed during fetal and neonatal life in studies conducted by the world's leading academic and government experts in the fields of endocrine disruption, endocrinology, neurobiology, reproductive biology, genetics, and metabolism, a relatively small number of studies reporting no adverse effects at low doses of BPA have continued to be promoted by the chemical industry and used by regulatory agencies (e.g., Ashby et al. 1999; Cagen et al. 1999; Tyl et al. 2002, 2008a). According to the U.S. FDA, these are accepted because they used GLP (U.S. EPA 2008), with the implication that studies not employing GLP are not reliable or valid (U.S. FDA 2008a).

GLP does not guarantee reliability or validity of scientific results. Unfortunately, although GLP creates the semblance of reliable and valid science, it actually offers no such guarantee. GLP specifies nothing about the quality of the research design, the skills of the technicians, the sensitivity of the assays, or whether the methods employed are current or out-of-date. (All of the above are central issues in the review of a grant proposal by an NIH panel.) GLP simply indicates that the laboratory technicians/scientists performing experiments follow highly detailed U.S. EPA requirements [or in the EU, Organization for Economic Co-operation and Development (OECD) requirements] for record keeping, including details of the conduct of the

experiment and archiving relevant biological and chemical materials (U.S. EPA 2008).

These record-keeping procedures in GLP were instituted because of widespread misconduct being committed by commercial testing laboratories (described above). These fraudulent results were possible because contract laboratory studies used in the regulatory process are rarely subject to the checks and balances that peer-reviewed, replicated scientific findings undergo. Without that acid test of reliability (replication by other independent scientists), other procedures were needed. Hence GLP was implemented, despite its severe limitations.

NIH-funded research subject to more stringent reviews than GLP. Although few NIH-funded investigators adhere to GLP-mandated record keeping, the procedures of GLP are actually surpassed by the procedures required for NIH-funded science published in peer-reviewed journals. NIH-funded studies pass through three phases of peer review that are far more challenging than GLP requirements. First, the principal scientists must have demonstrated competence to conduct the research, and experimental methods, assays, and laboratory environment must involve use of state-of-the-art techniques to be competitive for NIH funding. Second, results are published in peer-reviewed journals, with detailed evaluations by independent experts examining all aspects of the study. And third, the findings are challenged by independent efforts to replicate; for example, the initial findings concerning the stimulating effects of estrogenic chemicals on the mouse prostate (Nagel et al. 1997; vom Saal et al. 1997) were independently replicated and extended by Gupta (2000), which led to an editorial identifying "initial results confirmed" (Sheehan 2000).

Typically, within a laboratory, interesting findings are also followed by subsequent publications extending the prior findings; examples include the findings of BPA effects on β cells in the mouse pancreas (Alonso-Magdalena et al. 2005, 2006, 2008) and the effects of estrogenic chemicals and drugs on the developing mouse prostate that followed earlier findings (described above) from this same group (Timms et al. 2005; Richter et al. 2007b). In particular, independent replication by competent, respected scientists is the main criterion of acceptance of the findings as having been demonstrated to be reliable and having been validated by virtue of coming to the same conclusion using a variety of sophisticated techniques in multiple publications.

An important criticism of the approach taken by the U.S. FDA in its assessment of the now approximately 1,000 articles on BPA is that it appears to have made no attempt to connect the dots between replicated studies; instead, the U.S. FDA appears to have assessed each study without regard to whether it had been confirmed by other studies.

Thus, collectively, many phases used to verify the reliability and validity of NIH-funded published research have been completely ignored by the U.S. FDA, whereas industry-funded GLP research is rarely, if ever, subject to these central requirements and yet is accepted by regulatory agencies as reliable and valid.

The U.S. FDA's misguided gold standard. In this light, the U.S. FDA's reliance upon GLP as the gold standard is scientifically misguided. Furthermore, U.S. FDA administrators are ignoring published critiques of the GLP studies it considers reliable and valid, such as the study by Tyl et al. (2002) and two coordinated studies conducted at the same time by Ashby et al. (1999) and Cagen et al. (1999). Each was an industry-funded study conducted using GLP. Each was harshly criticized in peer-reviewed publications by academic scientists and government panels [Center for the Evaluation of Risks to Human Reproduction (CERHR) 2007; NTP 2001; vom Saal and Hughes 2005; vom Saal and Welshons 2006]. Yet, the U.S. FDA and EFSA panels still assert that these studies represent the gold standard in toxicologic research.

