CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: Bisphenol A

EC Number: **201-245-8**

CAS Number: **80-05-7**

Index Number: **604-030-00-0**

Contact details for dossier submitter: ANSES (on behalf of the French MSCA)

253 avenue du General Leclerc

F-94701 Maisons-Alfort Cedex

+33 1 56 29 19 30

cecile.michel@anses.fr

Version number: 2 **Date: 17/07/2013**

TABLE OF CONTENTS

PART A.

List of abbreviations

AGD: Anogenital Distance

AR: Androgen Receptor

ARC: Arcuate nucleus

ART: Assist Reproductive Technology

AUC: Aera Under the Curve

AVPV: Anterio Ventral PeriVentricular nucleus

BPA: Bisphenol A

BW: Body Weight

CLP: Classification Labelling and Packaging

DES: Diethylstilbestrol

DNA: Deoxyribonucleic Acid

DSD: Dangerous Substances Directive

ECN: Embryo Cell Number

EE: Ethynil Estradiol

EFS: Embryo Fragmentation Score

ER: Estrogen Receptor

ERR: Estrogen-Related Receptor

FAI: Free Androgen Index

FSH: Follicle-Stimulating Hormone

FTI: Free Testosterone Index

GD: Gestational Day

GnRH: Gonadotrophine Releasing Hormone

IV: Intravenous

IVF: *In Vitro* Fertilization

LH: Luteinizing Hormone

LOQ: Limit Of Quantification

PCOS: Polycystic Ovary Syndrome

PND: Post Natal Day

PVC: Polyvinyl chloride

RAC: Risk Assessment Comitee

RAR: Risk Assessment Report

SCC: Side Chain Cleavage

SCJP: Sertoli Cell Junction's Protein

SHBG: Sex Hormone Binding Globuline

TC C&L: Technical Committee for Classification & Labelling

TSH: Thydroïd Stimulating Hormone

VO: Vaginal Opening

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

The present CLH report deals with the toxicological properties of bisphenol A (BPA).

Table 1: Substance identity

Substance name:	***Bisphenol A***
EC number:	***201-245-8***
CAS number:	***80-05-7***
Annex VI Index number:	***604-030-00-0***

1.2 Harmonised classification and labelling proposal

Table 2: Current Annex VI entry and proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Repr. 2 H361f*** STOT SE 3 H335 Eye Dam. 1 H318 Skin Sens. 1 H317	Repr. Cat. 3; R62 Xi; R37-41 R43 R52
Current proposal for consideration by RAC	Repro 1B H360F	Repr. Cat. 2; R60
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Repro 1B H360F STOT SE 3 H335 Eye Dam. 1 H318 Skin Sens. 1 H317	Repr. Cat. 2; R60 Xi; R37-41 R43 R52

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None		None	Not evaluated
2.2.	Flammable gases	None		None	Not evaluated
2.3.	Flammable aerosols	None		None	Not evaluated
2.4.	Oxidising gases	None		None	Not evaluated
2.5.	Gases under pressure	None		None	Not evaluated
2.6.	Flammable liquids	None		None	Not evaluated
2.7.	Flammable solids	None		None	Not evaluated
2.8.	Self-reactive substances and mixtures	None		None	Not evaluated
2.9.	Pyrophoric liquids	None		None	Not evaluated
2.10.	Pyrophoric solids	None		None	Not evaluated
2.11.	Self-heating substances and mixtures	None		None	Not evaluated
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Not evaluated
2.13.	Oxidising liquids	None		None	Not evaluated
2.14.	Oxidising solids	None		None	Not evaluated
2.15.	Organic peroxides	None		None	Not evaluated
2.16.	Substance and mixtures corrosive to metals	None		None	Not evaluated
3.1.	Acute toxicity - oral	None		None	Not evaluated
	Acute toxicity - dermal	None		None	Not evaluated
	Acute toxicity - inhalation	None		None	Not evaluated
3.2.	Skin corrosion / irritation	None		None	Not evaluated
3.3.	Serious eye damage / eye irritation	None		Eye Dam. 1; H318	Not evaluated
3.4.	Respiratory sensitisation	None		None	Not evaluated
3.4.	Skin sensitisation	None		Skin Sens. 1 ; H317	Not evaluated
3.5.	Germ cell mutagenicity	None		None	Not evaluated
3.6.	Carcinogenicity	None		None	Not evaluated
3.7.	Reproductive toxicity	Repr. 1B; H360F	None	Repr. 2; H361f***	
3.8.	Specific target organ toxicity –single exposure	None		STOT SE 3; H335	Not evaluated

3.9.	Specific target organ toxicity – repeated exposure	None		None	Not evaluated
3.10.	Aspiration hazard	None		None	Not evaluated
4.1.	Hazardous to the aquatic environment	None		None	Not evaluated
5.1.	Hazardous to the ozone layer	None		None	Not evaluated

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Dgr
Pictogram codes: GHS07, GHS08, GHS05
Hazard statements: H317, H318, H335, H360F
Precautionary statements: not harmonised

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	None		None	Not evaluated
Oxidising properties	None		None	Not evaluated
Flammability	None		None	Not evaluated
Other physico-chemical properties *[Add rows when relevant]*	None		None	Not evaluated
Thermal stability	None		None	Not evaluated
Acute toxicity	None		None	Not evaluated
Acute toxicity – irreversible damage after single exposure	None		None	Not evaluated
Repeated dose toxicity	None		None	Not evaluated
Irritation / Corrosion	None		R37-41	Not evaluated
Sensitisation	None		R43	Not evaluated
Carcinogenicity	None		None	Not evaluated
Mutagenicity – Genetic toxicity	None		None	Not evaluated
Toxicity to reproduction – fertility	Repr. Cat. 2; R60		Repr. Cat. 3 R62	
Toxicity to reproduction – development	None			Not evaluated
Toxicity to reproduction – breastfed babies. Effects on or via lactation	None		None	Not evaluated
Environment	None		R52	Not evaluated

[1)] Including SCLs [2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger: Xn
R-phrases: R37-41-R43-R62-R52
S-phrases: -S26-S36/37-S39-S45-S53-S61

1.4 History of the previous classification and labelling

The classification of BPA is harmonised in Annex VI of CLP under the index number 604-030-00-0 as follows as a direct translation of TC C&L:

Repr. Cat. 3; R62 / Xi; R37-41 / R43 / R52

Classification of BPA was inserted in the 29th ATP (directive 2004/73) of Annexe I of Directive 67/548/EEC for the human health effects and in the 30th ATP (directive 2008/58) of Annexe I of Directive 67/548/EEC for the N; R52 classification.

