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

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CONTENTS

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’:

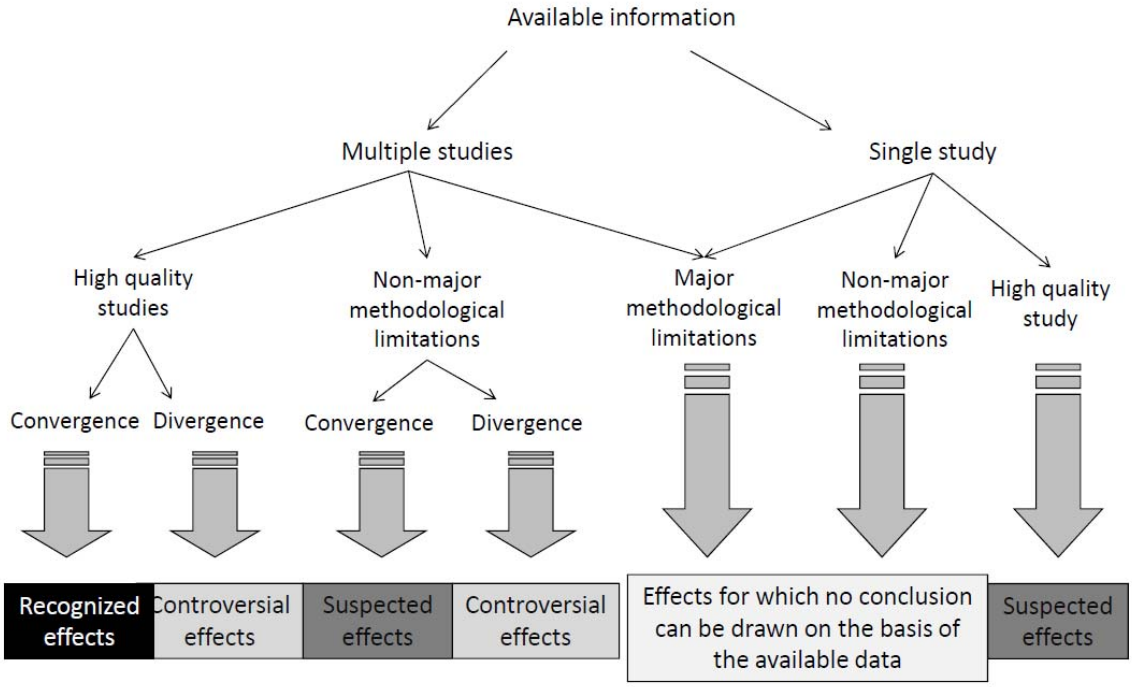
- converged, the effect was considered to be ‘suspected’,
- diverged, the effect was considered to be ‘controversial’.

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**.

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[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

5-HIAA	5-hydroxyindoleacetic acid (urinary metabolite of serotonin)
5-HT	5-hydroxytryptamine or serotonin
ACC	Acetyl-CoA carboxylase
ADH	Atypical ductal hyperplasia
ADI	Acceptable daily intake
AFSSA	French Food Safety Agency
AFSSET	French Agency for Environmental and Occupational Health Safety
AhR	Aromatic hydrocarbon receptor
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
APV/AVPV	Anteroventral periventricular nucleus
AR	Androgen receptor
ARC	Arcuate nucleus
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
BMI	Body mass index
BPA	Bisphenol A
BPA-GA	Bisphenol A glucuronide
BPA-SG	BPA specific-gravity
BPB	Bisphenol B
BPE	Bisphenol E
BPF	Bisphenol F
bw	Body weight
C/EBP-α	CCAAT enhancer binding protein α
CD-SD	Sprague-Dawley (SD) rats provided by the Charles River Laboratories
CERHR	Center for the Evaluation of Risks to Human Reproduction (USA)
CES	Expert Committee
CFSAN	Center for Food Safety and Applied Nutrition (USA)

CIS	Carcinoma *in situ* (ductal or lobular)
Cmax	Maximum serum concentration
DA	Dopamine
DAT	Dopamine transporter
DES	Diethylstilbestrol
DGPR	French Directorate General for Risk Prevention
DGS	French Directorate General for Health
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulphate
DHI	DHI Water Environment Health Consulting Engineers
DHT	Dihydrotestosterone
DIN	Ductal intraepithelial neoplasia
DMBA	Dimethylbenzanthracene
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DOPAC	3,4-dihydroxyphenylacetic acid (metabolite of dopamine)
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 α	Oestrogen receptor α
ER β	Oestrogen receptor β
ER	Oestrogen receptor
ERR γ	Oestrogen related receptor γ
EU	European Union
FAI	Free androgen index
FAO	Food and Agriculture Organization of the United Nations
FAS	Fatty acid synthase

FDA	Food and Drug Administration (USA)
FSH	Follicle stimulating hormone
GD	Gestational day
GFP	Green fluorescent protein
GHR	Growth hormone receptor
GLP	Good laboratory practices
GPCR	G-protein-coupled nonclassical membrane oestrogen receptor
GPR30	G-protein coupled receptor 30, transmembrane receptor involved in cell proliferation
HHG	Hypothalamo-hypophyseal-gonadal
HOMA	Homeostatic model assessment
Hpf	Hour post-fertilisation
HPLC	High-performance liquid chromatography
HRA	Health risk assessment
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods (USA)
IDF	International Diabetes Federation
Ig	Immunoglobulin
INSERM	French National Institute for Health and Medical Research
IP	Intraperitoneal
IVF	*In vitro* fertilisation
JRC	Joint Research Centre (EU)
LCIS	Lobular carcinoma *in situ*
LCIS	Lobular carcinoma *in situ*
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
MAP	Medically-assisted procreation
MIF	Macrophage migration inhibitory factor
MPO	Myeloperoxidase
MR	Hippocampal mineralocorticoid receptor

mRNA	Messenger ribonucleic acid
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)
OR	Oral route
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
TDI	Tolerable daily intake
TEB	Terminal end bud
TH	Thyroid hormone
TNBS	Trinitrobenzene sulphonic acid
TNF	Tumour necrosis factor
TPX	Polymethylpentene
TRV	Toxicity reference value
TRβ	Thyroid hormone receptor β (NR1A1)
TSH	Thyroid stimulating hormone
TTR	Transthyretin
UGT	Uridine diphosphate glucuronosyl transferase
Vtg	Vitellogenin
WHO	World Health Organization

Glossary

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.

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).

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.

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.

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).

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

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

MAIL RECEIVED
08 JUNE 2009
2186

253 avenue du Général Leclerc
94701 MAISONS-ALFORT CEDEX

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

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22 February 2010
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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).

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

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.

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.

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.

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.

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

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.

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.

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.

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)

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).

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

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.

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.

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

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).

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

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

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.

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

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.

Prenatal exposure period: Exposure that occurs only during pregnancy.

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

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/).

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.

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.

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.

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.

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.

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).

List of tables

List of figures

List of annexes

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

1.1. Context

Bisphenol A or BPA (2, 2-bis(4-hydroxyphenyl)propane), (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.

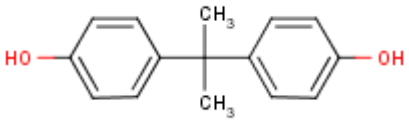
It was carried out in accordance with the French Standard NF X 50-110 “Quality in Expertise Activities” to ensure compliance with the following points: competence, independence and transparency, while at the same time ensuring traceability.

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 delayed 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 (CD-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).

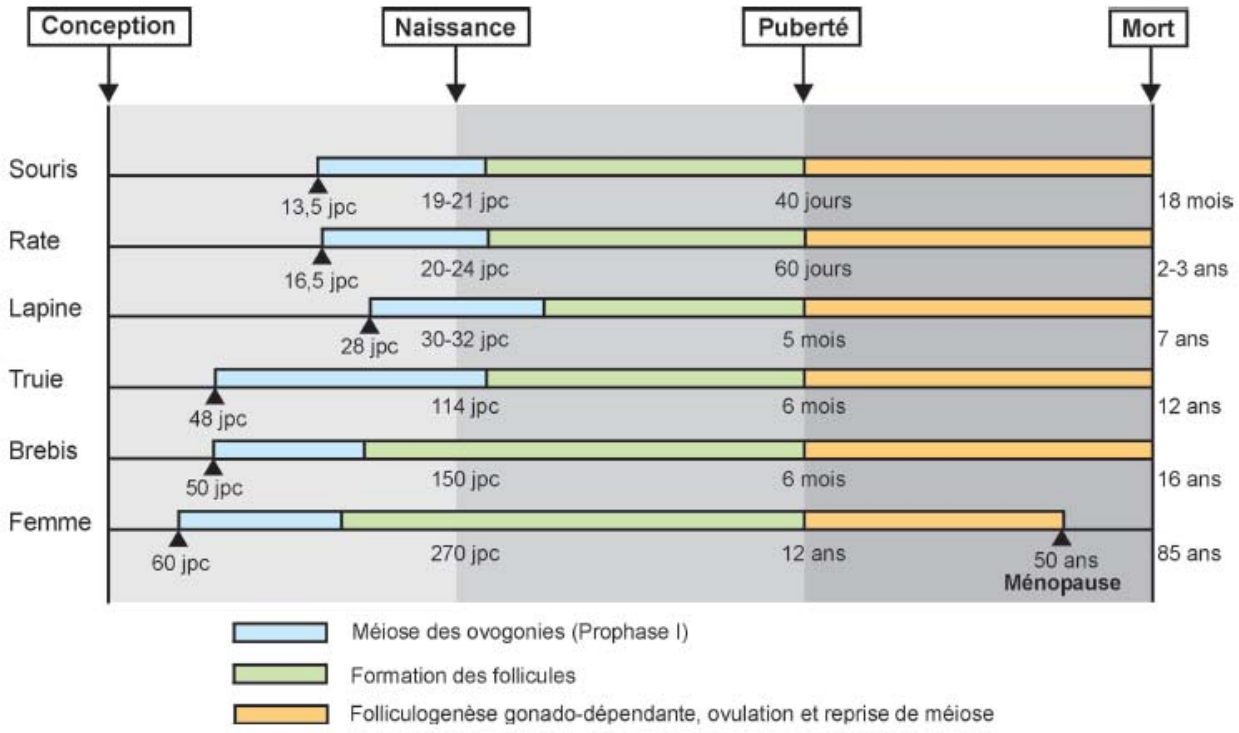
3.3. Transposition to humans

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-conceptionFigure 1: Comparison of ovarian differentiation periods in various mammals (INSERM, 2011)**

Legend:

4. French	5. English
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

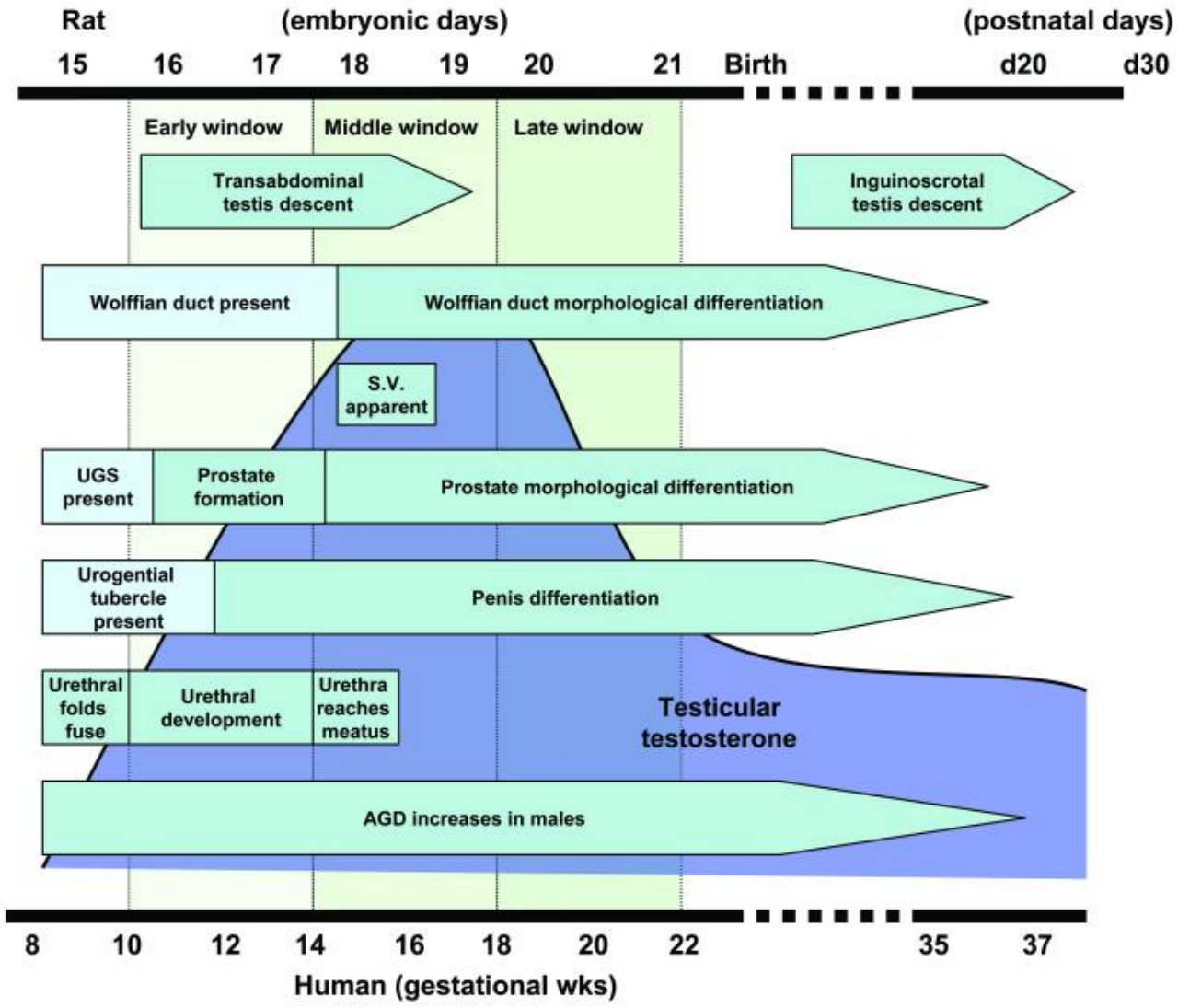


Figure 2: Diagram showing the main developmental periods of the male genital tract in humans and rats in relation to the level of testosterone production (INSERM, 2011) and according to (Welsh *et al.*, 2008)

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.

6. Methodology of evaluation

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):

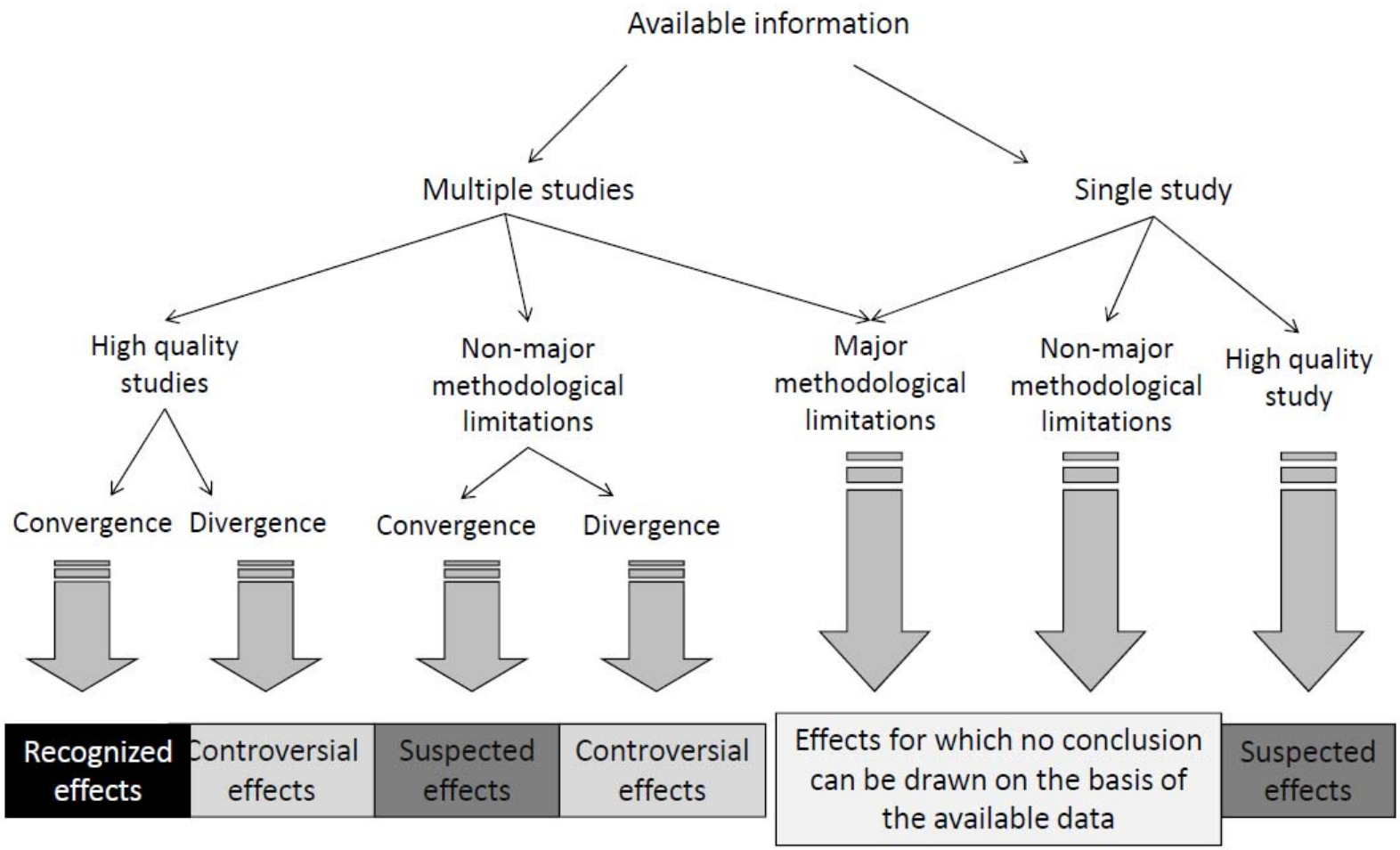


Figure 3: Decision tree

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.

7. 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.

7.1. Absorption

7.1.1. 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.

7.1.2. 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).

7.1.3. 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).

7.2. 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).

7.3. 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).

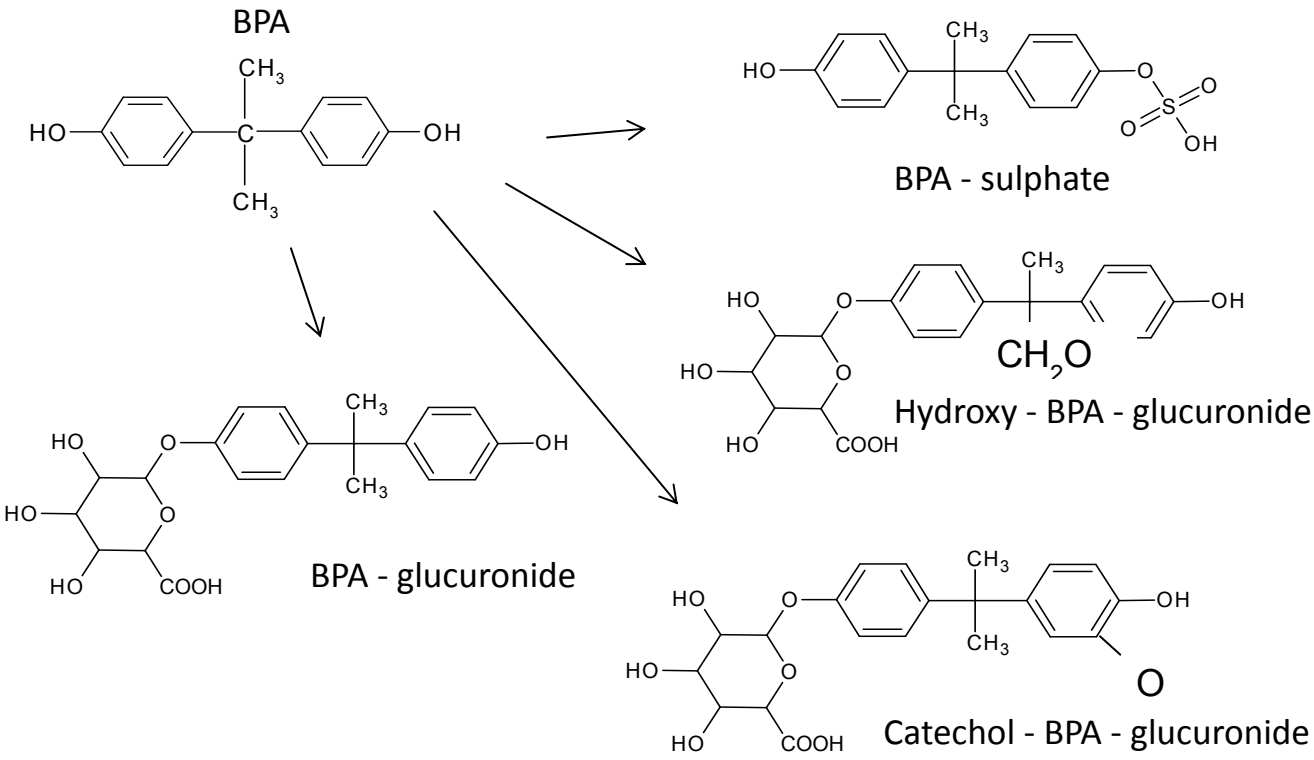


Figure 4: Major BPA metabolic pathways in mammals (INSERM, 2011)

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.

7.4. 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.

7.5. 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.

7.6. 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.