Specifically, the studies of Cagen et al. (1999) and Ashby et al. (1999) were recently rejected by the NTP CERHR panel on BPA as unusable for consideration in its evaluation of the health hazards posed by BPA (CERHR 2007). Both the Ashby et al. (1999) and Cagen et al. (1999) studies reported finding no effect of their positive control [the estrogenic drug diethylstilbestrol (DES)] on any outcome, although these failures were not acknowledged by the authors in either article. In experimental science, the failure of a positive control to show an effect indicates the experiment failed, which is the conclusion reached by the CERHR panel (CERHR 2007).

The Tyl et al. 2002 study, which the U.S. FDA still accepts as a major study for determination of the safety of BPA (U.S. FDA 2008a, 2008b), was criticized by an NTP panel that met in 2000 to examine the low-dose issue (NTP 2001), as well as in subsequent publications (vom Saal and Hughes 2005; vom Saal and Welshons 2006), for using an insensitive rat (the CD-SD rat) that requires extremely high doses (≥ 50 µg/kg/day) of the potent estrogenic drug ethinylestradiol to show effects such as those examined in the study by Tyl et al. (2002). This dose of ethinylestradiol is > 100 times higher than the approximately 0.3 µg/kg/day used by women in oral contraceptives. The fact that Tyl et al. (2002) adhered to GLP did not protect them from using insensitive animals. This led the NTP (2001) to state:

> Because of clear species and strain differences in sensitivity, animal model selection should be based on responsiveness to endocrine-active agents of concern (i.e., responsive to positive controls), not on convenience and familiarity.

Thus, when reviewed by other scientists, three prior major GLP studies of BPA have been found to be so flawed as to be useless for guiding regulatory agencies in decision making. A new GLP study has now been published by Tyl et al. (2008a). Close examination of this study also reveals fatal flaws which render it useless for regulatory purposes, even though it conforms to GLP.

Examples of Flaws Ignored by the U.S. FDA and EFSA in a Recent GLP Study of BPA

In summary, the flaws in Tyl et al. (2008a) are as follows:

- The high dose required for the positive control (estradiol) to cause an effect means the system used by Tyl et al. (2008a), at least in her laboratory, is relatively insensitive to exogenous estrogens and thus inappropriate for studying low-dose effects of estrogenic compounds such as BPA. The lack of response to low doses of estradiol or BPA in the Tyl laboratory is puzzling, in that the strain of mice used in these experiments (the CD-1 mouse) has been reported in > 20 other peer-reviewed publications to show adverse effects in response to very low doses of BPA (vom Saal 2008), as well as many other studies showing low-dose effects in response to the natural hormone estradiol, the estrogenic drugs ethinylestradiol and DES, and to other estrogenic chemicals.
- Tyl et al. (2008a) used insensitive, out-of-date protocols and assays that are incapable of finding many of the adverse effects reported by more sophisticated studies conducted by independent NIH-funded scientists as well as scientists funded by government agencies in other countries.
- In the specific case of testing for changes in prostate weight, Tyl et al. (2008a) reported an abnormally high prostate weight for control animals that exceeds by > 70% the prostate weights reported by other studies for animals of the same strain and similar age (e.g., Gupta 2000; Ruhlen et al. 2008). This suggests that the dissection procedures for the prostate in the Tyl laboratory included other nonprostatic tissues in the weight measurements, rendering them unusable for studying weight changes in the prostate in response to BPA or estradiol; neither chemical showed any effect on the selected end points, which directly contradicts other findings concerning opposite effects of low and high doses of estrogen on the prostate (Putz et al. 2001; Timms et al. 2005; vom Saal et al. 1997).

Aberrant insensitivity of CD-1 mouse to estrogens. Tyl et al. (2008a) used estradiol as a positive control. It was fed to female mice before and during pregnancy and lactation at 80–220 µg/kg/day; after weaning, estradiol was fed to offspring at doses of 80–100 µg/kg/day. Estradiol was used as a positive control because BPA is a man-made endocrine-disrupting estrogenic chemical.