A classification proposal for BPA was submitted by the UK CA at the TC C&L during its work for the Risk Assessment Report (RAR) on this substance. In 2002, BPA was classified as reprotoxic cat. 3. The initial proposal of the UK was to classify the BPA as Repr. Cat. 2; R60. Nevertheless as some member states stressed the fact that classifying the BPA as Repr. Cat. 2 is a borderline case and could create precedence, the member states choosed (after a divided vote) to rather classify the BPA as Repr. Cat. 3 for fertility and to discuss concerning the effects on development when more studies would be available. However, it seems that these new data have never been provided and then the classification was not discussed again later.

The conclusions of the RAR provided by UK in 2001 on this endpoint are available in annex 1. A complete version is available on IUCLID, together with the update of the document in 2008.

1.5 Short summary of the scientific justification for the CLH proposal

The last time the classification of BPA for human health was discussed and decided was in 2002. At the timebeing, the three guideline studies presented led UK to propose a Repr. Cat. 2; R60 (67/548) classification. Since then new studies have been published which have not been evaluated by the TC C&L. In 2011, ANSES (French Agency for food, environmental and occupational health and safety) published a report on the hazards of BPA demonstrating its effect on fertility. Therefore, the French Competent Authority considered that the classification for sexual function and fertility needs to be revised on the basis of the report of ANSES (ANSES, 2011). Indeed, the aim of this proposal is to harmonise the classification for fertility with the conclusion of the report of the French national mandated institute. This proposal is based on the studies presented in this French report (i.e. considered by the French experts as key studies, irrespective of their publication date) together with all the new data published since 2002 on fertility (bibliographical search stoped 31/12/2012). The key studies from the previous discussions (TC C&L from UK-RAR) are reported in the report in order to provide experts with the state of the previous European discussions. The bunch of data accumulated, together with the regulatory consequences of this classification are strong enough for justifying focusing on this endpoint only.

France welcomes any new classification proposal for other endpoints such as carcinogenicity, development or lactation but believes that the emergency for regulating BPA is high enough justifying targeted CLH report and ATP inclusion at the first place.

The ANSES'report highlights several effects on the reproductive system (ANSES, 2011 *in french*):

- In male animals a reduction of the sperm production after an exposure of adults to BPA for 5 weeks has been established (Chitra *et al*., 2003 and Herath *et al*., 2004)

- There are also suspected effects on the male reproductive system (decrease of plasmatic concentrations of testosterone, changes in sexual behaviour) following an exposure to BPA during the pubertal age (Della Seta *et al*., 2006).

- In female 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 should be considered as recognised: Increased occurrence of ovarian cysts; 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 female 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.

- In women, the effects of BPA on oocyte maturation (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) and another study having non-major methodological limitations (Fujimoto *et al*., 2010).

- There are few other epidemiological studies available for women butthey present methodological limitations (study population size, selection of participants, statistical analyses, confounding factors, etc.). Human data should therefore be considered as additional evidence in our weight of evidence of BPA toxicity. 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.

Studies used in this national report and its conclusions are used as the basis for this CLH proposal. Together with it, other new studies published since the discussion on the classification of the BPA

for human health at the TC C&L in 2002 are used for proposing this new CLH report on fertility of males and females.

1.6 Current harmonised classification and labelling

1.6.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

The classification of BPA is harmonised in Annex VI of CLP under the index number 604-030-00-0 as follows:

Table 3.1 (CLP)
Repr. 2 – H361f***
STOT SE 3 – H335
Eye Dam. 1 – H318
Skin Sens. 1- H317

1.6.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

The classification of BPA is harmonised in Annex VI of CLP under the index number 604-030-00-0 as follows:

Table 3.2 (67/548/EEC)
Repr. Cat. 3 ; R62
Xi ; R37-41
R43
R52

1.7 Current self-classification and labelling

Ox. Sol. 3 – H272
Asp. Tox. 1 – H304
Muta 1B – H340
Carc. 1B – H350
Repr. 2 – H361f***
STOT SE 3 – H335

Eye Dam. 1 – H318 Skin Sens. 1- H317 Aquatic Chronic 2 or 3

2 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

BPA has a harmonised classification and labelling in Annex VI of CLP that includes classification for reprotoxicity.

New relevant data on BPA reprotoxicity have been published in the past 10 years that have not been evaluated by the TC C&L.(see history of BPA classification in annex 1). The French Competent Authoritie considers that the classification for fertility within reprotoxicity needs to be revised on the basis of the new studies available.

Reprotoxicity as other CMR properties justifies a harmonised classification and labelling according to article 36 of CLP.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

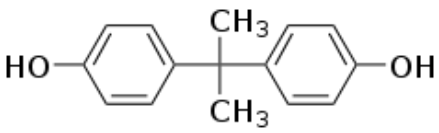
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	201-245-8
EC name:	4,4'-isopropylidenediphenol Synonyms: bisphenol A
CAS number (EC inventory):	80-05-7
CAS number:	80-05-7
CAS name:	
IUPAC name:	4,4'-dihydroxy-2,2-diphénylpropane
CLP Annex VI Index number:	604-030-00-0
Molecular formula:	$C_{15}H_{16}O_2$
Molecular weight range:	228.29 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Bisphenol A	99 – 99.8%	No information	

1.2.1 Composition of test material

Relevant information are given in the respective study summaries when available.