8. Health effects

8.1. 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 (Annex 7: Summary table of the epidemiological studies analysed in this report

				Effects on semen			
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**

(Li *et al.*, 2011) Urine bisphenol-A (BPA) level in relation to semen quality	Cohort study	Study population: workers Total N=218 → Sufficient population size	Urinary (free and conjugated BPA)	HPLC	Age: yes Sex: NA Medication: chronic disease Tobacco: yes BMI: yes Other contaminants: yes (prior exposure to heavy metals and other chemical substances) Other: alcohol, creatinine, education, professional history, marital status, age of 1st sexual relations, study location	Results: Statistically significant correlation between high urinary concentrations of BPA and reduced semen quality (concentration, motility, vitality, sperm count), No correlation between urinary BPA and semen volume or morphology In a sub-sample (n=87) of non-occupationally exposed subjects, a negative correlation between BPA and sperm concentration and sperm count Comments: Several subjects were lost to follow-up.	Study of high quali having no major methodological lim
(Meeker *et al.*, 2010b) Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic	Cross-sectional study nested in a study (2000-2004)	Study population: infertile men (18-55 years) N=190 men	Urinary (free and conjugated BPA) (<36.4 ng/mL)	HPLC/MS/MS	Age: yes Sex: NA Medication: NA Tobacco: yes BMI: yes Other contaminants: No Other: specific gravity	Results: association between a decline in sperm concentrations and percentage of typical forms, altered sperm characteristics, increased sperm nuclear DNA fragmentation and urinary concentrations of BPA Comments: Only one urine sample for half of the men in the study and only one semen sample for all the participants. It is important to note that some of the semen analysis parameters did not adhere to the WHO recommendations (WHO, 1999).	Study of high quali having no major methodological lim
(Mendiola *et al.*, 2010) Are Environmental Levels of Bisphenol A Associated with Reproductive Function in Fertile Men?	Cross-sectional study	Study population: fertile men (men having already had at least one child, partners of pregnant women) N=375 men → Sufficient population size	Urinary (free and conjugated BPA) (<0.4 -6.5 ng/mL)	HPLC/MS/MS	Age: yes Sex: NA Medication: no Tobacco: yes BMI: yes Other contaminants: No Other: ethnic group, urinary creatinine and sampling time	Results: - Inverse relationship between urinary concentrations of BPA and the Free Androgen Index (FAI) and the FAI/LH ratio - Significant positive association between urinary concentrations of BPA and SHBG - No association between urinary concentrations of BPA and sperm characteristics These results may reflect the oestrogen-mimetic effects of BPA. Comments: - selected population=fertile men only	Study of high quali having no major methodological lim

Effects on sex hormones							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Cha *et al.*, 2008) Influence of occupational exposure to bisphenol A on the sex hormones of male epoxy resin painters	Case-control study	Study population: professional population (male) N=25 cases (epoxy resin painters) vs 25 controls (non-painters) → Small population size	Urinary	HPLC	Age: yes Sex: NA Medication: no Tobacco: yes BMI: no Other contaminants: No	Results: Significant increase in LH and FSH Significant decrease in testosterone	Studynot taken into consideration since have major method limitations This study was exc light of the followin methodological we - small population - potential exposur several organic so - observations can extrapolated to the population, - the percentage o and consumers of beverages was too
(Galloway *et al.*, 2010) Daily Bisphenol A Excretion and Associations with Sex Hormone Concentrations : Results from the InCHIANTI Adult Population Study	Cross-sectional study nested in the Italian prospective study InCHIANTI	Study population: general adult population (20-74 years) N=715 → Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS/MS	Age: Yes Sex: yes Medication: no Tobacco: yes BMI: yes Other contaminants: No Other: number of study years, abdominal circumference, creatinine	Results: In men (n=307): significant association between daily BPA excretion and total testosterone concentrations (highly significant after adjustment). In women, no association between BPA and total testosterone and oestradiol concentrations. In all subjects: significant association between daily BPA excretion and fat measurements (BMI and abdominal circumference) Comments: - 24-hr. urine in plastic bottles. - No FSH measurement.	Study of high quali having no major methodological lim
(Meeker *et al.*, 2010a) Urinary Bisphenol A Concentrations in Relation to Serum Thyroid and Reproductive Hormone Levels in Men from an Infertility Clinic	Cross-sectional study	Study population: men consulting for fertility problems N=167 cases vs 190 controls	Urinary (free and conjugated BPA) (0.4 to 36.4 ng/mL)	HPLC/MS/MS	Age: Yes Sex: NA Medication: NA Tobacco: yes BMI: yes Other contaminants: No Other: season, sampling time, specific gravity	Results: - Positive association between urinary concentrations of BPA and FSH and the FSH/Inhibin B ratio - Negative association between urinary concentrations of BPA and the oestradiol/testosterone ratio, TSH, inhibin B and the free testosterone and oestradiol indices. Comments: Reservations can be issued regarding this study:	Study of high quali having no major methodological lim

						- it dealt with a specific population of men consulting for problems of infertility with their partners.	
(Mendiola *et al.*, 2010) Are Environmental Levels of Bisphenol A Associated with Reproductive Function in Fertile Men?	Cross-sectional study	Study population: fertile men (men having already had at least one child, partners of pregnant women) N=375 men → Sufficient population size	Urinary (free and conjugated BPA) (<0.4-6.5 ng/mL)	HPLC/MS/MS	Age: Yes Sex: NA Medication: no Tobacco: yes BMI: yes Other contaminants: No Other: ethnic group, urinary creatinine and sampling time	Results: - Inverse relationship between urinary concentrations of BPA and the Free Androgen Index (FAI) and the FAI/LH ratio - Significant positive association between urinary concentrations of BPA and SHBG - No association between urinary concentrations of BPA and sperm characteristics These results may reflect the oestrogen-mimetic effects of BPA. Comments: - selected population=fertile men only	Study of high quali having no major methodological lim
(Takeuchi *et al.*, 2004) Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction	Cross-sectional study	Study population: general population: women N=7 patients with hyper-prolactinemia, 21 with hypothalamic amenorrhea, 19 with PCOS (13 non-obese and 6 obese) vs 26 controls (7 obese and 19 non-obese) -> Small population size	Serum	ELISA (validation of the assay method by HPLC)	Age: no Sex: NA Medication: NA Tobacco: no BMI: no Other contaminants: no	Results: correlation between plasma concentrations of testosterone (free and total) and BPA firstly and BPA concentrations and Body Mass Index secondly: levels significantly increased in women with PCOS (obese or not) and obese women without ovulation dysfunction. Comments: The results remain difficult to interpret as is, due to the vagueness of the sampling plan, the lack of information on inclusion criteria and failure to take into account the pathologies of the control subjects in the results.	Study not taken int consideration since have major metho limitations This study was exc light of the followin methodological we - small population - statistical analysi detail, - the final comparis made in relation to obese women, with cycles (considered controls) - no adjustment for confounding factor - plasma BPA mea using the ELISA te (lower limit).

Effects on sexual function							
Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Li *et al.*, 2010a) Occupational exposure to bisphenol A and the risk of self-reported male sexual dysfunction	Cohort study (2004-2008)	Study population: professional population (male) N=230 exposed subjects (1 BPA production plant and 3 resin production plants) vs 404 non-exposed controls from 'several' plants (construction materials, water suppliers, textiles, electronics, commerce, etc.) in the same geographic sector as the BPA production plant (284 volunteers and 120 husbands of women working in these plants)	Urinary Air: The protocol included measurements of BPA in the atmosphere at the workplace, exposure history, individual monitoring, an inventory of protective equipment, hygiene measures and a survey of exposure to other products. Volunteers divided into sub-groups according to the above criteria, BPA measurements taken for each subject at the work station, otherwise average value for the workshop. Exposure measurements expressed in cumulative value.	HPLC (air) Method not specified for urinary assays	Age: yes Sex: NA Medication: chronic disease Tobacco: yes BMI: no Other contaminants: co-exposure to chemical compounds Other: education, marital status, professional history	Results: significantly increased risk of sexual dysfunction (erectile function, orgasmic function, sexual desire, overall satisfaction with sex life) Comments: Imprecision in the measurement of effect: the use of a questionnaire to assess the risk of erectile dysfunction entails a risk of over-estimation using the International Index of Erectile Function Inventory based on interviews (odds ratio analysis with $CI_{95\%}$)	Study of high qua having no major methodological li
(Li *et al.*, 2010b) Relationship between urine BPA level and declining men sexual function.	Cross-sectional study	Study population: professional population (male) (epoxy resin plant) Total N=427 (173 exposed vs 254 non-exposed) → Sufficient population size	Urinary (free and conjugated BPA)	HPLC/FD	Age: no Sex: NA Medication: chronic disease Tobacco: yes BMI: no Other contaminants: yes (other chemical products and heavy metals) Other: demographic characteristics, alcohol, professional history	Results: Correlation between urinary BPA measurements and decline in sexual function. Negative relationship between the highest urinary concentrations of BPA and decreased sexual desire, more difficulty having an erection, lower ejaculation strength, and lower overall satisfaction with sex life Comments: The very high difference in the frequency of sexual dysfunction between exposed and non-exposed subjects is worthy of note, but it could also be attributed to a bias; for example, the interviews were probably not blind in terms of the workers' exposure status and threshold values were used for sexual events estimated	Study of high qua having no major methodological li

						using a non-specified scale.	

Miscarriages / spontaneous abortions							
Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Sugiura-Ogasawara *et al.*, 2005) Exposure to bisphenol A is associated with recurrent miscarriage	Case-control study	Study population: general population: women having had at least 3 first-trimester miscarriages N=45 cases vs 32 controls (doctors, nurses, secretaries at the school of medicine) → Small population size	Serum	ELISA	Age: no Sex: no Medication: no Tobacco: no BMI: no Other contaminants: No	Results: - positive association with antinuclear antibodies but not with the other parameters - serum BPA levels higher in women having had at least 3 miscarriages.	Studies not taken in consideration since have major method limitations This study was exc due to the following methodological weaknesses: - small population s - questionable choi control group (no p attempted pregnan group), - limited list of conf factors to be consid - an analytical meth (ELISA) that does n distinguish betwee various forms of BP - other confounding for miscarriage, - an inadequate ana results (identical m serum levels in the groups) - inadequate choice statistical tools

Puberty and breast development							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Wolff *et al.*, 2008a) Environmental exposures and puberty in inner-city girls	Cross-sectional study	Study population: 9-year-old girls N=192 => 186 in the end → OK population size	Urinary	Not specified	Age: yes Sex: NA Medication: yes Tobacco: yes BMI: yes Other contaminants: yes Other: race, ethnic group, urinary creatinine, height, combined with a set of predictors identified through significant comparisons with a 20% threshold.	Results: No change in the age of puberty onset in the girls. Comments: the study's power is not known and the study size is not so large	Studies of high qua having no major methodological lim
(Wolff *et al.*, 2010) Investigation of Relationships between Urinary Biomarkers of Phytoestrogens, Phthalates, and Phenols and Pubertal Stages in Girls	Prospective cohort study	Study population: girls between the ages of 6 and 8 years N=1151 → Excellent population size	Urinary	Not specified	Age: yes Sex: NA Medication: yes (in particular, "endocrine medical conditions excluded") Tobacco: no BMI: yes Other contaminants: yes Other: race/ethnic group (for patients from Mount Sinai School of Medicine)	Results: No change in the age of puberty onset in the girls.	Studies of high qua having no major methodological lim

Effects on prematurity							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**
(Cantonwine *et al.*, 2010) Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study	Mexican, retrospective case-control study nested in a cohort study	Study population: pregnant women N=30 cases (delivery < 37 weeks of pregnancy) vs 30 controls (delivery > 38 weeks of pregnancy) → limited population size	Urinary	HPLC/MS/MS	Age: yes Sex: NA Medication: no Tobacco: NA (non-smoking women but passive smoking not taken into account) BMI: yes Other contaminants: yes (urinary phthalate metabolites) Other: maternal education, marital status, gender of children	Results: the 'premature' group (delivery < 37 weeks of pregnancy, n=12) had about twice as much BPA as the controls Comments: - Prematurity based solely on gestational age at delivery, no sonogram measurements. In light of the heterogeneity of this group (elective caesareans, spontaneous delivery, pre-eclampsia, etc.), it is difficult to pinpoint the hypothetical effect; - No measurements of lead or other contaminants; - Only one BPA measurement (one single spot urine sample), no repeated measurements, - No information about passive smoking or other risk factors for prematurity (obstetrical history)	Study having major methodological limitations This study was not taken consideration due to the limitations: - passive smoking not tak account, - other risk factors for pre not taken into account (ol history) - Mode of delivery not spe (caesarean? spontaneou births?) - Population size too sma have sufficient statistical determine the effect of lo environmental exposure. - In fact, this population s barely sufficient for the application of parametric statistical tests as underta the authors.

Ovarian effects							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Mok-Lin *et al.*, 2010) Urinary bisphenol A concentrations and ovarian response among women undergoing IVF	Prospective cohort study	Study population: women undergoing an ovarian stimulation protocol in the framework of IVF (21-44 years) N=84 women (112 IVF cycles) → Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS/MS	Age: yes Sex: NA Medication: no Tobacco: no BMI: yes Other contaminants: no Other: specific gravity, day-3 FSH	Results: urinary concentrations of BPA were associated with: - a decrease in the number of oocytes retrieved after stimulation - a decrease in peak serum oestradiol levels BPA was detected in the majority of women undergoing IVF Comments: - urine was sampled twice for BPA, a geometric mean was calculated for each subject - The urinary concentrations of BPA reflected exposure at the time of sampling and not during the period of follicular maturation, several months prior. - It is difficult to extrapolate results observed in sample of infertile women consulting for IVF to the general population.	Study of high quali having no major methodological lim
(Cobellis *et al.*, 2009) Measurement of Bisphenol A and Bisphenol B Levels in Human Blood Sera From Healthy and Endometriotic Women	Study in humans	Study population: fertile women consulting a gynaecological-obstetric service for chronic pelvic pain, dysmenorrhea or ovarian cysts N=58 cases (endometriosis) vs 11 controls (same population but without endometriosis) → Small control group	Serum Note: Bisphenol B was also measured	HPLC/ fluorescence	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other contaminants: no	Results: Absence of bisphenols in the control group BPA found in 30 sera (51.7%) Presence of at least one of the two bisphenols verified in endometriotic women (63.8%) Comments: This study mainly focused on analytical aspects, and particularly the assay techniques used to analyse serum BPA.	Studies not taken i consideration since have major metho limitations This study was exc to: - the small populat (only 11 controls), - the very limited d of results - simple descriptive analysis without ac
(Fujimoto *et al.*, 2011) Serum unconjugated bisphenol A concentrations in women may adversely influence oocyte quality during *in vitro* fertilization	Cohort study	Study population: couples undergoing IVF (infertile women undergoing ovarian stimulation and their male partners) N=58 women and 37 men	Serum (un-conjugated BPA)	HPLC/ESA coularray 5600 detector	Age: yes Sex: no Medication: no Tobacco: yes BMI: no Other contaminants: no Other: ethnic group	Results: Significant association between the serum BPA concentrations of the women and decreased oocyte fertilisation Comments: Patients who underwent both *in vitro* fertilisation procedures (with and without sperm microinjection) were considered as one single group. And yet male gamete quality was different in these two groups.	Studies of high qua having no major methodological lim

(Hiroi *et al.*, 2004) Differences in serum bisphenol a concentrations in premenopausal normal women and women with endometrial hyperplasia	Cross-sectional study	Study population: women N=19 female patients with endometrial hyperplasia (2 groups according to complexity: 10 with 'simple' hyperplasia and 9 with 'complex' hyperplasia) and 7 with an endometrial carcinoma vs 11 controls →Limited population size	Serum	ELISA	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other contaminants: no	Results: The correlation was the opposite of what was expected: the controls had more BPA than the cases (non-significant). Serum BPA concentration=2.9 ng/mL in women with simple hyperplasia vs 1.4 ng/mL in women with complex hyperplasia. Same inverse relationship observed in women with an endometrial carcinoma	Studies not taken i consideration since have major metho limitations This study was not consideration due following limitation - limited population - confounding facto taken into account
(Itoh *et al.*, 2007) Urinary bisphenol-A concentration in infertile Japanese women and its association with endometriosis: A cross-sectional study	Cross-sectional study	Study population: Female patients primarily complaining of infertility (endometriosis, 24-43 years) N=140 -> Sufficient population size	Urinary (total BPA)	HPLC/MS	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other contaminants: no Other: creatinine	Results: No significant association between urinary BPA levels (not adjusted and adjusted for creatinine) and the stage of endometriosis Comments: - urine testing for BPA reflects recent exposure and not long-term contamination. - no control group truly free from disease, - urinary samples stored in plastic tubes in a freezer for 5 years	Study of high quali having no major methodological lim
(Kandaraki *et al.*, 2011) Endocrine Disruptors and Polycystic Ovary Syndrome (PCOS): Elevated Serum Levels of Bisphenol A in Women with PCOS	Age- and BMI-matched cross-sectional study	Study population: women N=71 cases (women with PCOS) vs 100 controls → Sufficient population size	Serum	ELISA	Age: yes (via matching) Sex: NA Medication: NA Tobacco: NA BMI: yes (via matching) Other contaminants: no Other: via a multivariate analysis (anthropometric, hormonal and metabolic parameters)	Results: - Serum BPA concentrations significantly higher in women with PCOS (obese or not) compared to normal control women. - In women with PCOS (obese or not): significant increase in testosterone levels and the LH/FSH ratio while SHBG levels were lower than in the controls. - BPA concentrations were significantly correlated with testosterone and androstenedione concentrations and insulin resistance. - BPA concentrations were significantly correlated with the existence of PCOS.	Studies not taken i consideration since have major metho limitations This study was exc to: - an analytical met (ELISA) that does distinguish betwee various forms of B
(Takeuchi *et al.*, 2004) Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women	Cross-sectional study	Study population: general population: women N=7 patients with hyper-prolactinemia, 21 with hypothalamic	Serum	ELISA (validation of the assay method by HPLC)	Age: no Sex: NA Medication: NA Tobacco: no BMI: no Other contaminants: no	Results: correlation between plasma concentrations of testosterone (free and total) and BPA firstly and BPA concentrations and BMI secondly: levels significantly increased in women with PCOS (obese or not) and obese women without ovulation dysfunction.	Studies not taken i consideration since have major metho limitations This study was exc light of the followin methodological we - small population - statistical analysi

and women with ovarian dysfunction		amenorrhea, 19 with PCOS (13 non-obese and 6 obese) vs 26 controls (7 obese and 19 non-obese) -> Small population size				Comments: The results remain difficult to interpret as is, due to the vagueness of the sampling plan, the lack of information on inclusion criteria and failure to take into account the pathologies of the control subjects in the results.	detail, - the final comparis made in relation to obese women, witl cycles (considered controls) - no adjustment for confounding factor - plasma BPA mea using the ELISA te (lower limit).

Effects on child behaviour							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study q**
(Braun *et al.*, 2009) Prenatal Bisphenol A Exposure and Early Childhood Behavior	Prospective cohort study	Study population: Mothers and their 2-year-old children (included in the Health Outcomes and Measures of the Environment Study programme; use of an existing biobank, recruitment in 2003) N=249 mothers and their 2-year-old children -> Sufficient population size	Urinary (in mothers at 16 and 26 weeks of gestation and at birth), free and conjugated BPA	HPLC/MS/MS	Age: yes (age of the mother) Sex: NA Medication: NA Tobacco: yes BMI: NA Other contaminants: yes	Results: Positive association with externalising behaviour Comments: - no biological reliability - use of an existing biobank (recruitment in 2003) – the samples were stored for 4-5 years, *questionnaire - no direct urinary BPA measurements in children, The study was the subject of a highly critical analysis (Human Data on Bisphenol A and Neurodevelopment doi:10.1289/ehp.0901610) whose comments are clearly justified.	Study of high having no ma methodologic limitations
(Miodovnik *et al.*, 2011) Endocrine disruptors and childhood social impairment	Prospective cohort study	Study population: children between the ages of 7 and 9 years N=137 children	Urinary (in 404 mothers between the 25th and 40th weeks of pregnancy)	Not specified	Age: yes (maternal age and exact age of the child during the examination) Sex: yes (sex of children) Medication: no Tobacco: no BMI: no Other contaminants: no Other: urinary creatinine of children, marital status on the follow-up date, education of mothers, race, IQ of mothers and children	Results: 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.	Study of high having no ma methodologic limitations

Effects on metabolism / the cardiovascular system							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study q**
(Hong *et al.*, 2009) Community level exposure to chemicals and oxidative stress in adult population	Cross-sectional study	Study population: general adult population N=960 → Excellent population size	Urinary	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: yes BMI: no Other contaminants: yes Other: physical activity, medical and professional history, alcohol	Results: Significant positive relationship between urinary concentrations of chemical contaminants, particularly phthalates and BPA, and markers of oxidative stress in a simple regression analysis (not significant if multiple regression analysis for BPA) Subjects with the highest levels of BPA were prone to fasting hyperglycaemia but no association with insulin-resistance indices	Study of high having no ma methodologic limitations
(Lang *et al.*, 2008) Association of Urinary Bisphenol A Concentration With Medical Disorders and Laboratory Abnormalities in Adults	Cross-sectional study nested in the NHANES study (2003-2004)	Study population: general adult population (18-74 years) N=1455 adults -> Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: yes BMI: yes Other contaminants: no Other: urinary creatinine, ethnic group/race, education, financial resources, abdominal circumference	Results: positive association between the highest urinary concentrations of BPA (5 and 13 ng/mL) and cardiovascular disease, diabetes and levels of liver enzymes in the blood Comments: This study warrants particular attention because: - powerful study with a solid design, - the associations are extremely robust, - large sample size, - based on American national cohorts, However, the use of medication was not taken into account and contemporary exposure is not necessarily representative of past exposure, which was correlated with the observed effect Note: The studies by Melzer *et al.* and Lang *et al.*were undertaken 2 years apart with the same type of protocol.	Study of high having no ma methodologic limitations
(Melzer *et al.*, 2010) Association of Urinary Bisphenol A Concentration with Heart Disease: Evidence from NHANES 2003/06	Cross-sectional study nested in the NHANES study (2003-2006)	Study population: general adult population (18-74 years) N=1455 (2003/04) and 1493 (2005/06) -> Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: yes BMI: yes Other contaminants: no Other: urinary creatinine, ethnic	Results: - In 2005/2006: significant association between the highest urinary concentrations of BPA and coronary disease. No association between urinary concentrations of BPA and diabetes. - In 2003/06: significant association between the highest urinary concentrations of BPA and heart disease, diabetes, alkaline phosphatase and lactate dehydrogenase.	Study of high having no ma methodologic limitations

					group/race, education, financial resources, abdominal circumference	Comments: This study warrants particular attention because: - solid design and high power (80% for the 2003/2004 population and 74% for the 2005/2006 population) - the associations are robust, - large sample size, - based on American national cohorts, However, the use of medication was not taken into account and contemporary exposure is not necessarily representative of past exposure, which was correlated with the observed effect. Note: The studies by Melzer *et al.* and Lang *et al.* were undertaken 2 years apart with the same type of protocol.	
(Takeuchi *et al.*, 2004) Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction	Cross-sectional study	Study population: general population: women N=7 patients with hyperpro-lactinemia, 21 with hypothalamic amenorrhea, 19 with PCOS (13 non-obese and 6 obese) vs 26 controls (7 obese and 19 non-obese) -> Small population size	Serum	ELISA (validation of the assay method by HPLC)	Age: no Sex: NA Medication: NA Tobacco: no BMI: no Other contaminants: no	Results: correlation between plasma concentrations of testosterone (free and total) and BPA firstly and BPA concentrations and Body Mass Index secondly: levels were significantly higher in women with PCOS (obese or not) and obese women without ovulation dysfunction. Comments: The results remain difficult to interpret as is, due to the vagueness of the sampling plan, the lack of information on inclusion criteria and failure to take into account the pathologies of the control subjects in the results.	Studies not ta consideration they have ma methodologic limitations This study wa excluded in li following methodologic weaknesses: - small popul - statistical ar lacking detail - the final cor was made in non-obese w normal cycles (considered c - no adjustme confounding - plasma BPA measured us ELISA techni limit).