Many published findings reporting effects of very low doses of positive control estrogens and BPA in CD-1 mice demonstrate that the CD-1 mouse was somehow rendered insensitive in the test system used by Tyl et al. (2008a). The fact that a dose of 100–200 µg/kg/day estradiol was necessary to show an effect of the positive control predicts that Tyl et al. (2008a) should not detect effects of BPA < 10–100 mg/kg/day, far above the low-dose range relevant to human exposures that was supposedly of interest.

For nuclear estrogen receptor–mediated effects via regulation of gene activity (nuclear estrogen receptors are transcription factors whose activity is regulated by binding to estrogen), prior studies have typically shown a 1,000-fold lower activity for BPA relative to estradiol or potent estrogenic drugs, including DES and ethinylestradiol. For example, Richter et al. (2007b) reported an increase in androgen receptor gene activity to estradiol at 1 pM (0.28 pg/mL) in fetal CD-1 mouse prostatic mesenchyme cells in primary culture, and the same response was found for BPA at 1,000 pM (228 pg/mL); the *in vitro* response to estradiol was predicted by the response of the prostate to increasing free serum estradiol from 0.2 to 0.3 pg/mL in male mouse fetuses via estradiol administration to the mother (vom Saal et al. 1997). Other research showed that a significant effect on development of the male reproductive system in CF-1 mice occurred at a maternal dose of 0.002 µg/kg/day ethinylestradiol (Thayer et al. 2001), similar to effects observed with 2–20 µg/kg/day BPA (vom Saal et al. 1998). The research of Honma et al. (2002) showed accelerated puberty in CD-1 (ICR) mice at a DES dose of 0.02 µg/kg/day (the positive control), and the same response to BPA occurred at 20 µg/kg/day, again revealing a 1,000-fold difference between the positive control estrogen and BPA.

There are many other examples of findings where a higher dose of BPA was required to cause the same effect as the positive control estrogen (estradiol, ethinylestradiol, or DES) in studies where the effects were mediated by the classical nuclear estrogen receptors, in contrast to the more recently discovered rapid signaling estrogen response system where BPA and these positive control estrogens have equal potency, as described above. In summary, CD-1 mice have been used by a large number of academic and government investigators and have been reported in peer-reviewed publications to be sensitive to positive control estrogens within the range of human sensitivity based on *in vivo* and *in vitro* studies via the classical estrogen receptor α–mediated response mechanism. The CD-1 mouse is the animal model that has been used by the U.S. National Institute of Environmental Health Sciences (NIEHS) for decades, because it is considered the best animal model for predicting the effects of developmental exposure to estrogen in humans (Newbold 1995; Newbold et al. 2007).

The failure of traditional toxicologic studies conducted by Tyl et al. (2008a, 2008b) to detect the wide range of adverse effects of even relatively high doses of BPA or of low doses of estradiol that have been reported in numerous studies by academic and government scientists provides evidence that the GLP protocols established long ago by regulatory agencies to determine the toxicity of chemicals are inappropriate for detecting the endocrine-disrupting activities of chemicals such as BPA. Indeed, this was the premise of the congressional mandate in the Food Quality Protection Act (1996) for the U.S. EPA to establish a new set of assays for endocrine-disrupting chemicals, although this process has been systematically delayed and is > 8 years behind the congressionally mandated date of 2000 to have these new assays validated.

Citing Tyl et al. (2008a), the EFSA report on BPA (EFSA 2006) stated that "the positive control substance, 17β-estradiol, resulted in reproductive and developmental toxicity." This report failed to acknowledge that only a very high dose of the positive control was sufficient to elicit effects and that this meant that the experiments conducted in the Tyl laboratory were for some reason very insensitive to any estrogen and thus inappropriate for use in a study to examine low-dose estrogenic effects of BPA.

Based on the preliminary report released by the U.S. FDA regarding BPA (U.S. FDA 2008a), it appears that the U.S. FDA has followed the lead of the EFSA in its lack of understanding of the importance of the dose of the positive control estrogen required to cause adverse effects. The consequence is that the U.S. FDA has relied primarily on the study of Tyl et al. (2008a, 2008b), with the result that the U.S. FDA has assured Americans that BPA is safe at current human exposure levels.