1.3 Physico-chemical properties

Table 7: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Odourless (mild phenolic odour) solid (white crystals, flakes, prills)	HSDB (interrogation 2012) + Ashford (1994) (registration dossier)	Bisphenol A is a white solid at environmentally relevant temperatures
Melting/freezing point	melting point: 150-155°C	Study report (1989) HSDB (interrogation 2012) (registration dossier)	
Boiling point	at 1013hPa: 360 °C	Handbook physical and Thermodynamic Properties of Pure Chemicals Data Compilation (1994)	
	At 17hPa: 250-252°C with potential decomposition	IPCS (1994)	
Relative density	1.195 g/cm^3 (Air = 1) at 25° Density: 0.815 g/cm^3 at –20°C	HSDB (interrogation 2012) Sax NI, Lewis RJ (1996)	
Vapour pressure	1.61E-09 hPa at 20°C 4.12E-09 hPa at 25°C	Study report (1988)	
Surface tension	No data		
Water solubility	Moderately soluble in water: 146-173 m g/L at 25°C	QSAR estimate (US EPA, 2009)	
	300 mg/L at 25°C	Study report (1989)	Tests were conducted using bi-distilled water with 24 hours mixing. They were performed in triplicate. HPLC was used for analytical determina tion
Partition coefficient n-octanol/water	3.4 at 21.5°C	Study report (1989)	Experimental
	3.32 at 25°C	Hansch *et al*. (1995)	QSAR
Flash point	227°C @ 1013 hPa	Ullmann's Encyclopedia of Industrial Chemistry (1991)	Closed cup
Flammability	Bisphenol A is classified as not readily combustible solid	Study report (2010)	Tested according to UN Test Procedure N.1
Explosive properties	Waiving		
Self-ignition temperature	Auto-ignition temperature: 510°C @ 1013 hPa	HSDB (interrogation 2010)	

Oxidising properties	waiving		
Granulometry	Most of BPA granules are > 1mm in diameter. (1.25-2.0: 62.3-87.7%)	registrant data (2009)	Experimental data
Stability in organic solvents and identity of relevant degradation products	waiving		
Dissociation constant	pKa = 10.08 at 25°C	QSAR estimate (2009)	
Viscosity	Scientifically unjustified		

2 MANUFACTURE AND USES

2.1 Manufacture

Bisphenol A is made from phenol and acetone under acid catalysis in a continuous process. Hydrogen chloride or sulfonated cross-linked polystyrenes are used as catalysts which are usually arranged as a fixed bed over which the reaction mixture is passed. The reaction of phenol with acetone takes place at 50 - 90°C, the molar ratio phenol–acetone is up to 20:1. BPA crystallises as an adduct with 1 mol phenol after separation of the hydrogen chloride by distillation or neutralisation, as applicable. The use of ion exchangers as catalyst may however be preferred to processes that use hydrogen chloride because they are less corrosive. The yield is normally 80 – 95%. To isolate the BPA, the whole reaction mixture is fractionally distilled, whereby BPA itself is distilled over particularly carefully under high vacuum, separated from resinous byproducts, and subsequently recrystallised. Alternatively the reaction mixture is crystallised by cooling, where part of the phenol may first be removed by distillation. The adduct is molten and phenol is removed by distillation to obtain the final product. A very high purity product can be obtained if the BPA–phenol 1: 1 adduct is separated and again recrystallised from phenol or by meltcrystallisation of the final product.

2.2 Identified uses

BPA is a monomer mainly used in the production of polycarbonate plastics and epoxy resins.

The industrial/occupational identified uses are: manufacturing of BPA; repackaging of BPA (formulation) ; manufacturing polycarbonate, epoxy resins, polymers, coating materials, chemicals and thermal paper ; as anti-oxidant for processing PVC (Polyvinyl chloride) ; manufacturing epoxy resin hardeners (formulation) ; in epoxy resin hardeners (inclusion into or onto a matrix) ; as a laboratory reagent (R&D).

Out of many sources, the general public might be exposed viathermal paper; in articles made of PVC...

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1. Absorption

- Oral

In humans and other primates, BPA is rapidly absorbed from the gastrointestinal tract, consistent with its substantial aqueous solubility and lipophilicity. Analysis of the areas under the plasma concentration time curve (AUC) shows that gastrointestinal absorption is greater than 85% in rats and monkeys. The experiments conducted in adult human at relatively low doses (0.025 to 5 mg total) show that BPA is rapidly and completely absorbed from the gastrointestinal tract (Tsukioka et al, 2004. Volkel et al, 2002. Volkel and al., 2005). After a single dose, peak plasma concentrations are reached approximately 80 minutes after ingestion.
Indeed, BPA is well absorbed by gastrointestinal tractus after oral administration of 100 mg/kg of BPA in all the five species tested (mice, rats, dogs, ewes and pigs) (Farbos, 2012). Indeed, internal exposures to free BPA (also called aglycone BPA or unconjugated form) are remarkably similar for adult rodents, non-human primates and humans (Doergue *et al.*, 2010a), although some differences between BPA metabolism and disposition in rodents and primates will be described below.

- Sublingual

A recent study (Gayrard et al., 2013) with six dogs shows that the systemic bioavailability of BPA deposited sublingually is high (70-90%) and that BPA transmucosal absorption from the oral cavity led to much higher BPA internal exposure than obtained for BPA absorption from the gastro-intestinal tract after oral administration. This efficient systemic entry route of BPA may lead to far higher BPA internal exposures than known for BPA absorption from the gastro-intestinal tract. The

most difference between both exposures ways is that the conjugated BPA-Glucuronide : free BPA ratio is 100 times lower by sublingal route than that obtained after absorption from the gastro intestinal tract after oral absorption. The sublingual route of exposure bypasses the first-pass hepatic metabolism and may explain the much higher internal exposure to unconjugated form of BPA entry to the systemic circulation. Indeed, BPA human clearance of 30 ml/kg/min has been predicted from animals' clearance. This value reveals a major inconsistency between BPA concentrations reported in biomonitoring studies, and a BPA daily intake of 13 µg/kg reported by EFSA combined to its clearance (Farbos, 2012).

- Dermal

Calculations for estimating the skin absorption of BPA mention a value of 10% of the dose applied, which is confirmed by a study using a pig skin model (Kaddar *et al.*, 2008). A recent study (Demierre *et al*., 2012) stated that the dermal penetration contributes only in a negligible way to the total exposure (8.6% of penetration in human skin *in vitro*). Other studies contradict this statement: Zalko *et al.*, 2011 studied the distribution and metabolism of BPA in cultures of pig ear skin and human skin explants. This study, not guideline (not a study of penetration as recommended by the OECD guideline 428) and with limitations (the incubation time of 72 hours is well beyond the recommended 24 hours to preserve the integrity of the explants), shows that the BPA in the conditions of the experiment and 72 h incubation diffuse significantly across the two models of skin absorption of about 65% for pig ear skin and 46% for human skin explants. At low concentrations on human skin, about 40% of the dose which diffuses into the liquid receiver is as glucuronide and sulfate.