Effects on birth weight							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Padmanabhan *et al.*, 2008) Maternal bisphenol-A levels at delivery: a looming problem?	Cross-sectional study	Study population: general population: women at delivery N=40 pregnant women → Small population size	Plasma (in mothers) (free)	HPLC/ESI-MS/MS	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other contaminants: no Other: no	Results: No association between plasma concentrations of BPA and gestation period or birth weight Comments: - One single BPA measurement taken at birth and not at the start or middle of pregnancy	Studies not taken i consideration since have major method limitations This study was exc to the following methodological we - small population - no adjustment for confounding factor - no measurement conjugated BPA
(Wolff *et al.*, 2008b) Prenatal Phenol and Phthalate Exposures and Birth Outcomes	Prospective study	Study population: general population (women) N=367 → OK population size	Urinary	HPLC	Age: yes (gestational age) Sex: yes (sex of children) Medication: NA Tobacco: yes (during pregnancy) BMI: yes (pre-gestational) Other contaminants: yes Other: creatinine, race, maternal education	Results: no significant association between BPA and birth weight, infant size, head circumference or gestational age Comments: - Only one measurement taken, - Low plasma levels of BPA, - No association between plasma concentrations of BPA and effects on newborns	Studies of high qua having no major methodological lim

Effects on thyroid hormones							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study**
(Meeker *et al.*, 2010a) Urinary Bisphenol A Concentration in Relation to Serum Thyroid and Reproductive Hormone Levels in Men from an Infertility Clinic	Cross-sectional study	Study population: men consulting for fertility problems N=167 cases vs 190 controls	Urinary (free and conjugated BPA) (0.4 to 36.4 ng/mL)	HPLC/MS/MS	Age: yes Sex: NA Medication: NA Tobacco: yes BMI: yes Other contaminants: No Other: season, sampling time, specific gravity	Results: - Positive association between urinary concentrations of BPA and FSH and the FSH/Inhibin B ratio - Negative association between urinary concentrations of BPA and the oestradiol/testosterone ratio, TSH, inhibin B and the free testosterone and oestradiol indices. Comments: At least 2 and up to 4 urine samples	Studies not taken consideration sin major methodolo This study was e following reasons - it dealt with a sp population of me problems of infer partners. - thyroid aspects 'opportunistic', as undertaken in rel problem of fertilit protocol did not t features specific of thyroid functio - the significance correlation deper number of urine s calculate the geo (from 1 to 3 for e Yet only 75 patie repeated samplin examines the co between TSH an urinary BPA mea on the day of sa the patients (n=1 TSH at the first s and the geometri concentrations fr samples, limited patients who und sampling, no sig correlation can b

Effects on the immune system							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Clayton *et al.*, 2011) The Impact of Bisphenol A and Triclosan on Immune Parameters in the US Population, NHANES 2003-2006	Study nested in the NHANES study (2003-2006)	Study population: stratified general population with an over-representation of African Americans, Mexican Americans and Americans over the age of 60 years N=787 (CMV antibody) N=2133 (allergy)	Urinary	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: no BMI: yes Other contaminants: No Other: ethnic group, creatinine level, income, academic success	Results: For subjects ≥ 18 years: the highest concentrations of BPA were associated with higher CMV antibodies, For subjects < 18 years: negative relationship between urinary concentrations of BPA and CMV antibody concentrations Comments: A positive association with cytomegalovirus antibodies was observed, but the extent and causality of this relationship remain uncertain. Moreover, the authors worked in a population with "detected levels of BPA", which suggests that individuals with levels below the limit of detection were excluded, which is a truly questionable selection method.	Study of high quali having no major methodological lim

Breast cancer							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qual**
(Yang *et al.*, 2009) Effects of bisphenol A on breast cancer and its risk factors	Age-matched cross-sectional study	Study population: general population (women) N=70 cases (women with breast cancer) and 82 controls → The population size is difficult to assess as the expected difference is small	Blood (free and conjugated BPA) Conjugated BPA used as a biomarker (blood stored in Eppendorf tubes for over 10 years)	HPLC/FD	Age: Yes (age matching and adjustment) Sex: NA Medication: no Tobacco: yes BM: yes Other contaminants: No Other: age at menopause	Results: No significant difference in blood concentrations of BPA between the cases and controls.	Studies not taken int consideration since t have major methodo limitations This study was exclu the following reason - The population size difficult to assess as expected difference - blood samples stor Eppendorf tubes for years, - population recruite 1994-97, - BPA analysed only blood (without specif whether it was total plasma) in a single s - No urinary samplin

). This table includes a reference to the quality of these studies, as well as to the section(s) concerned.

8.1.1. 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.

8.1.2. 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 (Annex 7: Summary table of the epidemiological studies analysed in this report

Effects on semen							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Li *et al.*, 2011) Urine bisphenol-A (BPA) level in relation to semen quality	Cohort study	Study population: workers Total N=218 → Sufficient population size	Urinary (free and conjugated BPA)	HPLC	Age: yes Sex: NA Medication: chronic disease Tobacco: yes BMI: yes Other contaminants: yes (prior exposure to heavy metals and other chemical substances) Other: alcohol, creatinine, education, professional history, marital status, age of 1st sexual relations, study location	Results: Statistically significant correlation between high urinary concentrations of BPA and reduced semen quality (concentration, motility, vitality, sperm count), No correlation between urinary BPA and semen volume or morphology In a sub-sample (n=87) of non-occupationally exposed subjects, a negative correlation between BPA and sperm concentration and sperm count Comments: Several subjects were lost to follow-up.	Study of high quali having no major methodological lim
(Meeker *et al.*, 2010b) Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic	Cross-sectional study nested in a study (2000-2004)	Study population: infertile men (18-55 years) N=190 men	Urinary (free and conjugated BPA) (<36.4 ng/mL)	HPLC/MS/MS	Age: yes Sex: NA Medication: NA Tobacco: yes BMI: yes Other contaminants: No Other: specific gravity	Results: association between a decline in sperm concentrations and percentage of typical forms, altered sperm characteristics, increased sperm nuclear DNA fragmentation and urinary concentrations of BPA Comments: Only one urine sample for half of the men in the study and only one semen sample for all the participants. It is important to note that some of the semen analysis parameters	Study of high quali having no major methodological lim

						did not adhere to the WHO recommendations (WHO, 1999).	
(Mendiola *et al.*, 2010) Are Environmental Levels of Bisphenol A Associated with Reproductive Function in Fertile Men?	Cross-sectional study	Study population: fertile men (men having already had at least one child, partners of pregnant women) N=375 men → Sufficient population size	Urinary (free and conjugated BPA) (<0.4 -6.5 ng/mL)	HPLC/MS/ MS	Age: yes Sex: NA Medication: no Tobacco: yes BMI: yes Other contaminants: No Other: ethnic group, urinary creatinine and sampling time	Results: - Inverse relationship between urinary concentrations of BPA and the Free Androgen Index (FAI) and the FAI/LH ratio - Significant positive association between urinary concentrations of BPA and SHBG - No association between urinary concentrations of BPA and sperm characteristics These results may reflect the oestrogen-mimetic effects of BPA. Comments: - selected population=fertile men only	Study of high quali having no major methodological lim

Effects on sex hormones							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Cha *et al.*, 2008) Influence of occupational exposure to bisphenol A on the sex hormones of male epoxy resin painters	Case-control study	Study population: professional population (male) N=25 cases (epoxy resin painters) vs 25 controls (non-painters) → Small population size	Urinary	HPLC	Age: yes Sex: NA Medication: no Tobacco: yes BMI: no Other contaminants: No	Results: Significant increase in LH and FSH Significant decrease in testosterone	Studynot taken into consideration since have major method limitations This study was exc light of the followin methodological we - small population - potential exposur several organic so - observations can extrapolated to the population, - the percentage o and consumers of beverages was too
(Galloway *et al.*, 2010) Daily Bisphenol A Excretion and Associations with Sex Hormone Concentrations : Results from the InCHIANTI Adult Population Study	Cross-sectional study nested in the Italian prospective study InCHIANTI	Study population: general adult population (20-74 years) N=715 → Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS/MS	Age: Yes Sex: yes Medication: no Tobacco: yes BMI: yes Other contaminants: No Other: number of study years, abdominal circumference, creatinine	Results: In men (n=307): significant association between daily BPA excretion and total testosterone concentrations (highly significant after adjustment). In women, no association between BPA and total testosterone and oestradiol concentrations. In all subjects: significant association between daily BPA excretion and fat measurements (BMI and abdominal circumference) Comments: - 24-hr. urine in plastic bottles. - No FSH measurement.	Study of high quali having no major methodological lim
(Meeker *et al.*, 2010a) Urinary Bisphenol A Concentrations in Relation to Serum Thyroid and Reproductive Hormone Levels in Men from an Infertility Clinic	Cross-sectional study	Study population: men consulting for fertility problems N=167 cases vs 190 controls	Urinary (free and conjugated BPA) (0.4 to 36.4 ng/mL)	HPLC/MS/MS	Age: Yes Sex: NA Medication: NA Tobacco: yes BMI: yes Other contaminants: No Other: season, sampling time, specific gravity	Results: - Positive association between urinary concentrations of BPA and FSH and the FSH/Inhibin B ratio - Negative association between urinary concentrations of BPA and the oestradiol/testosterone ratio, TSH, inhibin B and the free testosterone and oestradiol indices. Comments: Reservations can be issued regarding this study:	Study of high quali having no major methodological lim

						- it dealt with a specific population of men consulting for problems of infertility with their partners.	
(Mendiola *et al.*, 2010) Are Environmental Levels of Bisphenol A Associated with Reproductive Function in Fertile Men?	Cross-sectional study	Study population: fertile men (men having already had at least one child, partners of pregnant women) N=375 men → Sufficient population size	Urinary (free and conjugated BPA) (<0.4-6.5 ng/mL)	HPLC/MS/MS	Age: Yes Sex: NA Medication: no Tobacco: yes BMI: yes Other contaminants: No Other: ethnic group, urinary creatinine and sampling time	Results: - Inverse relationship between urinary concentrations of BPA and the Free Androgen Index (FAI) and the FAI/LH ratio - Significant positive association between urinary concentrations of BPA and SHBG - No association between urinary concentrations of BPA and sperm characteristics These results may reflect the oestrogen-mimetic effects of BPA. Comments: - selected population=fertile men only	Study of high quali having no major methodological lim
(Takeuchi *et al.*, 2004) Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction	Cross-sectional study	Study population: general population: women N=7 patients with hyper-prolactinemia, 21 with hypothalamic amenorrhea, 19 with PCOS (13 non-obese and 6 obese) vs 26 controls (7 obese and 19 non-obese) -> Small population size	Serum	ELISA (validation of the assay method by HPLC)	Age: no Sex: NA Medication: NA Tobacco: no BMI: no Other contaminants: no	Results: correlation between plasma concentrations of testosterone (free and total) and BPA firstly and BPA concentrations and Body Mass Index secondly: levels significantly increased in women with PCOS (obese or not) and obese women without ovulation dysfunction. Comments: The results remain difficult to interpret as is, due to the vagueness of the sampling plan, the lack of information on inclusion criteria and failure to take into account the pathologies of the control subjects in the results.	Study not taken int consideration since have major metho limitations This study was exc light of the followin methodological we - small population - statistical analysi detail, - the final comparis made in relation to obese women, with cycles (considered controls) - no adjustment for confounding factor - plasma BPA mea using the ELISA te (lower limit).

Effects on sexual function							
Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Li *et al.*, 2010a) Occupational exposure to bisphenol A and the risk of self-reported male sexual dysfunction	Cohort study (2004-2008)	Study population: professional population (male) N=230 exposed subjects (1 BPA production plant and 3 resin production plants) vs 404 non-exposed controls from 'several' plants (construction materials, water suppliers, textiles, electronics, commerce, etc.) in the same geographic sector as the BPA production plant (284 volunteers and 120 husbands of women working in these plants)	Urinary Air: The protocol included measurements of BPA in the atmosphere at the workplace, exposure history, individual monitoring, an inventory of protective equipment, hygiene measures and a survey of exposure to other products. Volunteers divided into sub-groups according to the above criteria, BPA measurements taken for each subject at the work station, otherwise average value for the workshop. Exposure measurements expressed in cumulative value.	HPLC (air) Method not specified for urinary assays	Age: yes Sex: NA Medication: chronic disease Tobacco: yes BMI: no Other contaminants: co-exposure to chemical compounds Other: education, marital status, professional history	Results: significantly increased risk of sexual dysfunction (erectile function, orgasmic function, sexual desire, overall satisfaction with sex life) Comments: Imprecision in the measurement of effect: the use of a questionnaire to assess the risk of erectile dysfunction entails a risk of over-estimation using the International Index of Erectile Function Inventory based on interviews (odds ratio analysis with $CI_{95\%}$)	Study of high qua having no major methodological li
(Li *et al.*, 2010b) Relationship between urine BPA level and declining men sexual function.	Cross-sectional study	Study population: professional population (male) (epoxy resin plant) Total N=427 (173 exposed vs 254 non-exposed) → Sufficient population size	Urinary (free and conjugated BPA)	HPLC/FD	Age: no Sex: NA Medication: chronic disease Tobacco: yes BMI: no Other contaminants: yes (other chemical products and heavy metals) Other: demographic characteristics, alcohol, professional history	Results: Correlation between urinary BPA measurements and decline in sexual function. Negative relationship between the highest urinary concentrations of BPA and decreased sexual desire, more difficulty having an erection, lower ejaculation strength, and lower overall satisfaction with sex life Comments: The very high difference in the frequency of sexual dysfunction between exposed and non-exposed subjects is worthy of note, but it could also be attributed to a bias; for example, the interviews were probably not blind in terms of the workers' exposure status and threshold values were used for sexual events estimated	Study of high qua having no major methodological li

						using a non-specified scale.	

Miscarriages / spontaneous abortions							
Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Sugiura-Ogasawara *et al.*, 2005) Exposure to bisphenol A is associated with recurrent miscarriage	Case-control study	Study population: general population: women having had at least 3 first-trimester miscarriages N=45 cases vs 32 controls (doctors, nurses, secretaries at the school of medicine) → Small population size	Serum	ELISA	Age: no Sex: no Medication: no Tobacco: no BMI: no Other contaminants: No	Results: - positive association with antinuclear antibodies but not with the other parameters - serum BPA levels higher in women having had at least 3 miscarriages.	Studies not taken in consideration since have major method limitations This study was exc due to the following methodological weaknesses: - small population s - questionable choi control group (no p attempted pregnan group), - limited list of conf factors to be consid - an analytical meth (ELISA) that does n distinguish betwee various forms of BP - other confounding for miscarriage, - an inadequate ana results (identical m serum levels in the groups) - inadequate choice statistical tools

Puberty and breast development							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Wolff *et al.*, 2008a) Environmental exposures and puberty in inner-city girls	Cross-sectional study	Study population: 9-year-old girls N=192 => 186 in the end → OK population size	Urinary	Not specified	Age: yes Sex: NA Medication: yes Tobacco: yes BMI: yes Other contaminants: yes Other: race, ethnic group, urinary creatinine, height, combined with a set of predictors identified through significant comparisons with a 20% threshold.	Results: No change in the age of puberty onset in the girls. Comments: the study's power is not known and the study size is not so large	Studies of high qua having no major methodological lim
(Wolff *et al.*, 2010) Investigation of Relationships between Urinary Biomarkers of Phytoestrogens, Phthalates, and Phenols and Pubertal Stages in Girls	Prospective cohort study	Study population: girls between the ages of 6 and 8 years N=1151 → Excellent population size	Urinary	Not specified	Age: yes Sex: NA Medication: yes (in particular, "endocrine medical conditions excluded") Tobacco: no BMI: yes Other contaminants: yes Other: race/ethnic group (for patients from Mount Sinai School of Medicine)	Results: No change in the age of puberty onset in the girls.	Studies of high qua having no major methodological lim

Effects on prematurity							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**
(Cantonwine *et al.*, 2010) Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study	Mexican, retrospective case-control study nested in a cohort study	Study population: pregnant women N=30 cases (delivery < 37 weeks of pregnancy) vs 30 controls (delivery > 38 weeks of pregnancy) → limited population size	Urinary	HPLC/MS/MS	Age: yes Sex: NA Medication: no Tobacco: NA (non-smoking women but passive smoking not taken into account) BMI: yes Other contaminants: yes (urinary phthalate metabolites) Other: maternal education, marital status, gender of children	Results: the 'premature' group (delivery < 37 weeks of pregnancy, n=12) had about twice as much BPA as the controls Comments: - Prematurity based solely on gestational age at delivery, no sonogram measurements. In light of the heterogeneity of this group (elective caesareans, spontaneous delivery, pre-eclampsia, etc.), it is difficult to pinpoint the hypothetical effect; - No measurements of lead or other contaminants; - Only one BPA measurement (one single spot urine sample), no repeated measurements, - No information about passive smoking or other risk factors for prematurity (obstetrical history)	Study having major methodological limitations This study was not taken consideration due to the limitations: - passive smoking not tak account, - other risk factors for pre not taken into account (ol history) - Mode of delivery not spe (caesarean? spontaneou births?) - Population size too sma have sufficient statistical determine the effect of lo environmental exposure. - In fact, this population s barely sufficient for the application of parametric statistical tests as underta the authors.

Ovarian effects							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Mok-Lin *et al.*, 2010) Urinary bisphenol A concentrations and ovarian response among women undergoing IVF	Prospective cohort study	Study population: women undergoing an ovarian stimulation protocol in the framework of IVF (21-44 years) N=84 women (112 IVF cycles) → Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS/MS	Age: yes Sex: NA Medication: no Tobacco: no BMI: yes Other contaminants: no Other: specific gravity, day-3 FSH	Results: urinary concentrations of BPA were associated with: - a decrease in the number of oocytes retrieved after stimulation - a decrease in peak serum oestradiol levels BPA was detected in the majority of women undergoing IVF Comments: - urine was sampled twice for BPA, a geometric mean was calculated for each subject - The urinary concentrations of BPA reflected exposure at the time of sampling and not during the period of follicular maturation, several months prior. - It is difficult to extrapolate results observed in sample of infertile women consulting for IVF to the general population.	Study of high quali having no major methodological lim
(Cobellis *et al.*, 2009) Measurement of Bisphenol A and Bisphenol B Levels in Human Blood Sera From Healthy and Endometriotic Women	Study in humans	Study population: fertile women consulting a gynaecological-obstetric service for chronic pelvic pain, dysmenorrhea or ovarian cysts N=58 cases (endometriosis) vs 11 controls (same population but without endometriosis) → Small control group	Serum Note: Bisphenol B was also measured	HPLC/ fluorescence	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other contaminants: no	Results: Absence of bisphenols in the control group BPA found in 30 sera (51.7%) Presence of at least one of the two bisphenols verified in endometriotic women (63.8%) Comments: This study mainly focused on analytical aspects, and particularly the assay techniques used to analyse serum BPA.	Studies not taken i consideration since have major metho limitations This study was exc to: - the small populat (only 11 controls), - the very limited d of results - simple descriptive analysis without ac
(Fujimoto *et al.*, 2011) Serum unconjugated bisphenol A concentrations in women may adversely influence oocyte quality during *in vitro* fertilization	Cohort study	Study population: couples undergoing IVF (infertile women undergoing ovarian stimulation and their male partners) N=58 women and 37 men	Serum (un-conjugated BPA)	HPLC/ESA coularray 5600 detector	Age: yes Sex: no Medication: no Tobacco: yes BMI: no Other contaminants: no Other: ethnic group	Results: Significant association between the serum BPA concentrations of the women and decreased oocyte fertilisation Comments: Patients who underwent both *in vitro* fertilisation procedures (with and without sperm microinjection) were considered as one single group. And yet male gamete quality was different in these two groups.	Studies of high qua having no major methodological lim

(Hiroi *et al.*, 2004) Differences in serum bisphenol a concentrations in premenopausal normal women and women with endometrial hyperplasia	Cross-sectional study	Study population: women N=19 female patients with endometrial hyperplasia (2 groups according to complexity: 10 with 'simple' hyperplasia and 9 with 'complex' hyperplasia) and 7 with an endometrial carcinoma vs 11 controls →Limited population size	Serum	ELISA	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other contaminants: no	Results: The correlation was the opposite of what was expected: the controls had more BPA than the cases (non-significant). Serum BPA concentration=2.9 ng/mL in women with simple hyperplasia vs 1.4 ng/mL in women with complex hyperplasia. Same inverse relationship observed in women with an endometrial carcinoma	Studies not taken i consideration since have major metho limitations This study was not consideration due following limitation - limited population - confounding facto taken into account
(Itoh *et al.*, 2007) Urinary bisphenol-A concentration in infertile Japanese women and its association with endometriosis: A cross-sectional study	Cross-sectional study	Study population: Female patients primarily complaining of infertility (endometriosis, 24-43 years) N=140 -> Sufficient population size	Urinary (total BPA)	HPLC/MS	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other contaminants: no Other: creatinine	Results: No significant association between urinary BPA levels (not adjusted and adjusted for creatinine) and the stage of endometriosis Comments: - urine testing for BPA reflects recent exposure and not long-term contamination. - no control group truly free from disease, - urinary samples stored in plastic tubes in a freezer for 5 years	Study of high quali having no major methodological lim
(Kandaraki *et al.*, 2011) Endocrine Disruptors and Polycystic Ovary Syndrome (PCOS): Elevated Serum Levels of Bisphenol A in Women with PCOS	Age- and BMI-matched cross-sectional study	Study population: women N=71 cases (women with PCOS) vs 100 controls → Sufficient population size	Serum	ELISA	Age: yes (via matching) Sex: NA Medication: NA Tobacco: NA BMI: yes (via matching) Other contaminants: no Other: via a multivariate analysis (anthropometric, hormonal and metabolic parameters)	Results: - Serum BPA concentrations significantly higher in women with PCOS (obese or not) compared to normal control women. - In women with PCOS (obese or not): significant increase in testosterone levels and the LH/FSH ratio while SHBG levels were lower than in the controls. - BPA concentrations were significantly correlated with testosterone and androstenedione concentrations and insulin resistance. - BPA concentrations were significantly correlated with the existence of PCOS.	Studies not taken i consideration since have major metho limitations This study was exc to: - an analytical met (ELISA) that does distinguish betwee various forms of B
(Takeuchi *et al.*, 2004) Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women	Cross-sectional study	Study population: general population: women N=7 patients with hyper-prolactinemia, 21 with hypothalamic	Serum	ELISA (validation of the assay method by HPLC)	Age: no Sex: NA Medication: NA Tobacco: no BMI: no Other contaminants: no	Results: correlation between plasma concentrations of testosterone (free and total) and BPA firstly and BPA concentrations and BMI secondly: levels significantly increased in women with PCOS (obese or not) and obese women without ovulation dysfunction.	Studies not taken i consideration since have major metho limitations This study was exc light of the followin methodological we - small population - statistical analysi

and women with ovarian dysfunction		amenorrhea, 19 with PCOS (13 non-obese and 6 obese) vs 26 controls (7 obese and 19 non-obese) -> Small population size				Comments: The results remain difficult to interpret as is, due to the vagueness of the sampling plan, the lack of information on inclusion criteria and failure to take into account the pathologies of the control subjects in the results.	detail, - the final comparis made in relation to obese women, witl cycles (considered controls) - no adjustment for confounding factor - plasma BPA mea using the ELISA te (lower limit).