Several factors might account for the insensitivity of the CD-1 mouse in the Tyl et al. studies (2008a, 2008b) conducted at Research Triangle Institute (RTI), a testing facility that conducted these (as well as previous) studies funded by the American Chemistry Council. One possibility is that the diet used in these studies may have interfered with the results. The feed used by Tyl et al. (2008a) in this experiment (Purina 5002) has been shown by others to interfere with responses to exogenous estrogenic chemicals, blocking adverse effects documented on other diets. For example, a number of years ago, Thigpen et al. (2003) at the NIEHS recommended against the use of Purina 5002 in studies of endocrine-disrupting chemicals. Tyl et al. (2008a) measured some specific phytoestrogens in Purina 5002 feed by chemical analysis; however, in a report on NIH-sponsored meetings on this subject, Heindel and vom Saal (2008) pointed out that this is an insufficient control for total dietary estrogenic contaminants that can disrupt studies involving the effects of estrogenic chemicals.

A second possibility is that there are strain differences in sensitivity developed in the CD-1 mouse sold by the various Charles River Laboratories located in different regions. We consider this unlikely, because most laboratories regularly replace their CD-1 mouse breeder stock from Charles River Laboratories, and practices there make it unlikely that the sensitivity of this outbred stock to estrogens has changed dramatically over a very short period of time. Also, because RTI, where the Tyl studies were conducted, is very near the laboratories of the NIEHS, it is likely that the CD-1 mice used by these two programs were purchased from the same breeding facility.

Use of insensitive, out-of-date protocols and assays. Another serious concern about the two recent studies by Tyl et al. (2008a, 2008b) is the experimental approach used, thus raising questions about the validity of the studies. The study design used by Tyl et al. (2008a, 2008b) has been superseded by advances in both experimental design and analytical tools developed by NIH-funded scientists (and their counterparts in Europe and Asia) since the mid-1990s. The methods used by Tyl et al., primarily wet weight changes of tissues, gross histologic changes, and developmental landmarks such as vaginal opening, were established procedures by the 1950s. Thus, a major limitation of the Tyl studies is the failure to measure more meaningful and sensitive end points in order to detect the effects of low-dose BPA exposure, which are often not macroscopic in nature. Indeed, in 2001, the director of the reproductive division of the National Health and Environmental Effects Research Laboratory at the U.S. EPA stated that the inconclusive results concerning effects of BPA on reproductive toxicology can only be solved by understanding the mechanisms (Triendl 2001). With current GLP standards it is not possible to study mechanisms because they still rely on out-of-date assays.

As one example of a comparison between the approach by Tyl et al. (2008a) and independent government-funded academic scientists, extensive research has been conducted by Soto et al. (2008) and by other independent academic and government scientists

describing effects of exposure of female mice and rats to very low doses of BPA during perinatal development on the mammary glands (Jenkins et al. 2009). Although Tyl et al. (2008a) reported no low-dose effects of BPA on the mammary glands using conventional histologic analysis, there have been consistent findings of adverse effects of low doses of BPA from studies that used more sophisticated and sensitive analysis of whole mounted mammary glands to facilitate detection of microscopic lesions, coupled with immunostaining for regulatory proteins as well as techniques for determination of aberrant gene expression associated with progression to cancer. These peer-reviewed studies have reported detecting changes during embryonic development of mammary glands as well as abnormalities detected during adolescence through adulthood that are indicative of mammary gland cancer as well as other developmental abnormalities (Colerangle and Roy 1997; Durando et al. 2007; Jenkins et al. 2009; LaPensee et al. 2008; Markey et al. 2001, 2005; Moral et al. 2008; Munoz-de-Toro et al. 2005; Murray et al. 2007; Nikaido et al. 2004; Vandenberg et al. 2006, 2007b; Wadia et al. 2007).

Similar to the findings for the mammary gland, Ogura et al. (2007) reported that if tissues were analyzed by conventional histologic methods (staining with hematoxalin and eosin), prenatal exposure to low doses of BPA or DES showed no effects on prostate development, whereas if the sections were analyzed using antibodies that identified basal cells and basal cell squamous metaplasia, then significant effects were revealed. Squamous metaplasia of basal cells indicates abnormal proliferation and function of the prostate stem cell population that is thought to transform into neoplastic cells; Ho et al. (2006) reported that neonatal exposure to very low doses of BPA caused 100% of male rats to develop high-grade prostatic intraepithelial neoplastic lesions later in life. All of these studies were rejected by the U.S. FDA as not adequate for making regulatory decisions about the safety of BPA. Instead, the U.S. FDA relied upon Tyl et al. (2008a), even though the study used techniques that Ogura et al. (2007) showed lacked the sensitivity of 21st century experimental approaches.