Another recent guideline study, percutaneous absorption of BPA *in vivo* and *ex vivo* was determined in rats and *ex vivo* in humans after exposure for 24 hours (Marquet 2011). The permeability was found to be 12 times higher in rats than in humans. However, inter-individual variability was found in humans. The authors reported dermal flux values of 120 $ng.cm^{-2}.h^{-1}$ from explants of human skin samples exposed to 200 mg $BPA.cm^{-2}$. Finally, contrary to the study Zalko et al., 2011, the authors found a majority of BPA unchanged in the receptor fluid, which can be explained by the significantly higher dose of BPA applied to the skin samples in the study of Marquet and coworkers (Marquet et al. , 2011).

Athough varying in terms of permeability, the overall data show that dermal absorption occurs.

- Inhalation

There is no data on the toxicokinetics of BPA after exposure by inhalation. However, the changes in absolute organ weights highlighted in a study of repeated inhalation toxicity in rats exposed to 13 weeks show that absorption through the lungs occurs (Study report, 1988). (Decreased absolute liver weight in males at 10 or 150 mg/m^3, decreased absolute liver and kidney weights in females at 150 mg/m3, increased relative brain weights in females at 50 or 150 mg/m3, and increased relative lung weight in females at 150 mg/m3 were observed. In rats sacrificed 4 weeks after exposure,

males exposed to 150 mg/m3 BPA had increased relative brain weight. In rats sacrificed 12 weeks after exposure, decreased absolute kidney and lung weights were observed in males at 150 mg/m3 and decreased absolute and relative kidney weights were observed in females at 150 mg/m3. . This is in line with its octanol / water favorable (3.2) indicating that absorption through the lungs can occur.

However, in the absence of data, absoption by inhalation can not be quantified (EC, 2010b). For the characterization of the risk behavior in European 2008 report, the oral and inhalation absorption values are 100% and the dermal absorption value is 10% (EC, 2010b).

4.1.2. Distribution

Once absorbed, the BPA is rapidly distributed in all tissues withoutreal affinity of BPA for a particular organ. Although, in rodents, a few hours after oral administration of radiolabeled BPA, the highest concentrations are found in the liver and kidneys.

Following intraveinous bolus administration in adult mice, unconjugated d6-BPA is rapidly taken up into adipose tissues but does not exceed the initial measured serum level (Doerge, 2012).

Krotz et al., (2012) show that BPA does not accumulate (no BPA detected) in ovarian follicular fluid after a brief exposure to medical plastics during an IVF (in vitro fertilization) cycle in five women (small sample size). However, two previous studies contradict these results. In 2002, Ikezuki et al. measured BPA in non pregnant Japanese patients yielding follicular fluid levels averaged 2.4 ng/ml (n=32) and in 2005, Tsutsumi measured a follicular fluid levels ranged 1-2 ng/ml.

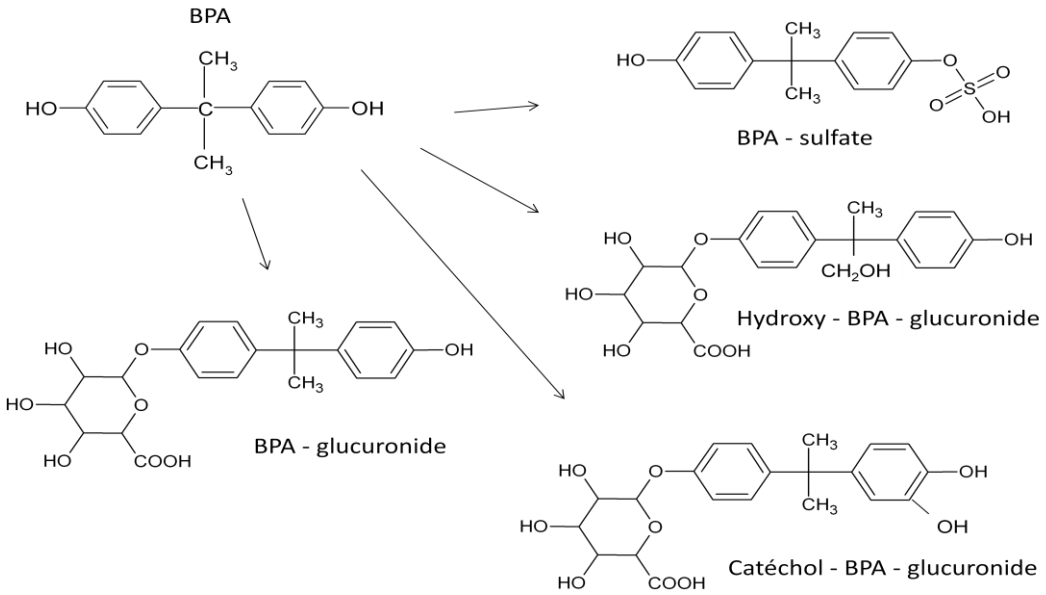
4.1.3. Metabolism

Toxicokinetic data obtained in rats and humans show a first-pass effect and indicate that the plasma residues are mainly (92-99%) as glucuronide. Several reports highlight the existence of an enterohepatic circulation in rats after the glucuronide hydrolysis in the intestine, which results in a relatively slow elimination of BPA in rats compared to humans (EC, 2010b; Ineris, 2010b; INFOSAN, 2009). This major difference in toxicokinetics between these 2 species has often been put forward to highlight the limitations of the rodent model in the risk assessment of BPA for humans (Mielke and Gundert-Remy, 2009; Ginsberg and Rice, 2009). Recent studies combining the use of tritium/deuterium-labeled BPA and specific and sensitive detection technics (LC-MS/MS) confirm the existence of an enterohepatic cycle in rodents unlike primates. However, they indicate that this cycle has very limited consequences on the clearance of BPA (Doerge et al, 2010a; Doerge et al, 2010b; Taylor et al, 2011) arguing for the relevance of the rodent model to humans for oral exposure to BPA.