Effects on child behaviour							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study q**
(Braun *et al.*, 2009) Prenatal Bisphenol A Exposure and Early Childhood Behavior	Prospective cohort study	Study population: Mothers and their 2-year-old children (included in the Health Outcomes and Measures of the Environment Study programme; use of an existing biobank, recruitment in 2003) N=249 mothers and their 2-year-old children -> Sufficient population size	Urinary (in mothers at 16 and 26 weeks of gestation and at birth), free and conjugated BPA	HPLC/MS/MS	Age: yes (age of the mother) Sex: NA Medication: NA Tobacco: yes BMI: NA Other contaminants: yes	Results: Positive association with externalising behaviour Comments: - no biological reliability - use of an existing biobank (recruitment in 2003) – the samples were stored for 4-5 years, *questionnaire - no direct urinary BPA measurements in children, The study was the subject of a highly critical analysis (Human Data on Bisphenol A and Neurodevelopment doi:10.1289/ehp.0901610) whose comments are clearly justified.	Study of high having no ma methodologic limitations
(Miodovnik *et al.*, 2011) Endocrine disruptors and childhood social impairment	Prospective cohort study	Study population: children between the ages of 7 and 9 years N=137 children	Urinary (in 404 mothers between the 25th and 40th weeks of pregnancy)	Not specified	Age: yes (maternal age and exact age of the child during the examination) Sex: yes (sex of children) Medication: no Tobacco: no BMI: no Other contaminants: no Other: urinary creatinine of children, marital status on the follow-up date, education of mothers, race, IQ of mothers and children	Results: 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.	Study of high having no ma methodologic limitations

Effects on metabolism / the cardiovascular system							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study q**
(Hong *et al.*, 2009) Community level exposure to chemicals and oxidative stress in adult population	Cross-sectional study	Study population: general adult population N=960 → Excellent population size	Urinary	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: yes BMI: no Other contaminants: yes Other: physical activity, medical and professional history, alcohol	Results: Significant positive relationship between urinary concentrations of chemical contaminants, particularly phthalates and BPA, and markers of oxidative stress in a simple regression analysis (not significant if multiple regression analysis for BPA) Subjects with the highest levels of BPA were prone to fasting hyperglycaemia but no association with insulin-resistance indices	Study of high having no ma methodologic limitations
(Lang *et al.*, 2008) Association of Urinary Bisphenol A Concentration With Medical Disorders and Laboratory Abnormalities in Adults	Cross-sectional study nested in the NHANES study (2003-2004)	Study population: general adult population (18-74 years) N=1455 adults -> Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: yes BMI: yes Other contaminants: no Other: urinary creatinine, ethnic group/race, education, financial resources, abdominal circumference	Results: positive association between the highest urinary concentrations of BPA (5 and 13 ng/mL) and cardiovascular disease, diabetes and levels of liver enzymes in the blood Comments: This study warrants particular attention because: - powerful study with a solid design, - the associations are extremely robust, - large sample size, - based on American national cohorts, However, the use of medication was not taken into account and contemporary exposure is not necessarily representative of past exposure, which was correlated with the observed effect Note: The studies by Melzer *et al.* and Lang *et al.*were undertaken 2 years apart with the same type of protocol.	Study of high having no ma methodologic limitations
(Melzer *et al.*, 2010) Association of Urinary Bisphenol A Concentration with Heart Disease: Evidence from NHANES 2003/06	Cross-sectional study nested in the NHANES study (2003-2006)	Study population: general adult population (18-74 years) N=1455 (2003/04) and 1493 (2005/06) -> Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: yes BMI: yes Other contaminants: no Other: urinary creatinine, ethnic	Results: - In 2005/2006: significant association between the highest urinary concentrations of BPA and coronary disease. No association between urinary concentrations of BPA and diabetes. - In 2003/06: significant association between the highest urinary concentrations of BPA and heart disease, diabetes, alkaline phosphatase and lactate dehydrogenase.	Study of high having no ma methodologic limitations

					group/race, education, financial resources, abdominal circumference	Comments: This study warrants particular attention because: - solid design and high power (80% for the 2003/2004 population and 74% for the 2005/2006 population) - the associations are robust, - large sample size, - based on American national cohorts, However, the use of medication was not taken into account and contemporary exposure is not necessarily representative of past exposure, which was correlated with the observed effect. Note: The studies by Melzer *et al.* and Lang *et al.* were undertaken 2 years apart with the same type of protocol.	
(Takeuchi *et al.*, 2004) Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction	Cross-sectional study	Study population: general population: women N=7 patients with hyperpro-lactinemia, 21 with hypothalamic amenorrhea, 19 with PCOS (13 non-obese and 6 obese) vs 26 controls (7 obese and 19 non-obese) -> Small population size	Serum	ELISA (validation of the assay method by HPLC)	Age: no Sex: NA Medication: NA Tobacco: no BMI: no Other contaminants: no	Results: correlation between plasma concentrations of testosterone (free and total) and BPA firstly and BPA concentrations and Body Mass Index secondly: levels were significantly higher in women with PCOS (obese or not) and obese women without ovulation dysfunction. Comments: The results remain difficult to interpret as is, due to the vagueness of the sampling plan, the lack of information on inclusion criteria and failure to take into account the pathologies of the control subjects in the results.	Studies not ta consideration they have ma methodologic limitations This study wa excluded in li following methodologic weaknesses: - small popul - statistical ar lacking detail - the final cor was made in non-obese w normal cycles (considered c - no adjustme confounding - plasma BPA measured us ELISA techni limit).

Effects on birth weight							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Padmanabhan *et al.*, 2008) Maternal bisphenol-A levels at delivery: a looming problem?	Cross-sectional study	Study population: general population: women at delivery N=40 pregnant women → Small population size	Plasma (in mothers) (free)	HPLC/ESI-MS/MS	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other contaminants: no Other: no	Results: No association between plasma concentrations of BPA and gestation period or birth weight Comments: - One single BPA measurement taken at birth and not at the start or middle of pregnancy	Studies not taken i consideration since have major method limitations This study was exc to the following methodological we - small population - no adjustment for confounding factor - no measurement conjugated BPA
(Wolff *et al.*, 2008b) Prenatal Phenol and Phthalate Exposures and Birth Outcomes	Prospective study	Study population: general population (women) N=367 → OK population size	Urinary	HPLC	Age: yes (gestational age) Sex: yes (sex of children) Medication: NA Tobacco: yes (during pregnancy) BMI: yes (pre-gestational) Other contaminants: yes Other: creatinine, race, maternal education	Results: no significant association between BPA and birth weight, infant size, head circumference or gestational age Comments: - Only one measurement taken, - Low plasma levels of BPA, - No association between plasma concentrations of BPA and effects on newborns	Studies of high qua having no major methodological lim

Effects on thyroid hormones							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study**
(Meeker *et al.*, 2010a) Urinary Bisphenol A Concentration in Relation to Serum Thyroid and Reproductive Hormone Levels in Men from an Infertility Clinic	Cross-sectional study	Study population: men consulting for fertility problems N=167 cases vs 190 controls	Urinary (free and conjugated BPA) (0.4 to 36.4 ng/mL)	HPLC/MS/MS	Age: yes Sex: NA Medication: NA Tobacco: yes BMI: yes Other contaminants: No Other: season, sampling time, specific gravity	Results: - Positive association between urinary concentrations of BPA and FSH and the FSH/Inhibin B ratio - Negative association between urinary concentrations of BPA and the oestradiol/testosterone ratio, TSH, inhibin B and the free testosterone and oestradiol indices. Comments: At least 2 and up to 4 urine samples	Studies not taken consideration sin major methodolo This study was e following reasons - it dealt with a sp population of me problems of infer partners. - thyroid aspects 'opportunistic', as undertaken in rel problem of fertilit protocol did not t features specific of thyroid functio - the significance correlation deper number of urine s calculate the geo (from 1 to 3 for e Yet only 75 patie repeated samplin examines the co between TSH an urinary BPA mea on the day of sa the patients (n=1 TSH at the first s and the geometri concentrations fr samples, limited patients who und sampling, no sig correlation can b

Effects on the immune system							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qua**
(Clayton *et al.*, 2011) The Impact of Bisphenol A and Triclosan on Immune Parameters in the US Population, NHANES 2003-2006	Study nested in the NHANES study (2003-2006)	Study population: stratified general population with an over-representation of African Americans, Mexican Americans and Americans over the age of 60 years N=787 (CMV antibody) N=2133 (allergy)	Urinary	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: no BMI: yes Other contaminants: No Other: ethnic group, creatinine level, income, academic success	Results: For subjects ≥ 18 years: the highest concentrations of BPA were associated with higher CMV antibodies, For subjects < 18 years: negative relationship between urinary concentrations of BPA and CMV antibody concentrations Comments: A positive association with cytomegalovirus antibodies was observed, but the extent and causality of this relationship remain uncertain. Moreover, the authors worked in a population with "detected levels of BPA", which suggests that individuals with levels below the limit of detection were excluded, which is a truly questionable selection method.	Study of high quali having no major methodological lim

Breast cancer							
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study qual**
(Yang *et al.*, 2009) Effects of bisphenol A on breast cancer and its risk factors	Age-matched cross-sectional study	Study population: general population (women) N=70 cases (women with breast cancer) and 82 controls → The population size is difficult to assess as the expected difference is small	Blood (free and conjugated BPA) Conjugated BPA used as a biomarker (blood stored in Eppendorf tubes for over 10 years)	HPLC/FD	Age: Yes (age matching and adjustment) Sex: NA Medication: no Tobacco: yes BM: yes Other contaminants: No Other: age at menopause	Results: No significant difference in blood concentrations of BPA between the cases and controls.	Studies not taken int consideration since t have major methodo limitations This study was exclu the following reason - The population size difficult to assess as expected difference - blood samples stor Eppendorf tubes for years, - population recruite 1994-97, - BPA analysed only blood (without specif whether it was total plasma) in a single s - No urinary samplin

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8.2. 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).

8.2.1. 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).

8.2.2. Animal data

8.2.2.1. 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.

8.2.2.2. 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.

8.2.2.3. 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.

8.2.2.4. 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).

8.2.2.5. 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).

8.2.3. 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.**

8.3. 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).

8.3.1. Human data

8.3.1.1. 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.

8.3.1.2. 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.).

8.3.1.3. 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.

8.3.2. Animal data

8.3.2.1. Prenatal, perinatal and pre-pubertal exposure

8.3.2.2. 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).

8.3.2.3. 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.

8.3.2.4. 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.

8.3.2.5. 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 10 or 1000 µg/kg bw/d in F1 ♀ after 3 months: ↗ 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

8.3.2.6. 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.

8.3.3. 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.

8.3.4. 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 effects of BPA on oocyte maturation in women, in a context of ART (Assisted Reproductive Technology), are 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.**

8.4. 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.

8.4.1. 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.

8.4.2. Animal data

8.4.2.1. Prenatal and perinatal exposure

8.4.2.2. 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.

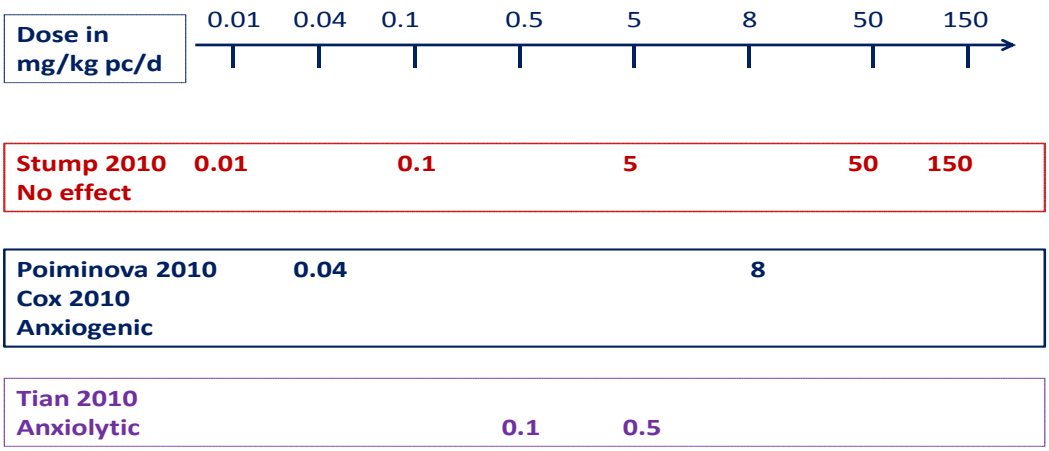


Figure 5: Effects of BPA on anxiety and exploratory behaviour according to exposure

8.4.2.3. 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).

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

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).

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.

8.4.2.4. 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 For systemic effects: NOAEL = 5.85 mg/kg bw/day for gestation and 13.1 mg/kg bw/day for lactation For neurotoxic effects: 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: - in ♂ F1 offspring: 'embracing' and 'social exploration' behaviours ↘ at 2 months and 'outward looking' behaviour ↗ at 2 and 3 months. - In mothers of ♂, '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

8.4.3. 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.**

8.5. 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).

8.5.1. 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).

8.5.2. Animal data

8.5.2.1. 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.

8.5.2.2. 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.

8.5.3. 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. Conclusion: Perinatal exposure to BPA. ↗Adipogenesis at weaning in ♀. In adult ♂, ↗ body weight observed if high-fat diet.

8.5.4. 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.**

8.6. 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).

8.6.1. 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).

8.6.2. Animal data

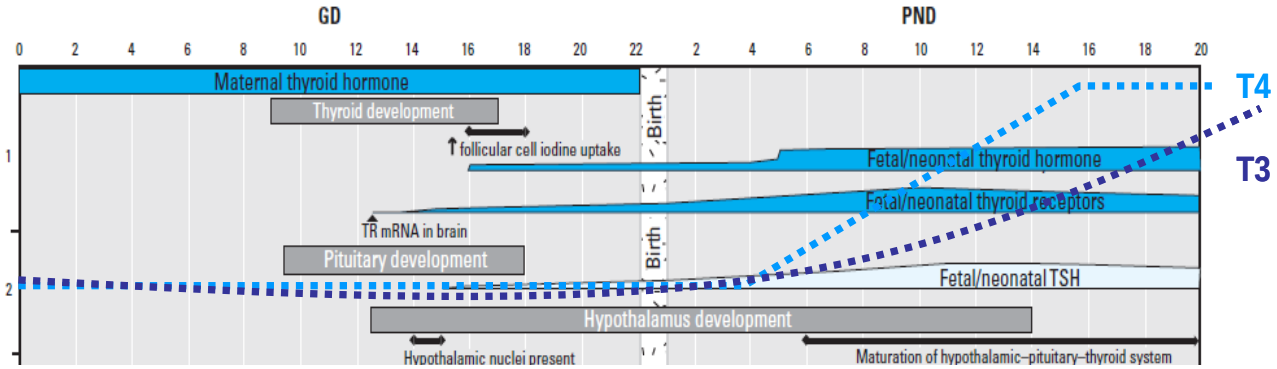
A first study was conducted in rats (Zoeller *et al.*, 2005). Exposure to BPA was initiated *in utero* (GD6) then continued throughout the neonatal period in female rats by the oral route in palatable biscuits made available in limited amounts to ensure consumption of the whole dose (negative control, 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).



According to Howdeshll 2002 and Parisault, 1975

Evaluation of the young and/or the mothers

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.

8.6.3. Data in vitro or in amphibians

8.6.3.1. 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.

8.6.3.2. 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.

8.6.3.3. 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

8.6.4. 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.**

8.7. Effects on the immune system

BPA is classified as a skin sensitiser, 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.

8.7.1. 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.

8.7.2. Animal data

8.7.2.1. 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).

[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 shortcomings, 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.

8.7.2.2. 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.

8.7.3. 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.

8.7.4. Conclusion

In conclusion, a positive association with anti-cytomegalovirus antibodies has been found in humans 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.

8.8. Effects on the intestine

8.8.1. Human data

No studies were found in humans.

8.8.2. 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 ♀).

8.8.3. Conclusion

No studies were found in humans.

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).**

8.9. 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 humans 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 humans.

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).

8.9.1. 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 humans.

- **Extrapolation to humans: interpretation issues**

In humans, 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.

8.9.2. 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.

8.9.3. 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.

8.9.4. 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

8.9.5. 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.**

8.10. 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.

8.10.1. 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.

8.10.2. 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.

8.10.3. 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.

8.10.4. 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.

8.10.5. 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).

8.10.6. 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.

8.10.7. 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 observed converge and the working group has therefore concluded that:

- accelerated structural maturation of the mammary gland at adulthood, 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.**

9. 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.

9.1. 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).

9.2. 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).

9.3. 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.

9.4. 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)

9.5. 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 (*Oryzias 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).

9.6. 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.

9.7. 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.

10. Discussion - conclusion

10.1. 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 guidelines 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 a 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 the oral route. 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.

- **No control during the study of the contamination with BPA** or **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)

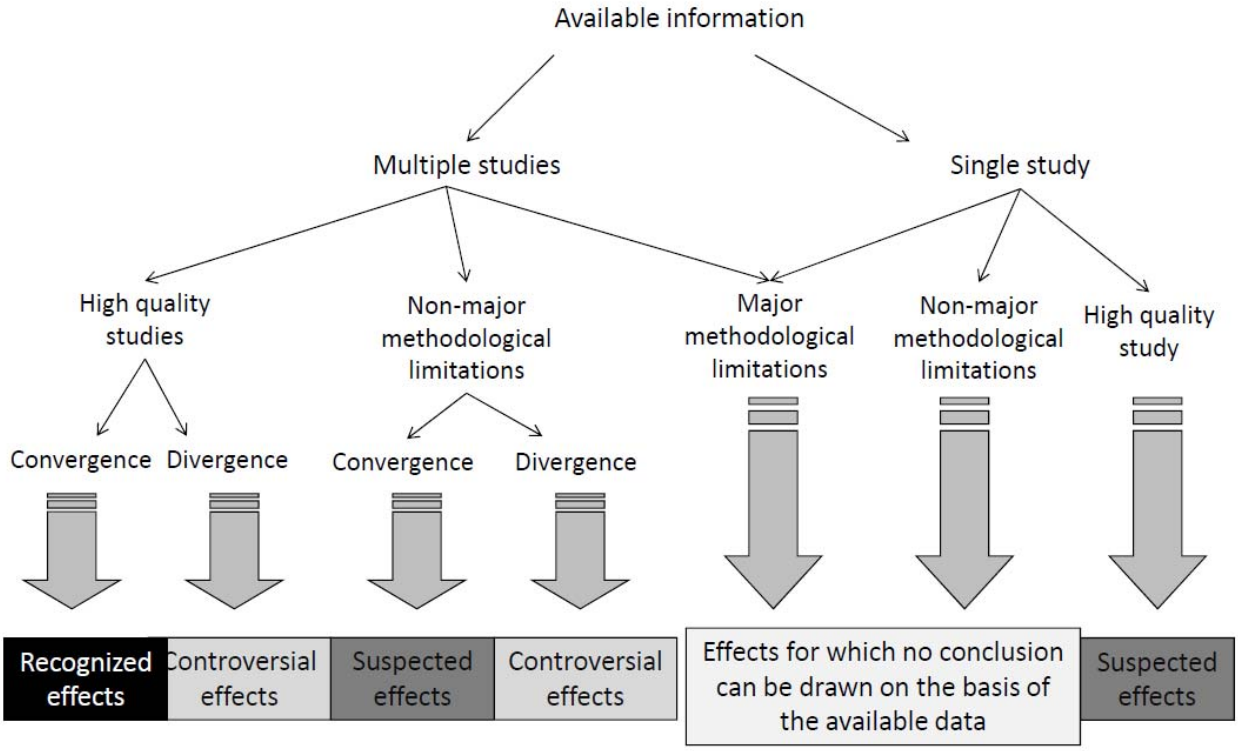


Figure 7: Decision tree

- **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.

10.2. Issues related to the toxicological evaluation of BPA

10.2.1. Experimental protocols

It should be pointed out that the OECD guidelines 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.**).

10.2.2. Windows of susceptibility

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).