Although findings regarding changes in brain structure, brain chemistry, and behavior represent the largest portion of the literature on low-dose BPA, Tyl et al. (2008a) did not examine any neurobehavioral end points. The NTP (2008) and the NIEHS conference consensus reports (vom Saal et al. 2007) both indicated concern about neurobehavioral effects of low doses of BPA. Thus, the absence of studies that included neurobehavioral end points is a glaring omission of Tyl et al. (2008a, 2008b).

Flawed prostate dissection. Data presented by Tyl et al. (2008a) raise questions about the adequacy of techniques used in their BPA studies. Specifically, Tyl et al. (2008a) reported that the prostate in 3.5-month-old control male CD-1 mice weighed > 70 mg [see Table 3 in Tyl et al. (2008a) for data on F_1 retained males]. This average control weight contrasts sharply with those reported from other laboratories. Specifically, the weight of the prostate in 2- to 3-month-old CD-1 mice using the dissection technique based on both Ruhlen et al. (2008) and Gupta (2000) and at the NIEHS (Newbold RR, personal communication) is about 40 mg. Several studies have reported that prenatal exposure to very low doses of BPA and positive control estrogens increased prostate size, prostatic androgen receptors, and prostate androgen receptor gene activity (Gupta 2000; Richter et al. 2007b; Thayer et al. 2001; Timms et al. 2005; vom Saal et al. 1997), but the enlarged prostate of experimental animals exposed to BPA in these laboratories weighed less than the prostates in the control animals of Tyl et al. (2008a). This raises serious questions about the procedures and/or animals used by Tyl et al. The weight of prostate reported by Tyl et al. (2008a) suggests that the technique used for dissecting the prostate resulted in nonprostatic tissue being weighed along with prostate. The seminal vesicle, coagulating gland, and dorsolateral prostate all merge together where the ejaculatory ducts enter the urethra, and there are also fat deposits on the prostate. This poses a challenge for those without proper training in distinguishing these different tissues during dissection in mice.

Alternatively, as male rodents age, they are prone to develop prostatitis. Although this inflammatory disease leads to an increase in prostate size and could thus account for the very large prostate weights reported by Tyl et al. (2008a), anyone familiar with the appearance of prostatitis would detect this abnormality upon histologic examination, which Tyl et al (2008a) supposedly conducted. Also, prostatitis is rare in young-adult mice or rats (Cowin et al. 2008), and the size of the prostates in the Tyl et al. (2008a) study were similar to those for middle-aged and old male mice.

The findings regarding effects of BPA on the prostate presented by Tyl et al. (2008a) are thus suspect and cannot be used as evidence that other earlier studies (Gupta 2000; Timms et al. 2005; vom Saal et al. 1997) are not replicable. Given these problems in prostate weight measurements, it is not surprising that even very high doses of BPA or estradiol reported by Tyl et al. (2008a) had no effect on the prostate, in sharp contrast to other studies that showed stimulation of the prostate at low doses of estrogen and inhibition at high doses (Putz et al. 2001; Timms et al. 2005).

In addition to the problem associated with the high prostate weight reported by Tyl et al. (2008a), in a separate measurement the authors combined the anterior prostate (coagulating gland) and seminal vesicle, presenting these two organs as one combined outcome measure. This is wrong and misleading. The coagulating glands emerge as the anterior ducts of the prostate from the dorsocranial region of the urogenital sinus, whereas the seminal vesicles bud from the proximal region of the Wolffian ducts. Elevated estrogen is associated with an increase in prostate size associated with an increase in prostate androgen receptors, whereas a decrease in seminal vesicle size is associated with a reduction in 5α-reductase, an enzyme that converts testosterone to the more potent androgen 5α-dihydrotestosterone (Nonneman et al. 1992). Low doses of BPA have been shown to decrease the size of organs that differentiate from the embryonic Wolffian ducts (epididymides and seminal vesicles) while increasing the size of regions of the prostate that develop from the urogenital sinus (vom Saal et al. 1998). Combining these different organs (it is technically not difficult to separate them) was thus inappropriate because they develop from different embryonic tissues that show markedly different responses to estrogenic chemicals during development. In fact, Ogura et al. (2007) reported that the anterior prostate (coagulating glands) showed the greatest expression of ER-α, and also showed the most pronounced indication of basal cell squamous metaplasia in response to developmental exposure to low doses of DES and BPA relative to other regions of the prostate.