In all species studied, the majority metabolic pathway is the combination of BPA with glucuronic acid to form the BPA-glucuronide (BPA-G) (Figure below). This combination takes place mainly in the liver and to a lesser extent in the intestine. It is catalyzed by UGT2B1 in rats, whereas humans are the UGT2B15 and UGT2B7 isoforms that are responsible for the glucuronidation (Mazur et al.,

2010). The genetic polymorphism of the UGT2B15 could result in individual differences in the ability to detoxify BPA (Hanioka et al, 2011; Inserm, 2011).
Human pharmacokinetic studies show that the urinary metabolites profile is composed of almost BPA-G. Monitoring studies conducted from urine samples collected from adults (Ye et al., 2005) indicate different proportions (9.5% BPA free, 69.5% BPA-glucuronide and 21% BPA-sulfate). Kim et al. analyzed the proportion of BPA and its metabolites in urine collected from 15 women and 15 men (Kim et al., 2003). The mean urinary composition among men was 29.1% BPA free, 66.2% BPA-glucuronide and 4.78% BPA-sulfate, whereas in women the proportions were 33.4% BPA free, 33.1% BPA-glucuronide and BPA sulfate 33.5%. The authors conclude that women have a better sulfation capacity than men (NTP-CERHR, 2008).
Other metabolites have also been identified from urine or bile samples in rodents or in incubations with hepatocytes in primary culture. These include BPA and BPA-hydroxy sulfate (Figure below). In total, these metabolites rarely exceed 5 to 10% of total metabolites in the urine of rodents. Other minor such as conjugated double or Metanephrine metabolites were also identified in rodents.

Several other metabolites formed by oxidation, have been identified in vitro from subcellular fractions (4-methyl-2 ,4-bis (p-hydroxyphenyl) pent-1-ene, isopropyl-hydroxyphenyl, glutathionyl-phenol, glutathionyl 4 isopropylphenol-and bisphenol A dimers), but to date they have not been described in vivo (INRS, 2010).



BPA-G and BPA sulfate represent detoxification pathways of BPA as they are not active on the estrogen receptor. Ginsberg et al. suggested that deconjugation could occur at specific tissue sites by the enzymes β glucuronidase and arylsufatase C transforming conjugates and sulfated metabolites into "free"-BPA active on the estrogen receptor (Ginsberg and Rice, 2009). The β-glucuronidase is present in the intestines but also in the placenta and fetal liver, which could result in exposure of the fetus to "free" BPA (Aschberger et al., 2010).

The route of administration affects the forms and circulating levels of BPA (Doerge et al, 2010b; Pottenger et al, 2000. Taylor et al, 2008). The data collected in rodents show significantly higher proportions of BPA free after oral administration than in the case of subcutaneous and intraperitoneal administration.
It is well established that ATP-Binding-Cassette (ABC) transporters play a fundamental role in the absorption, distribution, metabolism and excretion of endogenous and exogenous chemicals, and transporter membrane localization can directly influence these processes (Glavinas et al., 2004).
Mazur et al. (2012) show that in rat, possible transport preferences of BPA and BPA-G is into intestinal lumen and hepatobiliary excretion whereas in humans, BPA-G is preferably transported into the blood supply of the liver or portal blood supply of the small intestine.

4.1.4. Elimination

Orally administered BPA undergoes complete first-pass metabolism in the liver to BPA-glucuronide as major metabolite, which is rapidly excreted in the urine, with a half-life of less than 6 hours (Völkel *et al*., 2002, 2008). BPA-sulphate has been reported as a minor urinary metabolite of BPA in humans (Ye *et al*., 2005, 2006). Because this first-pass metabolism is effective, there is extremely low systemic availability of free BPA in humans after oral exposure. The conjugated forms of BPA have no endocrine activity (Snyder *et al*., 2000; Shimizu *et al*., 2002, Willhite *et al*., 2008). Therefore, these conjugation reactions represent detoxication pathways.
In rats, BPA is also predominantly glucuronidated, with sulphation representing a minor pathway (Pottenger *et al*., 2000), but the BPA-glucuronide formed is excreted from the liver via bile into the gastrointestinal tract, cleaved back to BPA and reabsorbed into the blood. Thus it undergoes enterohepatic recirculation resulting in slower elimination of BPA including its conjugate in rodents compared with humans (EFSA, 2006); this results in slow elimination (half-lives between 20 and 80 hours). The enterohepatic cycling and decreased first pass metabolism of BPA in rats results in higher plasma levels of unconjugated BPA in rats compared to humans given the same dose. There are differences in the molecular mass threshold for biliary elimination in rats and humans. The molecular mass of the BPA-glucuronide (484 D) is well above the threshold for rats (300 – 400 D) but below that of humans (500 - 600 D) (Hirom *et al*., 1976; Walton *et al*., 2001; Ghibellini *et al*., 2006).
Teeguarden et al (2011) show that after a 24 h period of dietary exposure to high dose of BPA in 20 humans, the total BPA concentrations serum are undetectable in 83% of the samples and when it is less than or equal to limit detection, BPA concentrations in serum is on average 42 times lower than urine concentrations. The rapid absorption and elimination kinetics of BPA observed in this study clearly demonstrate that spot urine sample reflect exposure in the prior meal or prior 4 h to 6 h period but not the full day’s exposure.
Although the main pharmacokinetic differences between 5 species (Farbos thesis, 2012) concern the elimination of BPA and BPA-G, they have not impact on the BPA plasmatic concentrations which are the reflection of the internal exposure of BPA.

4.1.5. Toxicokinetic of BPA during gestation and in foetuses.

Maternal exposure to BPA results in embryos and newborns receiving BPA via placental transfer and milk (Doerge *et al*., 2010). Concerning the toxicokinetics of BPA *in utero*, the presence of BPA in human fetal tissues at around the same concentrations as in maternal blood, demonstrates that BPA passes through the placenta. This is confirmed by Balakrishnan et al. (2010) on seven human placentae perfused *ex vivo* with 10 ng/mL (environmentally relevant concentration) of BPA for 180 minutes: the transfer percentage of BPA is 27.0% ± 1.88% and only 3.2% ± 1.6% of BPA in the fetal compartment is in the conjugated form. Thus BPA can transfer across the human placenta, mainly in active unconjugated form. Moreover, BPA have a high transplacental transfer rate much similar to passive diffusion according to the meta-analysis of data from human ex vivo placental perfusion studies which confirm that the placental barrier is not protective against exposures to BPA (Mose et al., 2012).