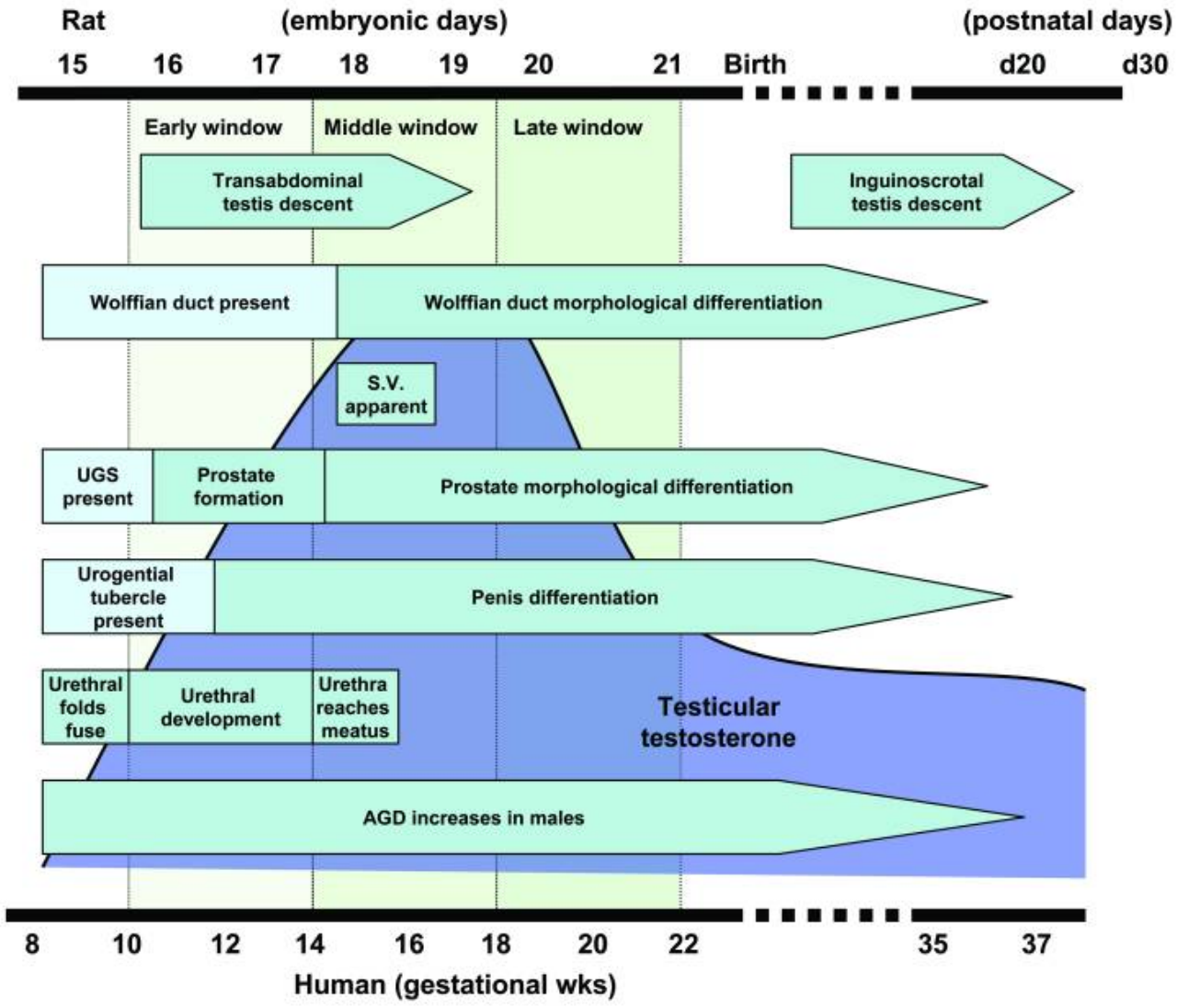


Figure 8: Diagram showing the main developmental periods of the male genital tract in humans and rats in relation to the level of testosterone production (Welsh *et al.*, 2008)

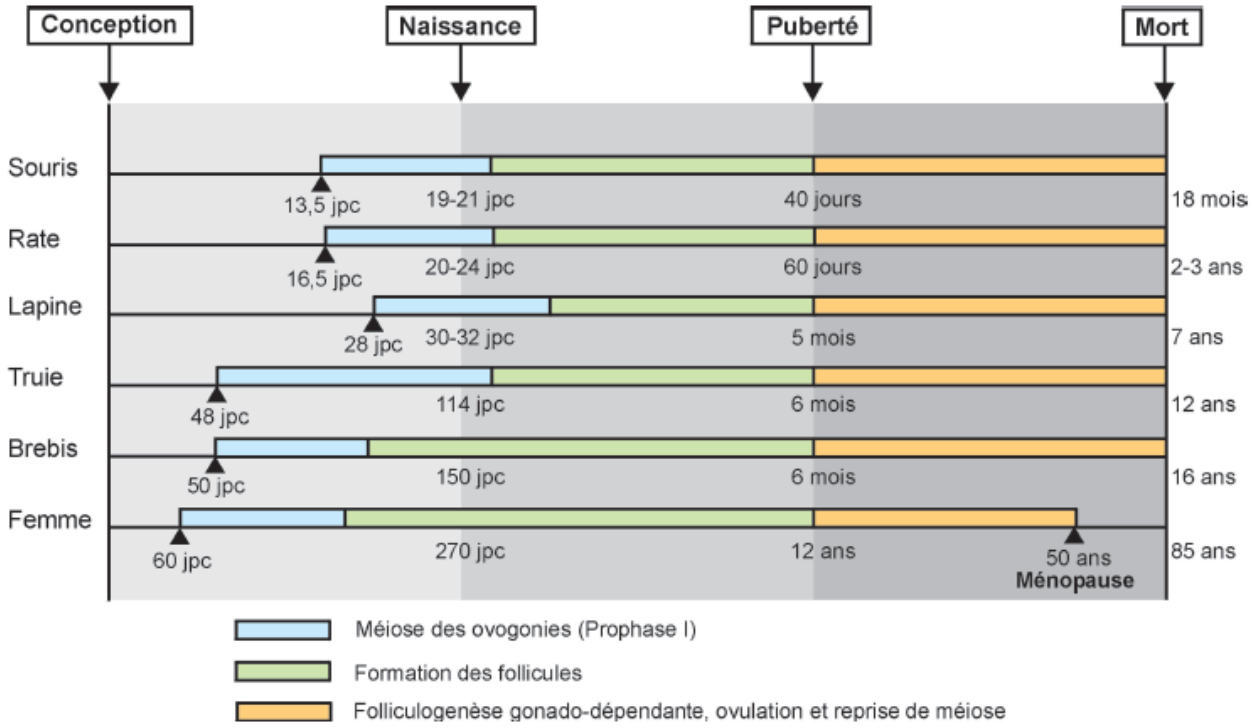
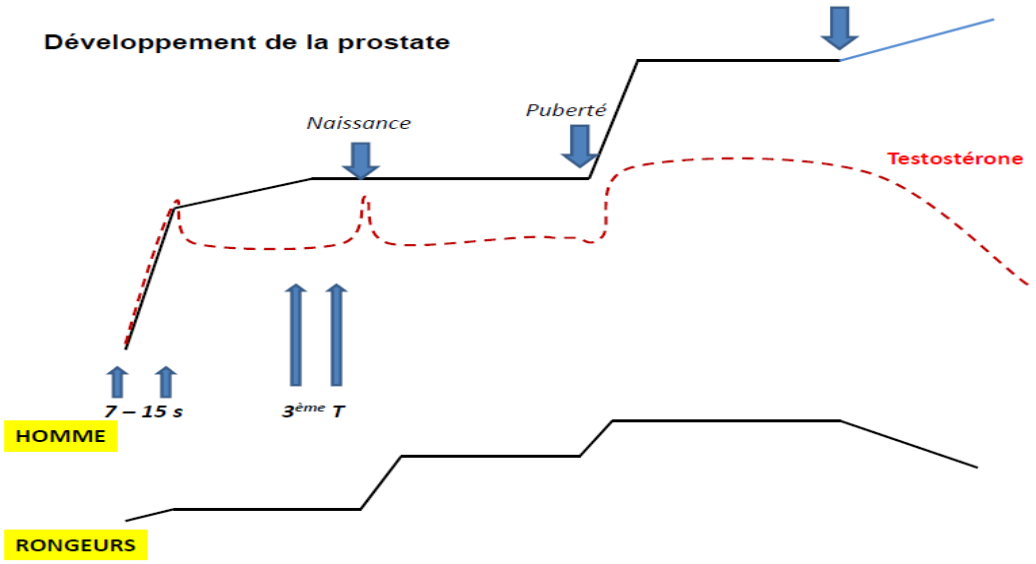


Figure 9: Comparison of ovarian differentiation periods in various mammals (INSERM, 2011)

Legend:

French	English
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

Legend:

French	*English*
Développement de la prostate	*Developent of prostate*
naissance	*birth*
puberté	*puberty*
Homme	*human*
Rongeurs	*rodents*

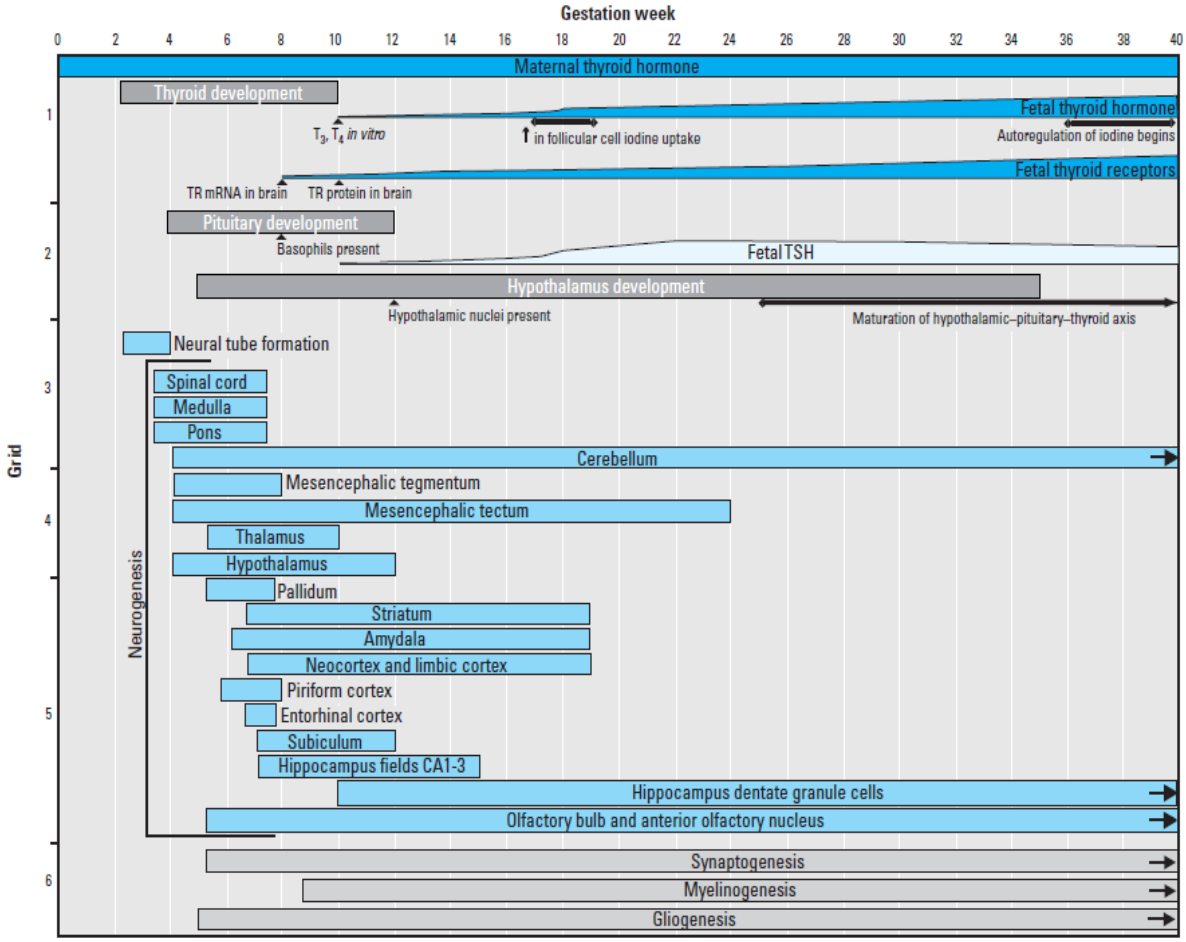


Figure 11: Thyroid and brain developmental periods from conception to birth (Bayer *et al.*, 1993; Howdeshell 2002)

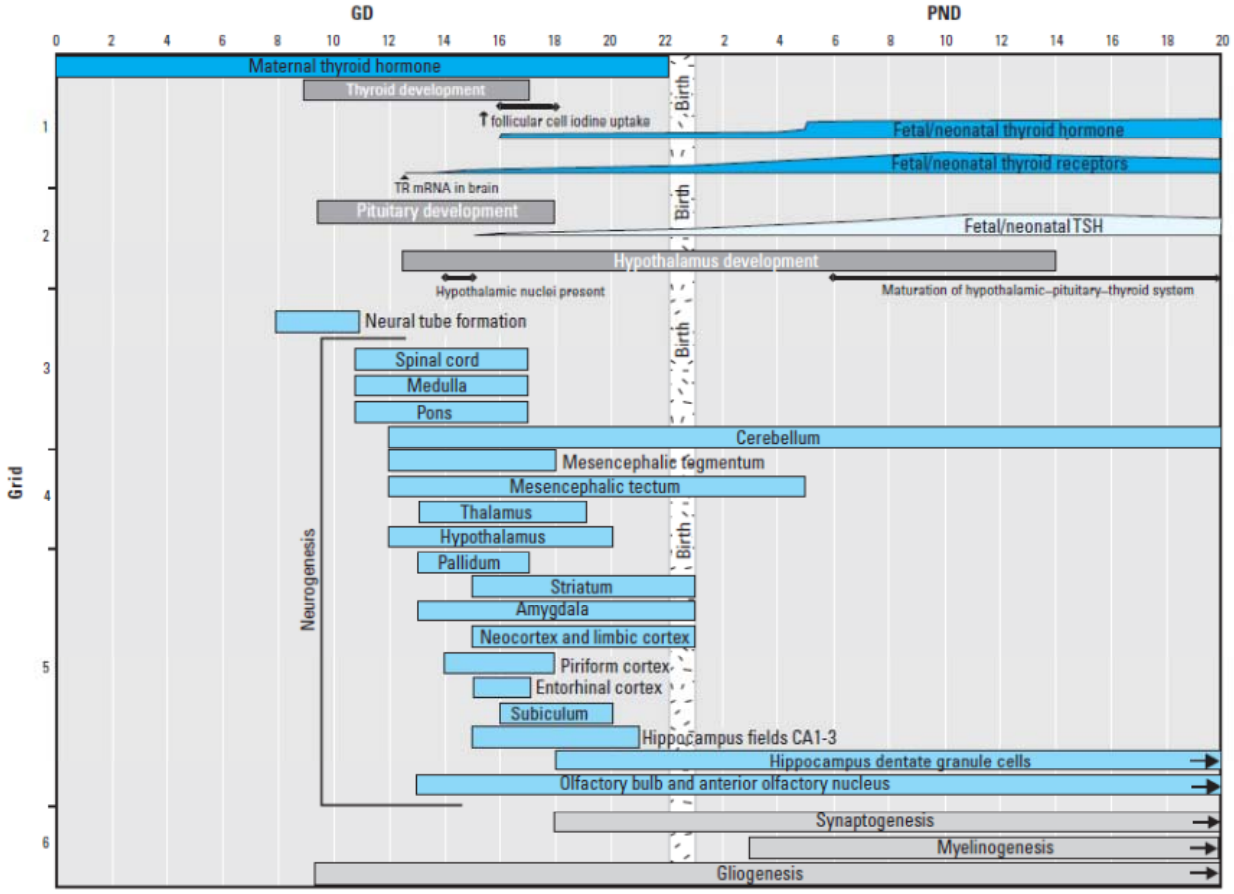


Figure 12: Thyroid and brain developmental periods from conception to PND20 in rats. GD0 indicates the date of conception, and PND1 indicates the date of birth

(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.

10.2.3. 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 available 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 limit direct 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 effect 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.

10.2.4. 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 no 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).

10.3. Summary of the conclusions of the report on health effects

10.3.1. Recognised effects

- In humans, the data available are insufficient 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; early onset of puberty due to pre- and postnatal exposure .
    - On cerebral development linked to 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 excess body weight elevated cholesterol levels and triglyceride levels, overexpression of lipogenic proteins following pre-and perinatal exposure in adults.

10.3.2. 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 levels, 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 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,* CIS) following perinatal exposure;
    - On the intestines: increased inflammatory response and reduction in intestinal permeability in adult females exposed in the perinatal period.

10.3.3. 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.

10.4. 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.

10.4.1. 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 spermatozoides) 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 exposure during puberty are suspected.

- Impairment of spermatogenesis is an effect to be taken into consideration in the health risk assessment.

10.4.2. 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 some doubts about the epidemiological data and consider 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,
- Early onset of due to early pre- and postnatal exposure.

Effects on the hypothalamic-pituitary-gonadal axis due to *in utero* exposure or early postnatal exposure resulting in changes in sex hormone levels, and the expression of these 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.

10.4.3. 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 cerebral 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).

10.4.4. 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.

10.4.5. 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 hazard 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.**

10.4.6. Effects on the immune system

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.

10.4.7. 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.

10.4.8. 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.

10.4.9. 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.

10.4.10. Information from ecotoxicological studies

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.

10.5. 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.

11. 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.

12. 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.

Annex 3: Developmental effects other than those involving the male and female reproductive system

Even if there is no evidence in humans of a BPA reprotoxic effect, the NTP-CERHR concluded in 2008 that studies in laboratory animals show that exposure to high doses of BPA during pregnancy or lactation can reduce survival of foetuses in rats and mice (> 500 mg/kg bw/day), pup weight at birth, and neonatal growth in rats and mice (> 300 mg/kg bw/day), and lead to delayed puberty in males (mice) and females (rats and mice) (> 50 mg/kg bw/day). These effects were observed at doses at which decreased body weight was also observed in treated dams. These doses are much higher than human exposure doses.

In its risk assessment (EC, 2003; EC, 2010b), effects on fertility and reproductive performance were evaluated. This assessment is particularly based on results of studies from Tyl *et al.* (2000), the Chemical Compound Safety Research Institute (2000) and the NTP (1985b). These were considered to be good quality studies. There is both a two-generation and a three-generation study conducted in rats (Chemical Compound Safety Research Institute, 2000 and Tyl *et al.*, 2000, respectively), and a continuous breeding study in mice (NTP, 1985b).

In the two-generation rat study, no adverse effects on fertility were observed up to the maximum dose tested of 200 µg/kg bw/day. Nevertheless, this is a relatively low dose.

In the three-generation rat study, using doses of 0.001 to 500 mg/kg bw/day, a statistically significant reduction in litter size was observed in the three generations at the highest dose (F1: 11.5 live pups per litter compared to 14.3 for controls; F2: 10.8 compared to 14.6; F3: 10.9 compared to 14.8). This reduction was not correlated with an increased number of dead pups per litter. At a dose of 500 mg/kg bw/d, systemic toxicity was observed in adults in all generations (reduced weight gain > 13% in both sexes and degeneration of renal tubules in females only).

In the mice study, significant reductions in the number of litters produced per F0 pair and in litter size (live pups per litter) were observed at middle and high doses (about 600 and 1300 mg/kg bw/day). These decreases in litter size were observed after each coupling and in a dose-related manner, but were not related to an increase in pup stillbirths. No effects were observed at the lowest dose (approximately 300 mg/kg bw/day).

Decreases in litter size were also observed in other "cross-over" experiments where only the females being treated engendered the most important effects. At high doses, general toxicity effects in the liver and kidneys of F0 parents were described but not graded (see ESR RAR for more details). No adverse effects on fertility were observed in the single litter tested by dose in the F1 generation in this study.

These data provided the basis for the proposed classification and labelling of bisphenol A as a reprotoxic compound, category 2, H-361f - specific target organ toxicity.

The expert panel convened under the auspices of the FAO/WHO (2010) took into account studies published since 2008. Based on studies in rodents after administration of low doses of BPA orally or subcutaneously (below 1 mg/kg bw/day), the expert panel found:

• sufficient evidence in favour of:

- the lack of teratogenic effects of BPA
- no effect on the survival of newborns and on birth weight
- no effect on the growth of newborns and their survival during lactation
- no effect on anogenital distance among males and females
- the lack of masculinising morphological effects in females or demasculinisation effects in males

• sufficient (but with a degree of uncertainty) evidence in favour of:

- the lack of a decrease in implantation rate, the lack of an effect on fertility or fecundity;

• controversial, the effects on:

- a change in the age of puberty
- changes in weight or histology of reproductive organs in exposed males and females
- changes in hormone levels in exposed males and females as well as among their descendants
- changes in weight and histology of reproductive organs or sperm parameters in male descendents

The experts estimate as significant the uncertainties about the effects of BPA in rodents at doses below 1 mg/kg bw/day by oral or dermal routes on conventional targets in relation to reproductive toxicity or development.

The experts point out the lack of data concerning:

- the effects of neonatal exposure to BPA on the development of reproductive function

- the effects of BPA on non-rodent animal models: rabbits, nonhuman primates, etc., that may be more relevant for certain types of effects (e.g., prostate)

Annex 4: Chronic effects

According to the EU-RAR 2003 and 2010 (EC, 2003; EC, 2010b), only four studies of chronic toxicity **by inhalation** are available in rats: Nitschke *et al.* (1985 and 1988; unpublished studies funded by Dow Chemical), Stasenkova *et al.* (1973; article in Russian) and Gage (1970). These studies in rats have shown:

- reversible changes of the nasal epithelium: a brownish color around the nose of rats beginning at 50 mg/m^3 and mild inflammation and hyperplasia of the epithelium at 50 and 150 mg/m^3 [Nitschke *et al.*, 1985 and 1988];

- perineal incontinence at 150 mg/m^3 [Nitschke *et al.*, 1985 and 1988];

- decreased body weight gain [Nitschke *et al.* 1988; Stasenkova *et al.*, 1973];

- effects on the kidneys and the liver: absolute change in weight of the kidneys and liver (decrease at 150 mg/m^3 in rats in the study by Nitschke *et al.*, 1988, and increase in the study of Stasenkova *et al.*, 1973), reduction in ascorbic acid in the liver and kidneys and in excretion of hippuric acid in urine [Stasenkova *et al.*, 1973].

Nitschke *et al.* (1988) noted an NOAEC of 10 mg/m^3 on which the SCOEL was based, in order to construct its 8h-OEL [SCOEL, 2004]. In 2008, the FDA reviewed the two studies by Nitschke *et al.* and considered them robust for the identification of potential target organs, but does not consider them useful in assessing the effects following oral exposure (FDA, 2008).

Via the **oral route**, the EU-RAR 2003 and 2010 (EC, 2003; EC, 2010b) identify different effects at doses much higher than 5 mg/kg bw/day, the NOAEL that was used to derive the current TDI:

- decreased body weight gain in rats (NTP, 1982: 2 weeks, 90 days, 2 years; Takahashi and Oishi, 2001; Tyl *et al.*, 1978) and mice (NTP, 1982 and 1985b);

- decreased body weight in rats (> 4000 ppm in males and 8000 ppm in females) (General Electric, 1976);

- a significant decrease in weight gain of organs in mice (> 650 mg/kg bw/day);

- hepatic effects: a decrease in weight (absolute and relative) of the liver (> 466 mg/kg) in rats (Takahashi and Oishi 2001); an increase in relative liver weight in dogs (270 mg/kg) (General Electric, 1976); changes in the size and state of hepatocyte nucleation in mice (NTP 1982, Furukawa *et al.*, 1994);

- a decrease in weight (absolute and relative) of the preputial gland (> 235 mg/kg) and prostate (> 950 mg/kg) in rats (Takahashi and Oishi 2001) and an increased relative weight of the brain, kidneys (females), and testes at 2500 ppm in rats (Tyl *et al.*, 1978); an absolute and relative increase in the weight of kidneys (> 2600 mg/kg) and ovaries (> 1300 mg/kg) in female mice (Furukawa *et al.*, 1994);

- hypertrophy of the caecum in rats (Tyl *et al.*, 1978, NTP 1982; 90 days);

- haematological changes in mice: decrease in the number of erythrocytes, haemoglobin (male) and haematocrit (4800 mg/kg in males and 650 mg/kg in females) (Furukawa *et al.*, 1994).

According to the EU-RAR (2010 (EC, 2010b), no study of chronic toxicity is available for the dermal route.