Conclusions

Because the control data of Tyl et al. (2008a) were not consistent with the prior published literature for prostate weight of young-adult CD-1 male mice and because their methods were inappropriate for revealing an extensive body of adverse effects detected using more sophisticated approaches, we deem the findings by Tyl et al. to be invalid. Hundreds of studies show adverse effects of BPA in animals, with many conducted at concentrations equivalent to current human levels of BPA exposure; thus, it is unlikely that academic scientists would bother to replicate the outdated approaches used by Tyl et al. (2008a, 2008b). This lack of replication is typical of GLP studies, which tend to involve unnecessarily large numbers of animals [Tyl et al. (2002) used > 8,000 rats], and reliability appears to be accepted because of the numbers of animals that were used. Although using excessive numbers of animals is accepted as good science by the U.S. FDA, the use of arbitrarily large numbers of animals per group (> 20 animals per treatment group is common) actually violates guidelines in the NIH *Guide for the*

Care and Use of Laboratory Animals (Institute of Laboratory Animal Research 1996) that govern research conducted by academic and government scientists. For research with animals to be approved by any university animal care and use committee, group sizes must be based on power analysis conducted using historic data. Based on this criterion in the NIH Guide, all of the studies by Tyl et al. were significantly over powered and thus in direct violation of federal guidelines for conducting animal research, a fact about which U.S. FDA regulators seem unaware.

Each of the four main industry-funded GLP studies of BPA (Ashby et al. 1999; Cagen et al. 1999; Tyl et al. 2008a, 2008b) is flawed and not appropriate for use in setting health standards. Clearly, meeting GLP standards is not a guarantee of reliable or valid science. It is of great concern that the U.S. and EU regulatory communities are willing to accept these industry-funded, antiquated, and flawed studies as proof of the safety of BPA while rejecting as invalid for regulatory purposes the findings from a very large number of academic and government investigators using 21st-century scientific approaches. The basis for these decisions by U.S. and EU regulatory agencies should be thoroughly investigated, particularly since the NTP (2008) concluded that BPA exposure to human infants was in the range shown to cause harm in experimental animals and since both the Canadian Ministry of Health and the Ministry of the Environment recently concluded that BPA was a toxic chemical (Environment Canada 2008).

Problems inherent with reliance on GLP as the standard for choosing data are compounded by the process used by federal agencies to determine membership on science advisory panels. Leading experts qualified by specific experience on the chemical or end points under consideration are often specifically excluded from membership. For example, the U.S. FDA's BPA review panel was identified as an expert panel, when in fact the panel was composed largely of scientists lacking any experience in research with BPA. This process, which appears to consider almost any scientist knowledgeable about a chemical to create bias, makes it vastly more difficult for the panel to integrate scientific data from the relevant literature, especially since, as with BPA, there are almost 1,000 relevant studies and the review panel is provided with very little time to become knowledgeable about the details. It means that the depth of knowledge present on this and similarly constituted government regulatory agency panels is unlikely to be sufficient to subject draft assessments to the scrutiny that peer review by experts normally entails. Combined with reliance on GLP data, this process has a high potential to yield flawed assessments that jeopardize public health.

We are not suggesting that GLP should be abandoned as a requirement for industry-funded studies. We object, however, to regulatory agencies implying that GLP indicates that industry-funded GLP research is somehow superior to NIH-funded studies that are not conducted using GLP. This argument demonstrates a lack of understanding of the profound difference between the use of replication as a mechanism to assess reliability and the methods used to assess validity for peer-reviewed published academic studies, whereas GLP was instituted with the expectation that this type of verification would not occur.