Nevertheless, the fetus has the capacity to deconjugate BPA into its "free" active form with the placental enzyme β-glucuronidase (Edlow *et al*., 2012). Free and total BPA were identified in both second and third trimester amniotic fluid. Thus, deconjugation of BPA by the placenta and limited capacity of the fetal liver to conjugate BPA, may increase fetal exposure to the active, endocrine-disrupting form (Edlow *et al*., 2012).

However, the study of Patterson (2013) done with 5 pregnant monkeys argues against the hypothesis that BPA conjugates are selectively deconjugateg by either the placenta or fetus,. Indeed, it is explained by the monotonic elimination of aglycone BPA from the fetal compartment accompanied by persistent conjugate levels. This study measures concurrently the pharmacokinetics of aglycone (active) and conjugated (inactive) deuterated BPA (d6) in 5 maternal and fetal rhesus monkey serum, amniotic fluid and placenta following intravenous injection in the dam. Internal exposures of the fetus to aglycone BPA is attenuated by maternal, placental, and fetal phase II metabolism to less than half that in the dam.

Exposure of human infants to BPA directly, in the absence of maternal transfer or excretion, also occurs through polycarbonate bottle feeding and/or infant formula feeding (AFSSA, 2010). The fetus and neonate may therefore be a sensitive and more highly exposed subpopulation deserving special attention.

4.1.6. Toxicokinetic of BPA for newborns.

There is contradicting results regarding the presence of BPA in milk. Indeed,Vandenberg have shown limited excretion of BPA in breast milk (Vandenberg et al., 2007a).

In a recent study, the level of BPA in milk was determined from experiments in rats exposed orally (Doerge et al., 2010b). Lactating rats (n = 5) were force-fed daily for a week with deuterated BPA (100 mg / kg bw) from the day of birth of newborns. A control group (n = 3) was treated with the vehicle only (ethanol / water, 1:9 v / v). The milk samples were performed after injection of oxytocin and take place exactly one hour after administration of BPA. Analyses of milk and serum were performed in LC / MS-MS. They were made to PND7 for milk and PND10 for serum (for

mothers and their young). Serum analyzes confirm the low percentage of aglycone (0.5%). The assays performed on milk indicate average concentrations of free and total BPA corresponding to 0.87 and 7.6 nM, a milk / serum 1.3 for BPA free and 0.062 for total BPA report.
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 are 300 times lower than in young mothers, the BPA free being undetectable in the offspring. The results, compared with previous data obtained by the same authors in rat pups at PND10 indicate that serum concentrations here are 500 times lower than those obtained when administered by gavage at a dose of 100 mg / kg bw this is to say that administered to mothers in this study.

In human neonates, several metabolic pathways, such as glucuronidation (2-5 times lower in preterm infants), and several excretory functions such as glomerular filtration rate (1.7 times lower) have a lower efficiency compared to adults; these functions reach their full capacity 1 month and 7 months after birth, respectively (EFSA, 2008). In 2008, EFSA was asked to review the toxicokinetics of BPA based on age and involvement in risk assessment and thus in the construction of the TDI. EFSA concluded that immaturity in glucuronidation capacity in newborns could be compensated by the presence of sulfo-transferases, which would result in an effective detoxification EPS (Aschberger et al, 2010;. EFSA, 2008). Contrary to the CGU, sulfotransferases (SULT), for which the substrates of UGT have high affinity, are active in the developing fetus and are functional at birth. These enzymes efficiently catalyze the formation of BPA-sulfate in vitro in humans. Finally, EFSA concluded that the ability of BPA biotransformation to inactive metabolites was sufficient in human neonates.

Studies in rats have shown that in infants, the glucuronidation pathway was more saturable than adults, which could lead to a greater concentration of BPA "free" in the target tissues. The ability of glucuronidation through the activity of UGT is also low after birth and remains low after weaning (Aschberger et al., 2010).
Studies in newborn rat and rhesus monkey confirm that toxicokinetic parameters for most of them significantly different from those determined in adults, particularly with regard to the total BPA (Doerge et al, 2010a.; Doerge et al., 2010b). However, with regard to maximum serum concentrations (Cmax) in BPA free, if they are significantly higher in the newborn rat (to NDP3 or PND10) in adults, it does not seem to be the same in monkeys.
These same authors also showed in adult rats and newborn that subcutaneous administration of BPA significantly altered the toxicokinetic parameters, but also the free BPA/ conjugated BPA ratio in serum in the neonatal rat (Doerge et al., 2010b).

4.2 Acute toxicity

Not evaluated in this dossier.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not evaluated in this dossier.

4.4 Irritation

Not evaluated in this dossier.

4.5 Corrosivity

Not evaluated in this dossier.

4.6 Sensitisation

Not evaluated in this dossier.

4.7 Repeated dose toxicity

Not evaluated in this dossier.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not evaluated in this dossier.

4.9 Germ cell mutagenicity (Mutagenicity)

Not evaluated in this dossier.

4.10 Carcinogenicity

Not evaluated in this dossier.

4.11 Toxicity for reproduction

Only effects on fertility from scientific literature produced since 2002 are presented thereafter.

4.11.1 Effects on female reproductive system in animals

4.11.1.1 *Non-human information*

4.11.1.1.1 *In utero* and lactational exposure

➔ In Mice

CD-1 mice

In the study by Newbold *et al.*, adult (18- months of age) females CD-1 mice who have been e exposed *in utero* from GD9 through GD16 up to 1000 µg/kg bw/day, displayed increased incidence of benign ovarian cysts was 67% ($p = 0.05$) in mice whom mother had been treated with 1 µg/kg bw/day BPA. More severe ovarian lesions were found in pups whom mother received 10, 100 or 1000 µg/kg bw/d by subcutaneous injection, including progressive proliferative lesions of the oviduct which occurrence seemed to increase following BPA exposure. Further, neoplasic lesions like cystadenomas were present at 10, 100 and 1000 µg/kg bw/d but not in the controls. In addition, malignant changes in the uterus like atypical hyperplasia which is considered a precursor lesion to estrogen-associated uterine adenocarcinoma, stromal polyps and sarcoma of the uterine cervix were also found. It is interesting to note that adverse effects in the reproductive tract were observed in all BPA-treated groups, but were more marked at the lowest dose (0.1 µg/kg bw/d) (Newbold *et al*., 2009).