Annex 5: Carcinogenic effects

One sole carcinogenicity study was conducted in 1982 by the NTP. F344 rats and B6C3F1 mice aged 5 weeks at initiation of treatment were exposed orally (administration in food) for 103 weeks (25 months). An increase in the incidence of leukaemias in male rats at a dose of 148 mg/kg bw/day and an increase in Leydig cells tumours and increased incidence of leukaemia in female rats were observed. However, these results lose their significance when adjusting for mortality based on life expectancy. In addition, as Leydig cell tumours are known to be very common in this strain of older rat (about 88%), this model is not appropriate for demonstrating this type of cancer. In mice, the increased number of leukaemias is not significant. The study has limitations in its protocol: limited number of animals per dose group, limited clinical observations, lack of data on organ weights, etc. Thus, the results are debatable: Health Canada (2008) considered these data unconvincing, the CFSAN's Cancer Assessment Committee (2009) considered that the observed cancers may not have been related to exposure to BPA, the EU-RAR in 2008 concluded that BPA did not significantly increase the number of tumours in this study; however, they feel it was well conducted (EC, 2010b)

Annex 6: Genotoxicity

The *in vitro* genotoxicity studies show no mutagenic or clastogenic effects in the cells of bacteria, fungi, or mammals: EU-RAR, 2002 (EC, 2003; EC, 2010b). However, the formation of DNA adducts (Tsuisui *et al.*, 1998), as well as damage in MCF-7 and MDA-MB-231 cells (Iso *et al.*, 2006) have been reported. In addition, an aneugenic effect of BPA is described, with the formation of micronuclei in the absence of metabolic activation in cultures of embryonic cells of Syrian hamsters and cultures of Chinese V79 hamster cells, respectively (NTP, 2007; CGS, 2003; Haighton *et al.*, 2002). BPA may disrupt the formation of microtubules in cells and cellular systems (Pfeiffer *et al.*, 1996 and 1997).

In vivo, a large number of standardised genotoxicity studies showed no mutagenic and clastogenic ability. Studies showing an aneugenic effect are more controversial, however. In fact, Hunt *et al.* (2003) found an increase in the failure of congression (capture of chromosomes by the kinetochore fibres and orientation at the equatorial plate in metaphase) and an increase in meiotic abnormalities. However, this study has methodological weaknesses, and the effects were not confirmed by Pacchietrotti *et al*., 2008, who studied the aneugenic effect on somatic cells of mice exposed to BPA orally.

Although the aneugenic effect is still debated, the various agencies (EC, 2010b; FAO/WHO, 2010; Health Canada, 2008) agree on the absence of mutagenic and clastogenic effects of BPA.

Annex 7: Summary table of the epidemiological studies analysed in this report

Effects on semen								
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Li *et al.*, 2011) Urine bisphenol-A (BPA) level in relation to semen quality	Cohort study	Study population: workers Total N=218 → Sufficient population size	Urinary (free and conjugated BPA)	HPLC	Age: yes Sex: NA Medication: chronic disease Tobacco: yes BMI: yes Other contaminants: yes (prior exposure to heavy metals and other chemical substances) Other: alcohol, creatinine, education, professional history, marital status, age of 1st sexual relations, study location	Results: Statistically significant correlation between high urinary concentrations of BPA and reduced semen quality (concentration, motility, vitality, sperm count), No correlation between urinary BPA and semen volume or morphology In a sub-sample (n=87) of non-occupationally exposed subjects, a negative correlation between BPA and sperm concentration and sperm count Comments: Several subjects were lost to follow-up.	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.2 Effects on the male reproductive system
(Meeker *et al.*, 2010b) Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic	Cross-sectional study nested in a study (2000-2004)	Study population: infertile men (18-55 years) N=190 men	Urinary (free and conjugated BPA) (<36.4 ng/mL)	HPLC/MS/MS	Age: yes Sex: NA Medication: NA Tobacco: yes BMI: yes Other contaminants: No Other: specific gravity	Results: association between a decline in sperm concentrations and percentage of typical forms, altered sperm characteristics, increased sperm nuclear DNA fragmentation and urinary concentrations of BPA Comments: Only one urine sample for half of the men in the study and only one semen sample for all the participants. It is important to note that some of	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.2 Effects on the male reproductive system

						the semen analysis parameters did not adhere to the WHO recommendations (WHO, 1999).		
(Mendiola *et al.*, 2010) Are Environmental Levels of Bisphenol A Associated with Reproductive Function in Fertile Men?	Cross-sectional study	Study population: fertile men (men having already had at least one child, partners of pregnant women) N=375 men → Sufficient population size	Urinary (free and conjugated BPA) (<0.4 -6.5 ng/mL)	HPLC/MS/ MS	Age: yes Sex: NA Medication: no Tobacco: yes BMI: yes Other contaminants: No Other: ethnic group, urinary creatinine and sampling time	Results: - Inverse relationship between urinary concentrations of BPA and the Free Androgen Index (FAI) and the FAI/LH ratio - Significant positive association between urinary concentrations of BPA and SHBG - No association between urinary concentrations of BPA and sperm characteristics These results may reflect the oestrogen-mimetic effects of BPA. Comments: - selected population=fertile men only	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.2 Effects on the male reproductive system

Effects on sex hormones								
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Cha *et al.*, 2008) Influence of occupational exposure to bisphenol A on the sex hormones of male epoxy resin painters	Case-control study	Study population: professional population (male) N=25 cases (epoxy resin painters) vs 25 controls (non-painters) → Small population size	Urinary	HPLC	Age: yes Sex: NA Medication: no Tobacco: yes BMI: no Other contaminants: No	Results: Significant increase in LH and FSH Significant decrease in testosterone	Studynot taken into consideration since they have major methodological limitations This study was excluded in light of the following methodological weaknesses: - small population size, - potential exposure to several organic solvents, - observations cannot be extrapolated to the general population, - the percentage of smokers and consumers of alcoholic beverages was too high.	6.1 Information from epidemiological studies
(Galloway *et al.*, 2010) Daily Bisphenol A Excretion and Associations with Sex Hormone Concentrations : Results from the InCHIANTI Adult Population Study	Cross-sectional study nested in the Italian prospective study InCHIANTI	Study population: general adult population (20-74 years) N=715 → Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS/MS	Age: Yes Sex: yes Medication: no Tobacco: yes BMI: yes Other contaminants: No Other: number of study years, abdominal circumference, creatinine	Results: In men (n=307): significant association between daily BPA excretion and total testosterone concentrations (highly significant after adjustment). In women, no association between BPA and total testosterone and oestradiol concentrations. In all subjects: significant association between daily BPA excretion and fat measurements (BMI and abdominal circumference) Comments:	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.2 Effects on the male reproductive system

						- 24-hr. urine in plastic bottles. - No FSH measurement.		
(Meeker *et al.*, 2010a) Urinary Bisphenol A Concentrations in Relation to Serum Thyroid and Reproductive Hormone Levels in Men from an Infertility Clinic	Cross-sectional study	Study population: men consulting for fertility problems N=167 cases vs 190 controls	Urinary (free and conjugated BPA) (0.4 to 36.4 ng/mL)	HPLC/MS/MS	Age: Yes Sex: NA Medication: NA Tobacco: yes BMI: yes Other contaminants: No Other: season, sampling time, specific gravity	Results: - Positive association between urinary concentrations of BPA and FSH and the FSH/Inhibin B ratio - Negative association between urinary concentrations of BPA and the oestradiol/testosterone ratio, TSH, inhibin B and the free testosterone and oestradiol indices. Comments: Reservations can be issued regarding this study: - it dealt with a specific population of men consulting for problems of infertility with their partners.	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.2 Effects on the male reproductive system 6.6 Effects on the thyroid
(Mendiola *et al.*, 2010) Are Environmental Levels of Bisphenol A Associated with Reproductive Function in Fertile Men?	Cross-sectional study	Study population: fertile men (men having already had at least one child, partners of pregnant women) N=375 men → Sufficient population size	Urinary (free and conjugated BPA) (<0.4-6.5 ng/mL)	HPLC/MS/MS	Age: Yes Sex: NA Medication: no Tobacco: yes BMI: yes Other contaminants: No Other: ethnic group, urinary creatinine and sampling time	Results: - Inverse relationship between urinary concentrations of BPA and the Free Androgen Index (FAI) and the FAI/LH ratio - Significant positive association between urinary concentrations of BPA and SHBG - No association between urinary concentrations of BPA and sperm characteristics These results may reflect the oestrogen-mimetic effects of BPA. Comments: - selected population=fertile men only	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.2 Effects on the male reproductive system
(Takeuchi *et al.*, 2004) Positive relationship between androgen and the	Cross-sectional study	Study population: general population: women	Serum	ELISA (validation of the assay method by HPLC)	Age: no Sex: NA Medication: NA Tobacco: no	Results: correlation between plasma concentrations of testosterone (free and total) and BPA firstly and BPA concentrations and Body Mass Index secondly: levels	Study not taken into consideration since they have major methodological limitations	6.1 Information from epidemiological studies 6.5 Effects on

endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction		N=7 patients with hyper-prolactinemia, 21 with hypothalamic amenorrhea, 19 with PCOS (13 non-obese and 6 obese) vs 26 controls (7 obese and 19 non-obese) -> Small population size			BMI: no Other contaminants: no	significantly increased in women with PCOS (obese or not) and obese women without ovulation dysfunction. Comments: The results remain difficult to interpret as is, due to the vagueness of the sampling plan, the lack of information on inclusion criteria and failure to take into account the pathologies of the control subjects in the results.	This study was excluded in light of the following methodological weaknesses: - small population size, - statistical analysis lacking detail, - the final comparison was made in relation to non-obese women, with normal cycles (considered as controls) - no adjustment for confounding factors, - plasma BPA measured using the ELISA technique (lower limit).	metabolism and the cardiovascular system

Effects on sexual function								
Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Li *et al.*, 2010a) Occupational exposure to bisphenol A and the risk of self-reported male sexual dysfunction	Cohort study (2004-2008)	Study population: professional population (male) N=230 exposed subjects (1 BPA production plant and 3 resin production plants) vs 404 non-exposed controls from 'several' plants (construction materials, water suppliers, textiles, electronics, commerce, etc.) in the same geographic sector as the BPA production plant (284 volunteers and 120 husbands of women working in these plants)	Urinary Air: The protocol included measurements of BPA in the atmosphere at the workplace, exposure history, individual monitoring, an inventory of protective equipment, hygiene measures and a survey of exposure to other products. Volunteers divided into sub-groups according to the above criteria, BPA measurements taken for each subject at the work station, otherwise average value for the workshop. Exposure measurements expressed in cumulative value.	HPLC (air) Method not specified for urinary assays	Age: yes Sex: NA Medication: chronic disease Tobacco: yes BMI: no Other contaminants: co-exposure to chemical compounds Other: education, marital status, professional history	Results: significantly increased risk of sexual dysfunction (erectile function, orgasmic function, sexual desire, overall satisfaction with sex life) Comments: Imprecision in the measurement of effect: the use of a questionnaire to assess the risk of erectile dysfunction entails a risk of over-estimation using the International Index of Erectile Function Inventory based on interviews (odds ratio analysis with $CI_{95\%}$)	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.2 Effects on the male reproductive system
(Li *et al.*, 2010b) Relationship between urine BPA level and	Cross-sectional study	Study population: professional population (male) (epoxy resin plant) Total N=427 (173	Urinary (free and conjugated BPA)	HPLC/FD	Age: no Sex: NA Medication: chronic disease Tobacco: yes	Results: Correlation between urinary BPA measurements and decline in sexual function. Negative relationship between the highest urinary concentrations of BPA and	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.2 Effects on the male reproductive

declining men sexual function.		exposed vs 254 non-exposed) → Sufficient population size			BMI: no Other contaminants: yes (other chemical products and heavy metals) Other: demographic characteristics, alcohol, professional history	decreased sexual desire, more difficulty having an erection, lower ejaculation strength, and lower overall satisfaction with sex life Comments: The very high difference in the frequency of sexual dysfunction between exposed and non-exposed subjects is worthy of note, but it could also be attributed to a bias; for example, the interviews were probably not blind in terms of the workers' exposure status and threshold values were used for sexual events estimated using a non-specified scale.		system

Miscarriages / spontaneous abortions								
Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Sugiura-Ogasawara *et al.*, 2005) Exposure to bisphenol A is associated with recurrent miscarriage	Case-control study	Study population: general population: women having had at least 3 first-trimester miscarriages N=45 cases vs 32 controls (doctors, nurses, secretaries at the school of medicine) → Small population size	Serum	ELISA	Age: no Sex: no Medication: no Tobacco: no BMI: no Other contaminants: No	Results: - positive association with antinuclear antibodies but not with the other parameters - serum BPA levels higher in women having had at least 3 miscarriages.	Studies not taken into consideration since they have major methodological limitations This study was excluded due to the following methodological weaknesses: - small population size, - questionable choice of control group (no proof of attempted pregnancy in this group), - limited list of confounding factors to be considered, - an analytical method (ELISA) that does not distinguish between the various forms of BPA, - other confounding factors for miscarriage, - an inadequate analysis of results (identical median serum levels in the two groups) - inadequate choice of statistical tools	6.1 Information from epidemiological studies 6.3 Effects on the female reproductive system

Puberty and breast development								
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Wolff *et al.*, 2008a) Environmental exposures and puberty in inner-city girls	Cross-sectional study	Study population: 9-year-old girls N=192 => 186 in the end → OK population size	Urinary	Not specified	Age: yes Sex: NA Medication: yes Tobacco: yes BMI: yes Other contaminants: yes Other: race, ethnic group, urinary creatinine, height, combined with a set of predictors identified through significant comparisons with a 20% threshold.	Results: No change in the age of puberty onset in the girls. Comments: the study's power is not known and the study size is not so large	Studies of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.3 Effects on the female reproductive system
(Wolff *et al.*, 2010) Investigation of Relationships between Urinary Biomarkers of Phytoestrogens, Phthalates, and Phenols and Pubertal Stages in Girls	Prospective cohort study	Study population: girls between the ages of 6 and 8 years N=1151 → Excellent population size	Urinary	Not specified	Age: yes Sex: NA Medication: yes (in particular, "endocrine medical conditions excluded") Tobacco: no BMI: yes Other contaminants: yes Other: race/ethnic group (for patients from Mount Sinai School of Medicine)	Results: No change in the age of puberty onset in the girls.	Studies of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.3 Effects on the female reproductive system

Effects on prematurity								
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Cantonwine *et al.*, 2010) Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study	Mexican, retrospective case-control study nested in a cohort study	Study population: pregnant women N=30 cases (delivery < 37 weeks of pregnancy) vs 30 controls (delivery > 38 weeks of pregnancy) → limited population size	Urinary	HPLC/MS/MS	Age: yes Sex: NA Medication: no Tobacco: NA (non-smoking women but passive smoking not taken into account) BMI: yes Other contaminants: yes (urinary phthalate metabolites) Other: maternal education, marital status, gender of children	Results: the 'premature' group (delivery < 37 weeks of pregnancy, n=12) had about twice as much BPA as the controls Comments: - Prematurity based solely on gestational age at delivery, no sonogram measurements. In light of the heterogeneity of this group (elective caesareans, spontaneous delivery, pre-eclampsia, etc.), it is difficult to pinpoint the hypothetical effect; - No measurements of lead or other contaminants; - Only one BPA measurement (one single spot urine sample), no repeated measurements, - No information about passive smoking or other risk factors for prematurity (obstetrical history)	Study having major methodological limitations This study was not taken into consideration due to the following limitations: - passive smoking not taken into account, - other risk factors for prematurity not taken into account (obstetrical history) - Mode of delivery not specified (caesarean? spontaneous births?) - Population size too small to have sufficient statistical power to determine the effect of low-dose environmental exposure. - In fact, this population size is barely sufficient for the application of parametric statistical tests as undertaken by the authors.	6.1 Information from epidemiological studies 6.3 Effects on the female reproductive system

Ovarian effects								
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Mok-Lin *et al.*, 2010) Urinary bisphenol A concentrations and ovarian response among women undergoing IVF	Prospective cohort study	Study population: women undergoing an ovarian stimulation protocol in the framework of IVF (21-44 years) N=84 women (112 IVF cycles) → Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS/MS	Age: yes Sex: NA Medication: no Tobacco: no BMI: yes Other contaminants: no Other: specific gravity, day-3 FSH	Results: urinary concentrations of BPA were associated with: - a decrease in the number of oocytes retrieved after stimulation - a decrease in peak serum oestradiol levels BPA was detected in the majority of women undergoing IVF Comments: - urine was sampled twice for BPA, a geometric mean was calculated for each subject - The urinary concentrations of BPA reflected exposure at the time of sampling and not during the period of follicular maturation, several months prior. - It is difficult to extrapolate results observed in sample of infertile women consulting for IVF to the general population.	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.3 Effects on the female reproductive system
(Cobellis *et al.*, 2009) Measurement of Bisphenol A and Bisphenol B Levels in Human Blood Sera From Healthy and Endometriotic Women	Study in humans	Study population: fertile women consulting a gynaecological-obstetric service for chronic pelvic pain, dysmenorrhea or ovarian cysts N=58 cases (endometriosis) vs 11 controls (same population	Serum Note: Bisphenol B was also measured	HPLC/ fluorescence	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other contaminants: no	Results: Absence of bisphenols in the control group BPA found in 30 sera (51.7%) Presence of at least one of the two bisphenols verified in endometriotic women (63.8%) Comments: This study mainly focused on analytical aspects, and particularly the assay techniques used to analyse serum BPA.	Studies not taken into consideration since they have major methodological limitations This study was excluded due to: - the small population size (only 11 controls), - the very limited description of results - simple descriptive statistical analysis without adjustment	6.1 Information from epidemiological studies 6.3 Effects on the female reproductive system

		but without endometriosis) → Small control group						
(Fujimoto *et al.*, 2011) Serum unconjugated bisphenol A concentrations in women may adversely influence oocyte quality during *in vitro* fertilization	Cohort study	Study population: couples undergoing IVF (infertile women undergoing ovarian stimulation and their male partners) N=58 women and 37 men	Serum (un-conjugated BPA)	HPLC/ESA coularray 5600 detector	Age: yes Sex: no Medication: no Tobacco: yes BMI: no Other contaminants: no Other: ethnic group	Results: Significant association between the serum BPA concentrations of the women and decreased oocyte fertilisation Comments: Patients who underwent both *in vitro* fertilisation procedures (with and without sperm microinjection) were considered as one single group. And yet male gamete quality was different in these two groups.	Studies of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.3 Effects on the female reproductive system
(Hiroi *et al.*, 2004) Differences in serum bisphenol a concentrations in premenopausal normal women and women with endometrial hyperplasia	Cross-sectional study	Study population: women N=19 female patients with endometrial hyperplasia (2 groups according to complexity: 10 with 'simple' hyperplasia and 9 with 'complex' hyperplasia) and 7 with an endometrial carcinoma vs 11 controls →Limited population size	Serum	ELISA	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other contaminants: no	Results: The correlation was the opposite of what was expected: the controls had more BPA than the cases (non-significant). Serum BPA concentration=2.9 ng/mL in women with simple hyperplasia vs 1.4 ng/mL in women with complex hyperplasia. Same inverse relationship observed in women with an endometrial carcinoma	Studies not taken into consideration since they have major methodological limitations This study was not taken into consideration due to the following limitations: - limited population size, - confounding factors not taken into account.	6.1 Information from epidemiological studies 6.3 Effects on the female reproductive system
(Itoh *et al.*, 2007) Urinary bisphenol-A concentration in infertile Japanese women and its association with endometriosis: A cross-sectional	Cross-sectional study	Study population: Female patients primarily complaining of infertility (endometriosis, 24-43 years)	Urinary (total BPA)	HPLC/MS	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other	Results: No significant association between urinary BPA levels (not adjusted and adjusted for creatinine) and the stage of endometriosis Comments: - urine testing for BPA reflects	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.3 Effects on the female reproductive system

study		N=140 -> Sufficient population size			contaminants: no Other: creatinine	recent exposure and not long-term contamination. - no control group truly free from disease, - urinary samples stored in plastic tubes in a freezer for 5 years		
(Kandaraki *et al.*, 2011) Endocrine Disruptors and Polycystic Ovary Syndrome (PCOS): Elevated Serum Levels of Bisphenol A in Women with PCOS	Age- and BMI-matched cross-sectional study	Study population: women N=71 cases (women with PCOS) vs 100 controls → Sufficient population size	Serum	ELISA	Age: yes (via matching) Sex: NA Medication: NA Tobacco: NA BMI: yes (via matching) Other contaminants: no Other: via a multivariate analysis (anthropometric, hormonal and metabolic parameters)	Results: - Serum BPA concentrations significantly higher in women with PCOS (obese or not) compared to normal control women. - In women with PCOS (obese or not): significant increase in testosterone levels and the LH/FSH ratio while SHBG levels were lower than in the controls. - BPA concentrations were significantly correlated with testosterone and androstenedione concentrations and insulin resistance. - BPA concentrations were significantly correlated with the existence of PCOS.	Studies not taken into consideration since they have major methodological limitations This study was excluded due to: - an analytical method (ELISA) that does not distinguish between the various forms of BPA.	6.1 Information from epidemiological studies 6.3 Effects on the female reproductive system
(Takeuchi *et al.*, 2004) Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction	Cross-sectional study	Study population: general population: women N=7 patients with hyper-prolactinemia, 21 with hypothalamic amenorrhea, 19 with PCOS (13 non-obese and 6 obese) vs 26 controls (7 obese and 19 non-obese) -> Small population size	Serum	ELISA (validation of the assay method by HPLC)	Age: no Sex: NA Medication: NA Tobacco: no BMI: no Other contaminants: no	Results: correlation between plasma concentrations of testosterone (free and total) and BPA firstly and BPA concentrations and BMI secondly: levels significantly increased in women with PCOS (obese or not) and obese women without ovulation dysfunction. Comments: The results remain difficult to interpret as is, due to the vagueness of the sampling plan, the lack of information on inclusion criteria and failure to take into account the pathologies of the control subjects in the results.	Studies not taken into consideration since they have major methodological limitations This study was excluded in light of the following methodological weaknesses: - small population size, - statistical analysis lacking detail, - the final comparison was made in relation to non-obese women, with normal cycles (considered as controls) - no adjustment for confounding factors, - plasma BPA measured	6.1 Information from epidemiological studies 6.3 Effects on the female reproductive system

							using the ELISA technique (lower limit).	