Public health decisions should be based on studies using appropriate protocols and the most sensitive assays. They should not be based on criteria that include or exclude data depending on whether or not the studies use GLP. Simply meeting GLP requirements is insufficient to guarantee scientific reliability and validity.

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Guest Blog

Commentary invited by editors of Scientific American

Opinion: FDA's decision on BPA exposes Catch 22

By Frederick S. vom Saal and Patricia A. Hunt | June 11, 2012 |

As scientists who have researched the health effects of bisphenol A (BPA) for over a decade, we are concerned that a recent Food and Drug Administration decision raises questions about the agency's ability to protect the health of Americans. The FDA's decision to reject a petition to ban BPA in food packaging exposes a serious Catch 22 in the federal mindset.

Used in plastic food containers and canned food linings, BPA leaches into food and beverages, exposing people to this estrogen-like substance. We have contributed to the hundreds of peer-reviewed studies of laboratory animals that link BPA exposure to cardiovascular disease, diabetes, obesity, neurobehavioral abnormalities, allergies and asthma, prostate and mammary gland cancer and a wide range of reproductive defects. Further, similar effects have been reported in a number of human studies.

Why doesn't the FDA consider these results evidence that BPA poses a risk to human health? The answer is simple: They have chosen to ignore most of this data, relying instead on a small number of studies conducted by FDA scientists and scientists funded by industries that manufacture BPA. In a statement released last March explaining its decision to reject the BPA petition, the FDA appeared to justify the omission of hundreds of independent studies from consideration because they used too few animals "to provide confidence in the conclusions." This statement is a jaw dropper because the FDA was previously criticized by its own Science Board for using exactly the same exclusion criterion in its 2008 assessment of BPA, which ultimately was rejected by the Science Board.

Decades ago, the National Institutes of Health published guidelines that restrict university scientists from using excessive numbers of animals in experiments. The NIH Guide mandates that only the smallest number of animals required to identify a

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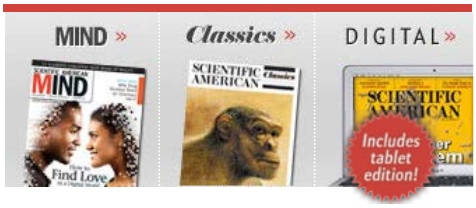

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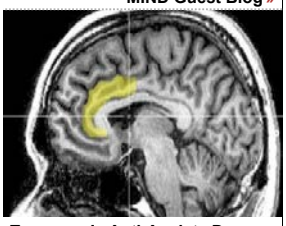

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significant effect and draw valid conclusions may be used in research conducted in academic settings. As university professors, we are required to follow NIH guidelines. The FDA should know that the strength of conclusions that can be drawn from data is not directly dependent on the number of animals used. The sensitivity of the methods employed, the precision of the measurements and the magnitude of the observed differences are the major factors.

The FDA's persistent rejection of virtually all the research from independent, university laboratories is disturbing. On one hand, NIH has established a data-based method (power analysis) to ensure that the fewest possible animals are used in research. At the same time, the FDA is rejecting NIH-funded research findings on the grounds that the studies did not use "enough" animals. This is a federal Catch 22 that does not serve the public interest.

In contrast, researchers working in industry-funded laboratories are not bound by NIH guidelines. In its decisions regarding BPA, the FDA has relied heavily on a single industry-funded study that involved killing more than 8,000 rats, even though the study is seriously flawed by use of insensitive methods and lack of appropriate controls.

Science evolves rapidly, and we should be using the very latest and most sensitive techniques to determine if BPA and similar chemicals affect the development of our babies and our long-term health. Much of the research discarded by the FDA has been conducted by leading scientists using state-of-the-art methods of analysis and published in peer-reviewed journals. The FDA's insistence that hundreds of these studies are not relevant is quite simply indefensible and puts the public health at risk.

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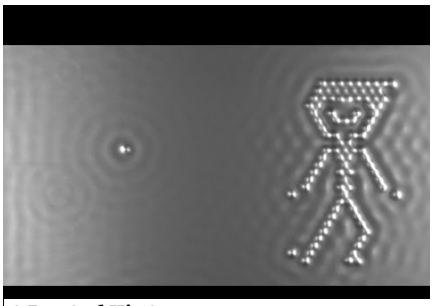

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