Administration of lower doses of BPA (25 and 250 ng/kg bw/d of BPA) was performed via osmotic pumps implanted into pregnant CD-1 mice dams from GD9 until PND4. With such low doses, the only route allowing such precision was the one used. It induces alterations in the genital tract of female offspring that are revealed during adulthood (3-months of age), including decreased weight of the vagina of 20 mg ($p < 0.01$), and decreased volume of the endometrial lamina propria of 11 mg ($p < 0.05$) observed at 250 ng/kg bw/d. These findings were assiocated at 250 ng/kg bw/d with an increase of the DNA synthesis in the uterine epithelium in the absence of any apoptotic change, suggesting modification of the balance proliferation/apopotosis leading to an increase number of new cells. (Markey *et al*., 2005). Although not physiological, this route of exposure render possible exposing animal to such small doses of xenobiotic.

Cabaton *et al.*, exposed via subcutaneous implant, female CD-1 mice to BPA at 0.025, 0.25 or 25 µg/kg bw/d from gestational day 8 through day 16 of lactation. Perinatal exposure to BPA resulted in a decline in reproductive capacity (i.e fertility and fecundity) in a forced-breeding protocol of 32 weeks starting at 2-months of age. Treatment with BPA at 25µg/kg induces a significant decrease in the number of pregnancies at the end of the protocol, (an average of 4.8 litters per dam in BPA group against 6.3 in control group; $p = 0.024$), indicating a decline in fertility. At 0.025 and 25 µg/kg, BPA induces a significant decrease in the cumulative number of pups born, a sign of impaired fecundity. The intermediate BPA dose, 0.25µg/kg, did not produce significant effects whereas both lower and higher doses did, suggesting a non-monotonic dose- type of dose-response

relationship (Cabaton *et al.*, 2010). However, further studies including a greater number of doses are needed to better characterize this dose-response relationship.

Cabaton *et al*. also observed that the female treated with BPA at 25 µg/kg showed a progressive decline in the number of pups delivered as the repetitive breeding experiment progessed, which suggests the possibility of an accelerated physiological reproductive decline. This could explain the lack of effect in other similar studies in which the observations were limited to the first pregnancy of the F1 females exposed perinatally, with no follow-up on potential effects in subsequent pregnancies. For instance, Honma *et al*. examine the reproductive ability of female mice after prenatal exposure from GD11 to GD17 to BPA at 2 or 20 µg/kg bw/d. Total number of pups per F1 mother and sex ratio were not affected but this study did not include repetitive breeding. Therefore the possibility of impairement of fertility induced by *in utero* BPA exposure might have been missed due to the examination of one pregnancy only (Honma *et al*., 2002).

CD-1 mice exposed *in utero* for four days from GD15 to GD 19 to 0.5 or 10 mg/kg/day of BPA via subcutaneous injections in pregnant dams displayed estrous cycle alteration characterized by an elongated cycle length of 3 days in average ($p < 0.01$) due to prolonged diestrus phase ($p < 0.01$) and reduced age at puberty (Nikaido *et al*., 2004).

The low doses used in these studies justify the route used (subcutaneous, osmotic pumps). Indeed, it is technically difficult to prepare, maintain and ensure administration of doses such as 0.1 µg/kg bw/d in feed.

Balbc-C mice

Signorile *et al*. (2010) found that exposure to 100 or 1000 µg/kg bw/d of BPA *in utero* and during 7 days after birth (mothers were treated by subcutaneous injections from GD1 through PND7) caused in 3-month old female offspring, endometriosis-like structures in the adipose tissue surrounding the genital tract (5% in controls, 30% at 100µg/kg and 35% at 1000µg/kg; $p = 0.024$). Histological analysis also demonstrated an increased incidence of cystic ovaries (10% in controls, 45% in the group treated with BPA at 100µg/kg and 50% at 1000µg/kg, $p = 0.008$), adenomatous hyperplasia with cystic endometrial hyperplasia, (10% in controls, and 25% in the BPA 100 and 1000µg/kg groups) and atypical hyperplasia ($p = 0.085$), such results that are in accordance with Newbold's findings. No cases of malignancy were found in this study, but animals were sacrified at 3 months of age and probably had no time to fully develop tumors (Signorile *et al*., 2010).

C57BL/6 mice

Susiarjo *et al*., implanted time-release BPA pellets designed to leach a dose of 20 µg/kg bw/d in pregnant C57BL/6 females at GD 11.5. To analyze meiotic prophase, ovaries were isolated from female fetuses at 18.5 day of gestation. Oocytes from exposed female fetuses displayed gross aberrations in meiotic prophase, including increased levels of recombination. In the mature female,

these aberrations were translated into an increase in aneuploid eggs and embryos. These synaptic and recombination defects typically result in the loss of a significant proportion of oocytes prior to sexual maturation, reducing the pool of oocytes in the adult female. Thus, in addition to reducing the genetic quality of their eggs and embryos, BPA may adversely influence the reproductive lifespan of exposed females (Susiarjo *et al*., 2007).