Effects on child behaviour								
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Braun *et al.*, 2009) Prenatal Bisphenol A Exposure and Early Childhood Behavior	Prospective cohort study	Study population: Mothers and their 2-year-old children (included in the Health Outcomes and Measures of the Environment Study programme; use of an existing biobank, recruitment in 2003) N=249 mothers and their 2-year-old children -> Sufficient population size	Urinary (in mothers at 16 and 26 weeks of gestation and at birth), free and conjugated BPA	HPLC/MS/MS	Age: yes (age of the mother) Sex: NA Medication: NA Tobacco: yes BMI: NA Other contaminants: yes	Results: Positive association with externalising behaviour Comments: - no biological reliability - use of an existing biobank (recruitment in 2003) – the samples were stored for 4-5 years, *questionnaire - no direct urinary BPA measurements in children, The study was the subject of a highly critical analysis (Human Data on Bisphenol A and Neurodevelopment doi:10.1289/ehp.0901610) whose comments are clearly justified.	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.4 Effects on the brain and behaviour
(Miodovnik *et al.*, 2011) Endocrine disruptors and childhood social impairment	Prospective cohort study	Study population: children between the ages of 7 and 9 years N=137 children	Urinary (in 404 mothers between the 25th and 40th weeks of pregnancy)	Not specified	Age: yes (maternal age and exact age of the child during the examination) Sex: yes (sex of children) Medication: no Tobacco: no BMI: no Other	Results: 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.	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.4 Effects on the brain and behaviour

					contaminants: no Other: urinary creatinine of children, marital status on the follow-up date, education of mothers, race, IQ of mothers and children			

Effects on metabolism / the cardiovascular system								
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Hong *et al.*, 2009) Community level exposure to chemicals and oxidative stress in adult population	Cross-sectional study	Study population: general adult population N=960 → Excellent population size	Urinary	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: yes BMI: no Other contaminants: yes Other: physical activity, medical and professional history, alcohol	Results: Significant positive relationship between urinary concentrations of chemical contaminants, particularly phthalates and BPA, and markers of oxidative stress in a simple regression analysis (not significant if multiple regression analysis for BPA) Subjects with the highest levels of BPA were prone to fasting hyperglycaemia but no association with insulin-resistance indices	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.5 Effects on metabolism and the cardiovascular system
(Lang *et al.*, 2008) Association of Urinary Bisphenol A Concentration With Medical Disorders and Laboratory Abnormalities in Adults	Cross-sectional study nested in the NHANES study (2003-2004)	Study population: general adult population (18-74 years) N=1455 adults -> Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: yes BMI: yes Other contaminants: no Other: urinary creatinine, ethnic group/race, education, financial resources, abdominal circumference	Results: positive association between the highest urinary concentrations of BPA (5 and 13 ng/mL) and cardiovascular disease, diabetes and levels of liver enzymes in the blood Comments: This study warrants particular attention because: - powerful study with a solid design, - the associations are extremely robust, - large sample size, - based on American national cohorts, However, the use of medication was not taken into account and contemporary exposure is not necessarily representative of past exposure, which was correlated with the observed effect	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.5 Effects on metabolism and the cardiovascular system

						Note: The studies by Melzer *et al.* and Lang *et al.*were undertaken 2 years apart with the same type of protocol.		
(Melzer *et al.*, 2010) Association of Urinary Bisphenol A Concentration with Heart Disease: Evidence from NHANES 2003/06	Cross-sectional study nested in the NHANES study (2003-2006)	Study population: general adult population (18-74 years) N=1455 (2003/04) and 1493 (2005/06) -> Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: yes BMI: yes Other contaminants: no Other: urinary creatinine, ethnic group/race, education, financial resources, abdominal circumference	Results: - In 2005/2006: significant association between the highest urinary concentrations of BPA and coronary disease. No association between urinary concentrations of BPA and diabetes. - In 2003/06: significant association between the highest urinary concentrations of BPA and heart disease, diabetes, alkaline phosphatase and lactate dehydrogenase. Comments: This study warrants particular attention because: - solid design and high power (80% for the 2003/2004 population and 74% for the 2005/2006 population) - the associations are robust, - large sample size, - based on American national cohorts, However, the use of medication was not taken into account and contemporary exposure is not necessarily representative of past exposure, which was correlated with the observed effect. Note: The studies by Melzer *et al.* and Lang *et al.* were undertaken 2 years apart with the same type of protocol.	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.5 Effects on metabolism and the cardiovascular system

(Takeuchi *et al.*, 2004) Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction	Cross-sectional study	Study population: general population: women N=7 patients with hyperpro-lactinemia, 21 with hypothalamic amenorrhea, 19 with PCOS (13 non-obese and 6 obese) vs 26 controls (7 obese and 19 non-obese) -> Small population size	Serum	ELISA (validation of the assay method by HPLC)	Age: no Sex: NA Medication: NA Tobacco: no BMI: no Other contaminants: no	Results: correlation between plasma concentrations of testosterone (free and total) and BPA firstly and BPA concentrations and Body Mass Index secondly: levels were significantly higher in women with PCOS (obese or not) and obese women without ovulation dysfunction. Comments: The results remain difficult to interpret as is, due to the vagueness of the sampling plan, the lack of information on inclusion criteria and failure to take into account the pathologies of the control subjects in the results.	Studies not taken into consideration since they have major methodological limitations This study was excluded in light of the following methodological weaknesses: - small population size, - statistical analysis lacking detail, - the final comparison was made in relation to non-obese women, with normal cycles (considered controls) - no adjustment for confounding factors, - plasma BPA measured using the ELISA technique (lower limit).	6.1 Information from epidemiological studies 6.3 Effects on the female reproductive system 6.5 Effects on metabolism and the cardiovascular system

Effects on birth weight								
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Padmanabhan *et al.*, 2008) Maternal bisphenol-A levels at delivery: a looming problem?	Cross-sectional study	Study population: general population: women at delivery N=40 pregnant women → Small population size	Plasma (in mothers) (free)	HPLC/ESI-MS/MS	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other contaminants: no Other: no	Results: No association between plasma concentrations of BPA and gestation period or birth weight Comments: - One single BPA measurement taken at birth and not at the start or middle of pregnancy	Studies not taken into consideration since they have major methodological limitations This study was excluded due to the following methodological weaknesses: - small population size, - no adjustment for confounding factors, - no measurement of conjugated BPA	6.1 Information from epidemiological studies 6.5 Effects on metabolism and the cardiovascular system
(Wolff *et al.*, 2008b) Prenatal Phenol and Phthalate Exposures and Birth Outcomes	Prospective study	Study population: general population (women) N=367 → OK population size	Urinary	HPLC	Age: yes (gestational age) Sex: yes (sex of children) Medication: NA Tobacco: yes (during pregnancy) BMI: yes (pre-gestational) Other contaminants: yes Other: creatinine, race, maternal education	Results: no significant association between BPA and birth weight, infant size, head circumference or gestational age Comments: - Only one measurement taken, - Low plasma levels of BPA, - No association between plasma concentrations of BPA and effects on newborns	Studies of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.5 Effects on metabolism and the cardiovascular system

Effects on thyroid hormones								
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Meeker *et al.*, 2010a) Urinary Bisphenol A Concentration in Relation to Serum Thyroid and Reproductive Hormone Levels in Men from an Infertility Clinic	Cross-sectional study	Study population: men consulting for fertility problems N=167 cases vs 190 controls	Urinary (free and conjugated BPA) (0.4 to 36.4 ng/mL)	HPLC/MS/MS	Age: yes Sex: NA Medication: NA Tobacco: yes BMI: yes Other contaminants: No Other: season, sampling time, specific gravity	Results: - Positive association between urinary concentrations of BPA and FSH and the FSH/Inhibin B ratio - Negative association between urinary concentrations of BPA and the oestradiol/testosterone ratio, TSH, inhibin B and the free testosterone and oestradiol indices. Comments: At least 2 and up to 4 urine samples	Studies not taken into consideration since they have major methodological limitations This study was excluded for the following reasons: - it dealt with a specific population of men consulting for problems of infertility with their partners. - thyroid aspects appear as 'opportunistic', as the study was undertaken in relation to a problem of fertility and the protocol did not take into account features specific to investigation of thyroid function. - the significance of this correlation depends on the number of urine samples used to calculate the geometric mean (from 1 to 3 for each individual). Yet only 75 patients underwent repeated sampling. If the analysis examines the correlation between TSH and the single urinary BPA measurement taken on the day of sampling in all of the patients (n=167), or between TSH at the first sampling time and the geometric mean of BPA concentrations from the 3 samples, limited to the 75 patients who underwent multiple sampling, no significant correlation can be highlighted.	6.1 Information from epidemiological studies 6.2 Effects on the male reproductive system 6.6 Effects on the thyroid

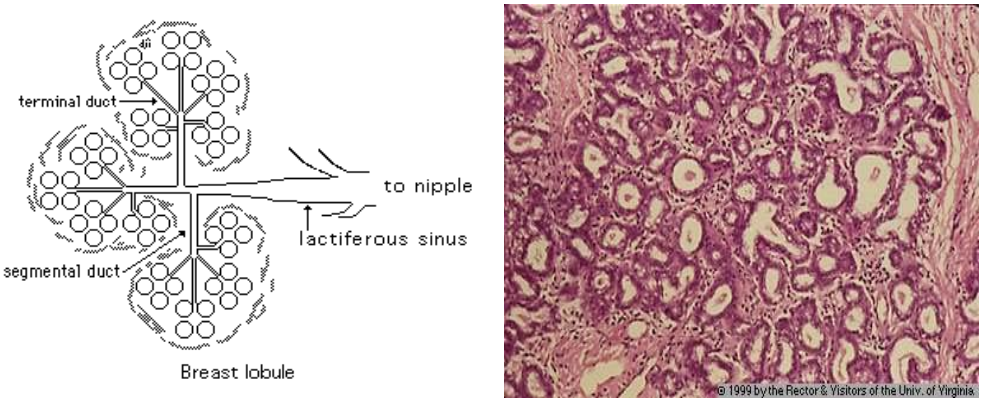
Effects on the immune system								
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Clayton *et al.*, 2011) The Impact of Bisphenol A and Triclosan on Immune Parameters in the US Population, NHANES 2003-2006	Study nested in the NHANES study (2003-2006)	Study population: stratified general population with an over-representation of African Americans, Mexican Americans and Americans over the age of 60 years N=787 (CMV antibody) N=2133 (allergy)	Urinary	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: no BMI: yes Other contaminants: No Other: ethnic group, creatinine level, income, academic success	Results: For subjects ≥ 18 years: the highest concentrations of BPA were associated with higher CMV antibodies, For subjects < 18 years: negative relationship between urinary concentrations of BPA and CMV antibody concentrations Comments: A positive association with cytomegalovirus antibodies was observed, but the extent and causality of this relationship remain uncertain. Moreover, the authors worked in a population with ”detected levels of BPA”, which suggests that individuals with levels below the limit of detection were excluded, which is a truly questionable selection method.	Study of high quality or having no major methodological limitations	6.1 Information from epidemiological studies 6.7 Effects on the immune system

Breast cancer								
Reference Article title	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Yang *et al.*, 2009) Effects of bisphenol A on breast cancer and its risk factors	Age-matched cross-sectional study	Study population: general population (women) N=70 cases (women with breast cancer) and 82 controls → The population size is difficult to assess as the expected difference is small	Blood (free and conjugated BPA) Conjugated BPA used as a biomarker (blood stored in Eppendorf tubes for over 10 years)	HPLC/FD	Age: Yes (age matching and adjustment) Sex: NA Medication: no Tobacco: yes BM: yes Other contaminants: No Other: age at menopause	Results: No significant difference in blood concentrations of BPA between the cases and controls.	Studies not taken into consideration since they have major methodological limitations This study was excluded for the following reasons: - The population size is difficult to assess as the expected difference is small, - blood samples stored in Eppendorf tubes for over 10 years, - population recruited in 1994-97, - BPA analysed only in the blood (without specifying whether it was total blood or plasma) in a single sample - No urinary sampling.	6.1 Information from epidemiological studies 6.11 Effects on the breasts

Annex 7: State of knowledge concerning preneoplastic lesions of the breast - Discussion Points

The frequency with which pathologists and clinicians are faced with preneoplastic or precancerous lesions of the breast has increased significantly with the widespread use of mammographic screening and, to a lesser extent, with the improvement in the management of pathological surgical specimens.

Precancerous lesions of the breast correspond to atypical epithelial proliferations that develop within the lactiferous tree and are of two types: ductal and lobular. These two types are distinguished not by their location but by their constituent cell type. Indeed, in 1994, Wellings *et al.* demonstrated that most pre-invasive breast lesions begin in the ***terminal duct lobular unit*** (TDLU), very sensitive to hormonal factors and located at the termination of the lactiferous ducts and their junction with the lobules (Wellings and Alpers, 1994).



TDLU: High power view showing the two-cell layer epithelium

Figure 1: anatomy of the breast and terminal duct lobular unit

Both types, ductal and lobular, are characterised at the molecular level by the presence at the cytoplasmic membranes of an intercellular junction complex protein: E-cadherin.

Histological diagnosis of precancerous lesions is difficult, and **inter-pathologist reproducibility** is **poor**, as evidenced by a number of studies. The diagnosis seems slightly improved with the contribution of immunohistochemistry in lesions that have a specific profile (MacGrogan *et al.*, 2008).

To facilitate the diagnosis and management of precancerous lesions, new terminology was proposed in the early 2000s and adopted by the WHO in 2003. It is superimposed on the traditional terminology and is **based on morphological,** non-molecular criteria.

2003 WHO classification of precancerous lesions

It is based on the terms describing *intraepithelial neoplasia*: ductal or DIN: lobular or LIN. The ductal epithelial proliferations are divided into five categories and lobular proliferations into three categories.

Intraepithelial neoplasia, ductal type

1. Atypical cylindrical metaplasia (ACM): DIN-1A
2. Atypical ductal hyperplasia (ADH): DIN-1B
3. Ductal carcinoma *in situ* (DCIS) low-grade: DIN-1C
4. DCIS intermediate grade: DIN-2
5. DCIS high-grade: DIN-3

The first two categories correspond to precancerous lesions.

Ductal carcinoma *in situ* (DCIS) is not a **precancerous lesion** but a **pre-invasive** cancerous lesion. In the US, DCIS accounts for nearly 20% of detected cancers (1 case of DCIS for 1300 screening mammograms) (Ernster *et al.*, 2002).

Mammary intraepithelial neoplasia, lobular type

1. Atypical lobular hyperplasia (ALH): LIN-1
2. ALH/LCIS: LIN-2
3. Lobular carcinoma *in situ* (LCIS): LIN-3, which includes three different types

This new terminology has led to improved management of precancerous lesions. For example, for lobular lesions, the recommendations of the International Agency for Research on Cancer (IARC), published in November 2009, advocate simple monitoring for LIN-1 and LIN-2, and when the histological examination reveals LIN-3, the initial treatment is based on surgical excision.

Preneoplastic breast lesions: risk of transformation to invasive cancer

When left in place, preneoplastic or precancerous lesions may transform into pre-invasive carcinoma or carcinoma *in situ*, which can itself progress to invasive carcinoma (Figure 2).

The introduction of the concept of the terminal duct lobular unit has led to the concept of malignant transformation – non-obligatory – passing through various stages, similar to colon cancer. Normal cells located in the terminal duct lobular unit are transformed to atypical hyperplasia, and ductal carcinoma *in situ* to invasive cancer (Figure 2 below).

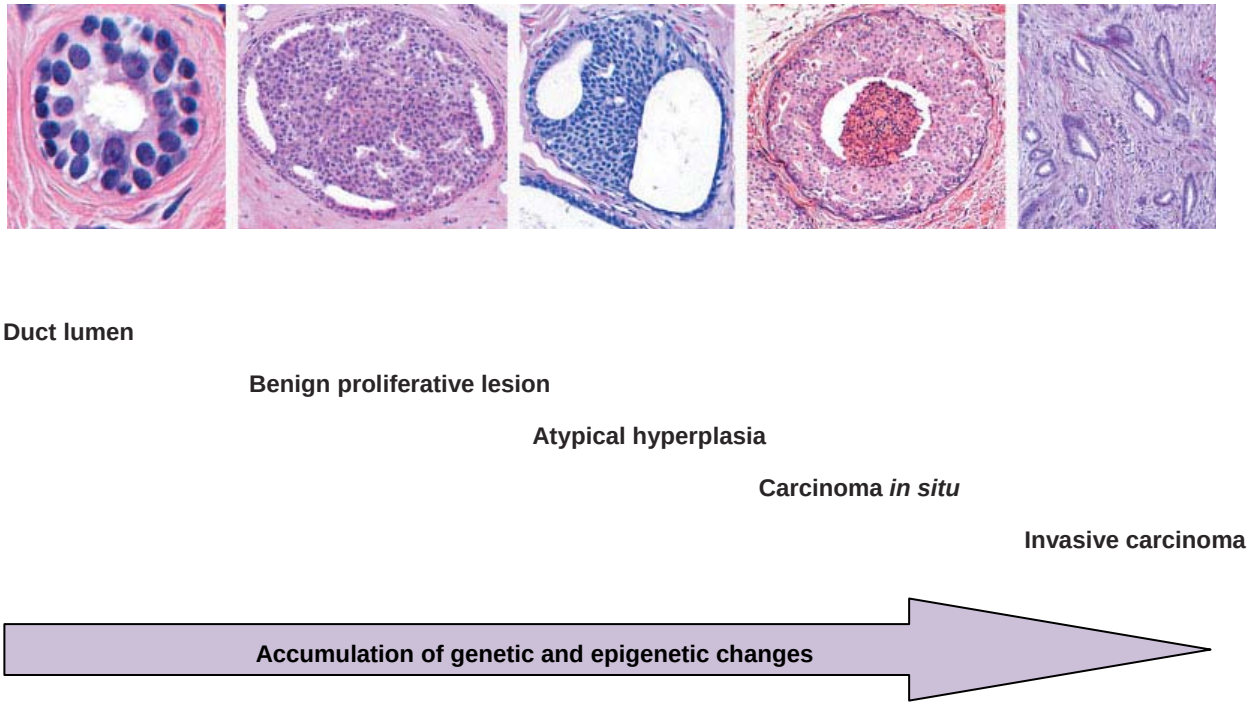


Figure 2: Pathological slides illustrating the transformation of the normal mammary gland to invasive cancer (Figure taken from Burstein *et al.*, 2004)

The hypothesis of the existence of a continuum between the normal mammary gland and invasive breast cancer, although it may seem too simplistic, is based on direct and indirect arguments (Antoine *et al.*, 2010).

Historical studies since the early 19th century have observed that benign lesions were more frequently found in patients with breast cancer. More recent epidemiological studies have shown that women with a history of benign breast lesions have an increased risk of breast cancer.

Until the early 1990s, pathologists and clinicians referred to earlier works, notably those of Dupont and Page (Dupont and Page, 1985) who calculated the risk of developing subsequent breast cancer in patients with benign lesions, often found as palpable lesions before the era of mammography screening.

The natural history of low-grade ductal carcinoma *in situ* (DCIS) was determined by long-term monitoring studies in women who underwent diagnostic biopsy without further treatment before the era of organised mass screening. After 10 years of follow-up, 14 to 60% of these women were diagnosed with invasive cancer in the same breast (Page *et al.*, 1995). The demonstration of this risk of invasion has also led to the present attitude of actively treating these lesions. The natural history of high-grade DCIS or clinically palpable DCIS is, however, not well characterised, because in most cases, the tumour is removed completely by surgery, which is also the case for atypical ductal hyperplasia (ADH) lesions.

The significant increase in biopsies done on the basis of subclinical images and recent data provided by the molecular study of lesions have shed new light on the transformation risk of hyperplastic lesions to cancer.

During the transformation of hyperplastic lesions to carcinoma *in situ* and then invasive cancer, imbalances are observed at the chromosomal level, with a loss of heterozygosity in 70% of high-grade carcinomas *in situ*, compared to nearly 40% cases of atypical hyperplasia and 0% in healthy breast tissue (Aubele *et al.*, 2000). Molecular markers of breast tumour transformation have been identified. The oestrogen receptor expressed by normal epithelial breast cells is expressed by more than 70% of ductal carcinoma *in situ*. The HER2/neu proto-oncogene is overexpressed in half of DCIS but not in atypical hyperplasia (Allred *et al.*, 1992).

Published work from the early 2000s on the molecular pathways involved have shown that breast cancer is not a single disease, but rather a set of different diseases occurring in the same anatomical structures (TDLU). These molecular biology techniques have also shown that **precancerous and pre-invasive lesions** are as heterogeneous as invasive cancers.

Different models of progression according to the **histological grade** (low-grade or high-grade) and the presence or absence of **oestrogen receptors** have been proposed (see figures below taken from the publication of (Lopez-Garcia *et al.*, 2010)).

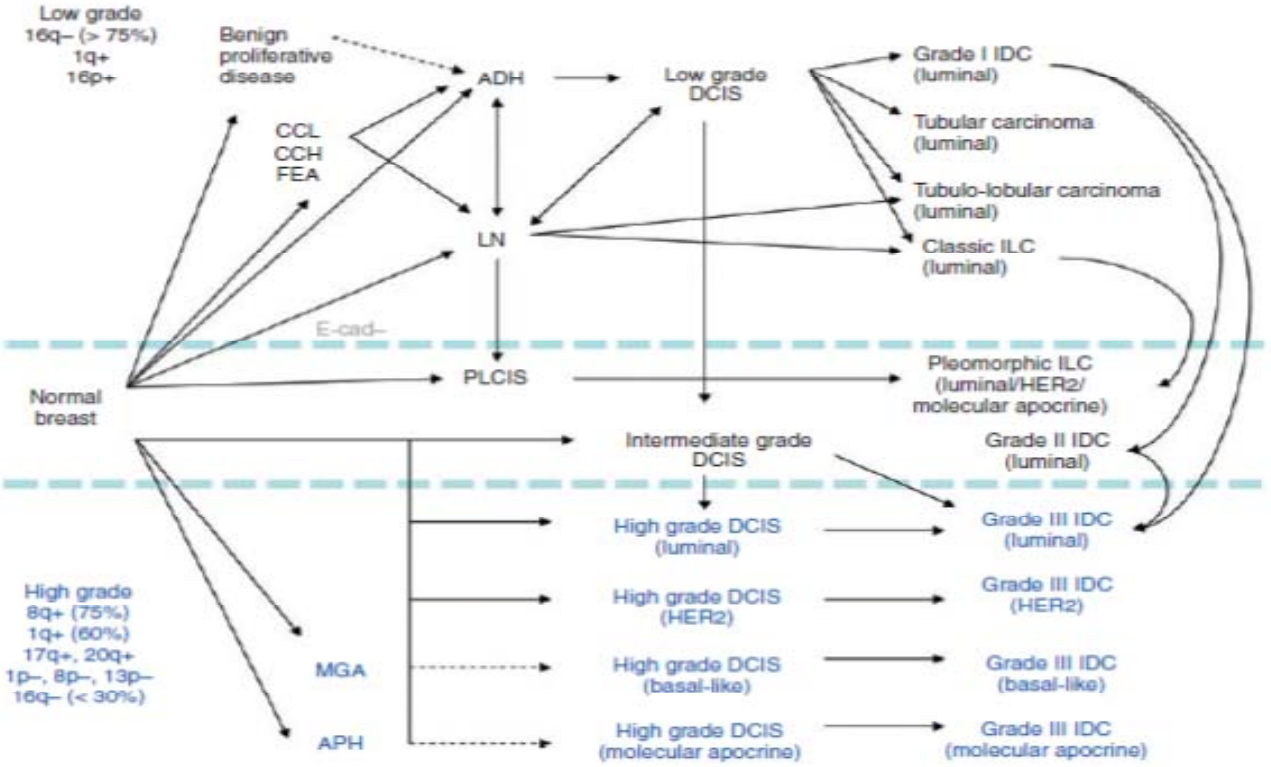


Figure 3. Low- and high-grade multistep model of breast cancer progression based on morphological, immunophenotypical and molecular features. Connectors drawn with continuous lines represent links between morphological entities which are demonstrated by morphological and/or molecular data. Connectors 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*.

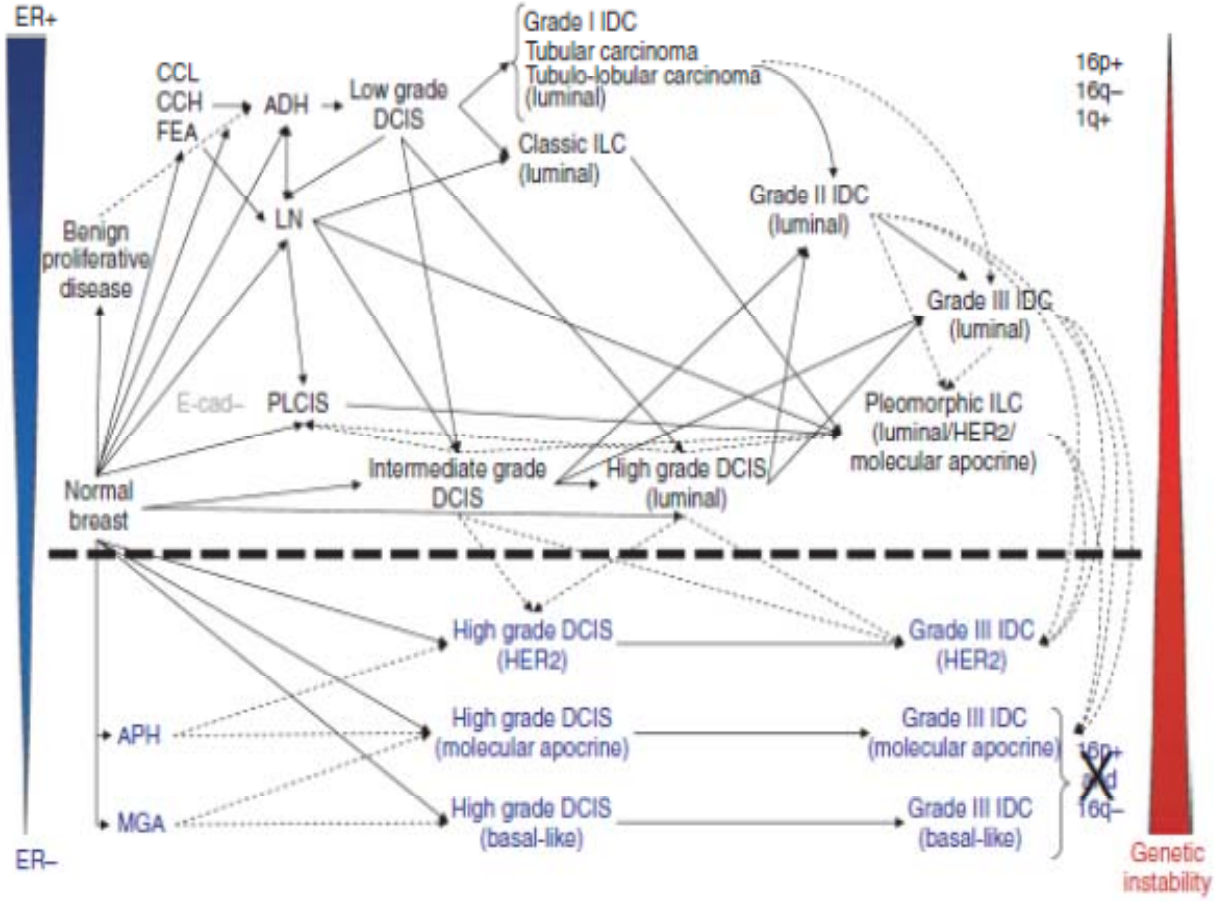


Figure 4. Revised multistep model of breast cancer evolution based on oestrogen receptor (ER)-status. Note that the two main pathways are defined by the expression of ER and ER-regulated genes. In this model, the ER-positive arm encompasses most of the precursor lesions and a range of invasive lesions 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*.

Moreover, recent data show that the epithelial atypias represent not only a **risk factor for secondary carcinogenesis**, but also a **risk marker for concomitant cancer in the surrounding area** (from Mascarel *et al.*, 2007).

Conclusion

Improved knowledge provided by immunohistochemistry, cytogenetics, and molecular biology has improved the classification of precancerous lesions and the understanding of the relationship between epithelial atypias and breast cancer. These elements have helped to refine the diagnosis, to better determine the risk of progression to invasive cancer, and thus to optimise patient treatment.

Annex 8: Summary of breast cancer in rodents

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-independent 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 approaches or exceeds 50% in the chronic studies (e.g., NTP historical data, 2008). There are strains with increased susceptibility to mammary carcinogenesis: the Wistar-Furth strain is very susceptible to 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 between the lesions observed in humans and those induced in rodents were described, but differences also exist.

- The similarities include:
  - A multi-stage process of development
  - The majority 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* are regarded as a morphological progression towards breast carcinoma, beginning with intraductal proliferative lesions.

Table XVII: 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
Hyperplasias	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 often affected in rats, as compared to humans.

Annex 9: Presentation of differing positions

Position expressed by member Ms Véronique Ezratty

A proposal made by one of the members of the Working Group was not accepted by the Working Group. This alternative proposal consisted in establishing a single conclusion, integrating both human and animal data, while indicating respectively for each of the two categories their level of quality as well as the plausibility of a mechanism of action, along the lines of IARC classifications of carcinogenic substances, and carcinogens, mutagens, and reprotoxic agents (CMRs) of the European Union. This proposal also judged that it would be preferable to avoid speaking about recognised effects when they were only observed in animals without available human data. In fact, separating but using the same terminology for humans and animals, as was decided by the WORKING GROUP, could pose a risk of misinterpretation, if certain elements of the conclusion were excerpted during the report's release.

Annex 10: Summary of experts' public declarations of interest in the field of the request

This section provides a summary of interests reported by experts in the field of the request, and specifies on one hand how these interests have been analysed, and also how they were managed in regard to a potential conflict of interest.

Public statements of interests are updated by the experts at each change of situation. During the expert appraisals, related interests are reviewed in light of the agenda.

REVIEW OF THE SECTIONS OF THE PUBLIC DECLARATION OF INTEREST

IP-A	Occasional services: other
IP-AC	Occasional services: consulting activities
IP-CC	Occasional services: conferences, seminars, training activities
IP-RE	Occasional services: expert reports
IP-SC	Occasional services: scientific research, testing, etc.
LD	Lasting or permanent relationship (work contract, regular payment, etc.)
PF	Financial interest in the capital of a company
SR	Other unpaid relationships (Relatives employed in companies referred to above)
SR-A	Other special unpaid relationships (Participation on Boards of Directors, scientific boards in an organisation, company or professional body)
VB	Activities resulting in a payment to the budget of an organisation

SUMMARY OF PUBLIC DECLARATIONS OF INTEREST OF MEMBERS OF THE EXPERT COMMITTEE (CES) IN THE FIELD OF THE REQUEST

Last name **ANSES analysis:**	**First name** ***Sections of the PDI*** Description of the interest *if declared relationship*	**Date of the declaration of interest**
BELZUNCES	**Luc** ***LD***	10/02/2010 28/01/2011

	University of Avignon: Teaching (since 1998) (Occasional) University of Angers: Teaching (since 2004) (Occasional) University of Aix-Marseille 3: Teaching (since 2000) (Occasional) Mediterranean Agronomic Institute of Chania, Crete: Teaching (01/11/2005 to 10/11/2010) (Salary) ***IP*** ADAPi (Association for the Development of Provençal Apiculture): Conferences (2000-2006) (Personal compensation) UNAF (National Union of French Beekeeping): Conferences (2000-2006) (Personal compensation) and consulting (2011) (Occasional personal compensation) ADARA: Conferences (2000-2006) (Personal compensation) GIE Apiculture Pays de Loire: Training in bee toxicology (02/02/2011) (Personal compensation) Beekeeping organisations: Regular conferences (Permanent) (Personal compensation) ***VB*** Bayer: Effects of imidacloprid in the honeybee, resulting in payment to the INRA (50% of the laboratory budget where the expert is Director of Research) (contract ended in 2001) Aventis-BASF: Activity of sunflower secretions in the honeybee, resulting in payment to the INRA (50% of the laboratory budget) (2002)	

ANSES analysis:	Aventis: Mechanism of action of acetamiprid, resulting in payment to the INRA (50% of the laboratory budget) (2001-2003) ***SR*** BASF: youth intern (2007) No potential conflict of interest	
BONVALLOT **ANSES analysis:**	**Nathalie** ***Resigned 31 March 2011*** ***LD*** EHESP: fixed-term contract (Oct. 2008-Oct 2011) ***IP*** PBE+ (performance Bretagne environnement), an association of Breton companies from all sectors: PBE+ Days, Toxicology (April 2008) (No compensation) EDF GDF: Training QHRA (June, each year) (No compensation) ***SR*** OBERTHUR: Spouse is Manager for Health, Safety and Environment /	09/02/2010 25/01/2011
BOURGEOIS	**Damien** ***IF*** Rhodia: Company savings plan, some stocks ***SR***	18/02/2010 24/01/2011

ANSES analysis:	Environmental Maintenance Department: Spouse is engineer (since 01/12/2010) No potential conflict of interest	
CASSIER-CHAUVAT **ANSES analysis:**	**Corinne** ***LD*** CNRS/CEA: CNRS researcher in the UMR CEA-CNRS (since 1983) ***SR*** CEA: Spouse is Researcher, Laboratory Head (since 1985) No potential conflict of interest	26/02/2010 01/02/2011
EMPEREUR-BISSONNET **ANSES analysis:**	**Pascal** ***SR*** SERVIER International Research Institute: Spouse is Operational Support Director (1990-2011) No potential conflict of interest	01/02/2010 26/01/2011
ENRIQUEZ **ANSES analysis:**	**Brigitte** No declared relationship /	01/02/2010 02/02/2011
GUENOT	**Dominique** ***IP*** Second edition of the event "To your health!" ("*A votre santé !*") Health and Medical Research Month in Alsace: Lecture/discussion following film screening (1 day in March 2010) (No compensation)	18/02/2010 02/02/2011

ANSES analysis:	No potential conflict of interest	
GUERBET **ANSES analysis:**	**Michel** ***IP*** AFSSAPS: expert in the preclinical group (2006-2012) and on the MA committee for medicinal products (2010-2013) (No compensation) No potential conflict of interest	24/02/2010 02/02/2011
HUYNH **ANSES analysis:**	**Cong Khan** No declared relationship /	19/01/2010 28/01/2011
KRISHNAN **ANSES analysis:**	**Kannan** ***IP*** Regulatory checkbook: Invited speaker and panelist, Naphthalene: State of the Science Symposium and Workshop (3 days in 2006) (Paid 3 days, travel costs) ***VB*** ExxonMobil in conjunction with the Natural Sciences and Engineering Research Council of Canada: Research Grant for "An integrated fugacity-pharmacokinetic model" resulting in payment to the University of Montreal, Trent University and University of Quebec at Montreal (<10% of budget) (2007-2010) No potential conflict of interest	31/03/2009 02/03/2011
LAFON	**Dominique** ***LD*** Archives of occupational diseases: copyright (since 1995)	15/02/2010 25/01/2011

ANSES analysis:	Dassault Falcon service: Occupational health physician on permanent contract (since 1995) ***IP*** AFSSA: CES MCDA (2000-2006) (Occasional) AFSSAPS: Cosmetology Commission (2010) No potential conflict of interest	
LAGADIC-GOSSMANN **ANSES analysis:**	**Dominique** No declared relationship /	25/02/2010 30/01/2011
LAUDET **ANSES analysis:**	**Annie** No declared relationship /	23/02/2010 17/03/2011
MENETRIER **ANSES analysis:**	**Florence** ***IP*** ANR: Call for projects: Environmental Health/Occupational Health (June 2006-August 2006) (No compensation) No potential conflict of interest	23/02/2010 26/01/2011
PRAT **ANSES analysis:**	**Odette** No declared relationship /	27/01/2010 30/01/2011
SCHROEDER	**Henri** ***IF*** Air France, Rexel, BNP: stocks Investment funds, LCL Protection: Financial	27/02/2010 28/01/2011

ANSES analysis:	products ***VB*** Food industry: Contract concerning functional foods, resulting in payment to the University Henri Poincaré (50% of the laboratory where the expert is Research Professor, Head of Studies) (2001-2006) ANSES: Literature monitoring (author of updates) resulting in payment to the University Henri Poincaré (15% of the laboratory budget) (2009-2011) No potential conflict of interest	
SECRETAN-LAUBY	**Béatrice** ***LD*** IARC: Employee (since 2001) ***IP*** Encyclopedia of Cancer (Springer Verlag): Article on "UV radiation" (December 2007 to February 2008) (No compensation) AFTIM (French Association of Safety Engineers and Occupational Health Physicians): AFTIM-ADHYS Day, Paris (2004) (No compensation) ADHYS (Association for the Development Of Health And Safety In Research Institutions Or Higher Education): 22nd ADHYS Day (2005) (No compensation) BTP: 28th National BTP Day (2005) (No compensation) DRASS (Departmental Directorate of Health And Social Affairs): Day of exchanges on professional practice concerning CMRs (2005) (No	02/03/2010 03/02/2011

	compensation) TSR (Télévision Suisse romande): Broadcast: "A bon entendeur," regarding benzene in table drinks (2006) (No compensation) AFSSET (French Agency for Environmental and Occupational Health Safety): Substitution: an issue for CMRs (2007) (No compensation) University of Grenoble: CME Day: What's New in Oncology (2008) (No compensation) University Claude Bernard Lyon 1er : Training day for the SMST Company, Lyon: Occupational cancer (2010) (No compensation) University of Grenoble: Teaching (1 day in 2006, 2007, 2010) (No compensation) No potential conflict of interest	
ANSES analysis:		
TISSOT	**Sylvie** ***Resigned 14 January 2011*** No declared relationship	05/02/2010 27/01/2011
ANSES analysis:	/	

Summary of experts' public declarations of interest in the field of the request

LAST NAME	**First name** ***Sections of the PDI*** Description of the interest *if declared relationship*	**Date of the declaration of interest**
ANSES analysis:		

ANTIGNAC	**Jean-Philippe**	22/02/2010 10/03/2011

	IP INSERM expert report on reprotoxic substances (since 2009) (Personal compensation) **ANSES analysis:** No potential conflict of interest	
BELZUNCES	**Luc** (Member of CES on Assessment of the risks related to chemical substances) ***LD*** University of Avignon: Teaching (since 1998) (Occasional) University of Angers: Teaching (since 2004) (Occasional) University of Aix-Marseille 3: Teaching (since 2000) (Occasional) Mediterranean Agronomic Institute of Chania, Crete: Teaching (01/11/2005 to 10/11/2010) (Salary) ***IP*** ADAP (Association for the Development of Provençal Apiculture): Conferences (2000-2006) (Personal compensation) UNAF (National Union of French Beekeeping): Conferences (2000-2006) (Personal compensation) and consulting (2011) (Occasional personal compensation) ADARA: Conferences (2000-2006) (Personal compensation) GIE Apiculture Pays de Loire: Bee Toxicology	10/02/2010 28/01/2011

	Training (02/02/2011) (Personal compensation) Beekeeping organisations: Regular conferences (Permanent) (Personal compensation) ***VB*** Bayer: Effects of imidacloprid in the bee, resulting in payment to the INRA (50% of the laboratory budget where the expert is Director of Research) (contract ended in 2001) Aventis-BASF: Activity of sunflower secretions in the bee, resulting in payment to the INRA (50% of the laboratory budget) (2002) Aventis: Mechanism of action of acetamiprid, resulting in payment to the INRA (50% of the laboratory budget) (2001-2003) ***SR*** BASF: youth intern (2007)	
ANSES analysis:	No potential conflict of interest	
BENAHMED	**Mohamed** ***IP*** Galderma: Expert appraisal (December 2009) BASF-Galderma: Expert Report (January 2010) BASF-Galderma: Training on the utility of new markers (in progress) (Personal compensation) ECETOC: Workshop (Personal compensation) ***VB*** BAYER, GALDERMA: Cooperation contracts, conventions on endocrine disruptors, resulting in payment to the INSERM (5% of budget)	04/03/2010

ANSES analysis:	BASF, BAYER: CIFRE contracts on hormone disrupters, reprotoxicity resulting in payment to the INSERM (5% of budget) No potential conflict of interest	
BERTRAND **ANSES analysis:**	**Nicolas** ***LD*** Employee of the INRS (2007-2011) No potential conflict of interest	17/03/2010 09/03/2011
BLANCHARD **ANSES analysis:**	**Olivier** ***LD*** Employee of the INERIS (until 2009) No potential conflict of interest	22/02/2010 28/01/2011
CLAUW	**Martine** ***LD*** Sanofi-Aventis: External member of the Ethics Committee for the Protection of Laboratory Animals, Toulouse (since 2009) (No compensation) ***IP*** Nutreco (The Netherlands): Research agreement on the digestive impact of thermal stress (since 2010) Royal Canin, SAS (Aymargues, France): Research agreement on canine geriatrics and stress (since 2010) ***VB*** Training levy for Pierre Fabre medications and	16/03/2010 02/02/2011

ANSES analysis:	Sanofi-Aventis for the benefit of the Veterinary School of Toulouse (0.01% of budget) No potential conflict of interest	
CRAVEDI	**Jean-Pierre** ***IF*** Total, Arcelor Mittal: Stocks ***LD*** EFSA: Expertise (Contaminants panel) (since 2003) (Personal compensation) ***IP*** DANONE: Analysis of water samples (from October 2010), leading to compensation of the parent organisation (INRA) L'OREAL: Bioaccumulation in fish (2008-2009) (Occasional) DANONE: Detection of endocrine disrupters in water (2009-201) (Occasional) ***VB*** Pierre FABRE: Thesis grant resulting in payment to the INRA (5% of budget) DANONE: Analysis of xenobiotics in the water, resulting in payment to the INRA (1% of budget)	19/11/2009 18/04/2011
ANSES analysis:	No potential conflict of interest	
DUPUPET	**Jean-Luc** ***Resigned 22 October 2010*** ***LD***	31/03/2010

ANSES analysis:	CCMSA: National Medical Officer /	
ELEFANT **ANSES analysis:**	**Élisabeth** No declared relationship ***/***	11/03/2010
EMOND **ANSES analysis:**	**Claude** ***IP*** Health Canada: Expert review (2006-2008) (Personal compensation) US EPA, USA: Development of a PBPK model for PBDEs (2007) and consulting work for the reassessment of dioxin exposure levels (2009) (Personal compensation) No potential conflict of interest	24/02/2010 14/07/2011
EUSTACHE **ANSES analysis:**	**Florence** No declared relationship ***/***	08/03/2010
EZRATTY	**Véronique** ***IF*** EDF, Gaz de France-Suez: Stocks ***LD*** EDF: Employee (since 1998) ***IP*** RSEIN: Analysis of scientific articles (since 2007)	10/02/2010 29/03/2011

ANSES analysis:	(Personal compensation) WHO: Author of three chapters on QAI guidelines (September 2008) (Personal compensation) No potential conflict of interest	
LABADIE **ANSES analysis:**	**Pierre** ***Resigned 12 November 2010*** ***IP*** ONEMA: Scientific Monitoring Committee for national action plan on PCBs (No compensation) (December 2009) /	22/02/2010
LE MAGUERESSE-BATTISTONI **ANSES analysis:**	**Brigitte** No declared relationship ***/***	26/02/2010 09/03/2011
LEMARCHAND **ANSES analysis:**	**Frédérick** No declared relationship ***/***	01/02/2010 15/03/2011
MANDIN	**Corinne** ***LD*** Employee of the CSTB ***IP*** Occasional work at the Higher Institute of Health	15/06/2009 11/03/2010 08/03/2011

ANSES analysis:	and Bioproducts of Angers (once a year) (Personal compensation) Analysis of scientific articles for the update newsletter for the RSEIN network of INERIS (once to twice a year) (Personal compensation) ***SR*** Spouse is Sales Director for Taiki Europe No potential conflict of interest	
MINIER **ANSES analysis:**	**Christophe** No declared relationship /	10/03/2010 09/05 /2011
MULTIGNER **ANSES analysis:**	**Luc** No declared relationship ***/***	03/03/2010
PASCUSSI **ANSES analysis:**	**Jean-Marc** ***Resigned 11 January 2011*** ***IP*** SERVIER: Course on CAR and PXR xenoreceptors (Personal compensation) (May 2009) ***VB*** SERVIER: CIFRE grant for thesis (2006-2009) resulting in payment to the parent organisation (ONSERM) (3% of budget) /	22/01/2010
SANCHEZ	**Wilfried**	04/03/2010

ANSES analysis:	***LD*** Employee of the INERIS No potential conflict of interest	
STEENHOUT	**Anne** ***LD*** ULB (Free University of Brussels): Department Head and Director of Research (since 1990) ***IP*** US Environmental Protection Agency: Scientific work resulting in payment to the parent organisation (ULB) Health Council of Belgium: Expert report on dichloromethane resulting in payment to the parent organisation (2000) European Commission: Participation, speaker at meetings, WGs, committees resulting in payment to the parent organisation (since 2002) CSTC (Centre for Science and Technology of Construction) / BATIBOUW (International Building, Renovation and Design Fair): participation in WGs and conferences (no compensation) (2003, 2004-2005) ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals): Project evaluation resulting in payment to the parent organisation (2002) Schools of architecture: lecturer, training resulting in payment to the parent organisation (2001-2005) Ministry, French Community of Belgium: lecturer, training resulting in payment to the parent organisation (2006)	25/02/2010 07/03/2011

	Scientific societies: Speaker (No compensation) ***VB*** Various Ministries / Brussels region, the French Community: scientific research in environment and health resulting in payment to the parent organisation LRI (Long-range research initiative), CEFIC (European Chemical Industry Council): scientific research and statement, resulting in payment to the parent organisation	
ANSES analysis:	No potential conflict of interest	
TAKSER	**Larissa** No declared relationship	02/02/2010 06/05/2011
ANSES analysis:	***/***	
THONNEAU	Patrick No declared relationship	02/03/2010 28/05/2011
ANSES analysis:	***/***	
VIGUIE	Catherine No declared relationship	18/02/2010 24/06/2011
ANSES analysis:	***/***	

Notes