More recently, Brannick *et al.* showed that prenatal exposure to BPA increases pituitary gonadotroph development in C57BL/6 females: pregnant mice were administered 0.5 µg/kg/day BPA, 50 µg/kg/day BPA, or vehicle beginning on GD 10.5 trough 18.5 by gavage. At parturition, pituitaries from female offspring exposed in utero to either dose of BPA had increased proliferation, as assessed by mKi67 mRNA levels and immunohistochemistry. Coincidently, gonadotroph number also increased in both treated females LHβ (p=0.029, p=0.049) and FSHβ (p=0.020, p=0.002) immunoreactive cells as the average percent of positive cells /to total cells in the anterior pituitary compared to vehicle controls. However, they observed a dichotomy between mRNA levels of Lhb and Fshb (gene encoding for the beta subunit of their respective hormone LHand FSH). Female mice exposed to 0.5µg/kg/day BPA had increased mRNA levels of gonadotropins and the GnRH receptor (Gnrhr), which mediates GnRH regulation of gonadotropin production and release. In contrast, mice treated with 50µg/kg/day BPA had decreased gonadotropin mRNA levels, Gnrhr and Nr5a1, a transcription factor required for gonadotroph differentiation. No other pituitary hormones were altered on the day of birth in response to in utero BPA exposure and male pituitaries showed no change in the parameters tested. These data show that it is difficult to describe precise mode of action of BPA, which effect is implicated in multiple pathways regulated by numerous key players.

CF-1 mice

Prenatal treatment of CF-1 mice from GD11 to GD17 with BPA at 2.4µg/kg bw/d had no effect on the age at vaginal opening but significantly reduced the number of days between the opening and the onset of first estrus. However, this study underlines clearly a major limitation of the rodent model: the fetal hormone exposure levels and thus the responsiveness of the fetuses to sex hormone and BPA are largely influenced by the intrauterine position. Therefore, mouse fetuses positioned between two males are exposed to the lowest levels of BPA and are not affected by the treatment while fetuses located next to female fetuses are exposed to the highest levels (Howdeshell *et al.*, 1999).

Berger *et al* used a wide range of subcutaneous doses, from 0,0005 to 10,125 mg/animal/day to determine the levels of exposure required to terminate pregnancy in mice. The results show that only the two higher doses impacted the pregnancy outcome in mice: the 3.375 mg/day and 10.125 mg/day doses resulted in a significant decrease in the average number of pups ($p < 0.05$ and $p < 0.01$, respectively) and there was a significant reduction by approximately 70% ($p < 0.001$) in the number of females that gave birth at 10.125 mg/animal/day only. This reduction in pregnancies was observed both when females were allowed to give birth and when sites of implantation were

inspected via uterine histology on day 6 after insemination. Then they treated mice via oral route with diet contaminated with either 3 or 6% of BPA. None of the animals treated with the 6% contaminated food (corresponding to a dose level of 68 mg BPA/animal/day) was parturient although in controls animals, 11 of 12 were parturient ($p < 0.001$) (Berger *et al*., 2007).

In 2008, Berger assessed the impact of acute and repeated subcutaneous administration of BPA upon intrauterine implantation of fertilized ova and urinary levels of 17β-estradiol and progesterone in inseminated female mice. Daily subcutaneous doses of 6.75 and 10.125 mg/animal/day (approximately 200 mg/kg/d and 300 mg/kg/d, respectively) from days 1 to 4 of gestation clearly reduced the number of uterine implantation sites: at 6.25 mg no animals showed any implantation sites, while only one animal did at the 10.125 mg dose ($p < 0.0001$). This disruption in implantation coincided with a decrease in urinary progesterone levels seen from day 2 to 5 of pregnancy at 10.125 mg ($p < 0.0005$). A single injection of 10.125 mg/animal when given on day 0 or 1, and a single injection of 6.75 mg/animal on day 0 were sufficient to disrupt implantation, indicating effects of just one exposure. However, lower environmentally relevant doses did not affect pregnancy, implantation, or hormonal output. These results indicate that previously observed reductions in litter size in response to BPA may be mediated by a disruption of intrauterine blastocyst implantation rather than post-implantation effects (Berger *et al*., 2008).

In the third study, exposure from GD1 to GD4 to subcutaneous injections of BPA at 3.375, 6.75 and 10.125 mg/animal/day equivalent to approximately 100, 200 and 300 mg/kg respectively, also induces a significant decrease in blastocyst implantation ($p < 0.001$) associated with an alteration of the uterine morphology, including increase of the average luminal area, and a decreased expression of ERα and Progesterone receptors. This changes in uterine morphology and steroid receptor expression would have affect the uterine receptivity for blastocyst implantation (Berger *et al.*, 2010).

ICR mice

Administration of BPA at a lower dose than those used in Berger's studies (10 mg/kg bw /d) to ICR dams from GD0 to GD7 leads to reductions in the number of embryos and uterine weight on GD10 and GD12 and influences placentation, especially the development of the deciduas basalis and trophoblastic layers. In addition, BPA decreased the number of neonates and increased their mortality (Tachibana *et al.*, 2007).

➔ In Rats

Wistar rats

Oral exposure in drinking water of females Wistar rats during gestation and lactation from GD6 through PND21 to BPA at 1.2 mg/kg bw/d induces in the 4 months old female offsprings a reduction in apoptotic cells in uterin epithelium ($p < 0.001$) which is associated with an increase thickness of both uterine epithelia and stroma ($p < 0.05$). A modification of the estrous cyclicity and

a down regulation of the protein Estrogen Receptor α (ERα) in uterin cells on estrus day were also described by immunohistochemistry (Mendoza-Rodríguez *et al.*, 2011).

Long Evans rats

Pregnant Long Evans rats were gavaged with vehicle, EE2 (0.05–50 mg/kg/day), or BPA (2, 20, and 200 mg/kg/day) from day 7 of gestation to postnatal day (PND) 18, and the female offspring were studied. BPA had no effect on AGD, pup body weight, age at vaginal opening, F1 fertility, nor F2 litter sizes. (Ryan et al. 2010)

Sprague Dawley rats

Rubin *et al.* exposed female Sprague-Dawley rats via drinking water to approximately 0.1 or 1.2 mg /kg bw/day of BPA from day 6 of pregnancy through the end of the lactation period. Results in some female offspring exposed perinatally to the highest dose of BPA (1.2 mg/kg bw/d) revealed intermittent extended periods of diestrus, whereas other exhibited extended periods of proestrus and/or estrus. Besides altered patterns of estrous cycle in approximately 80% of females, the offspring of the high dose BPA females also revealed decreased levels of plasma luteinizing hormone (LH) (-18%) in adulthood. In contrast, many parameters examined in this study (anogenital distance, age of vaginal opening, macroscopic observation of the genital tract) were not significantly affected by perinatal exposure to BPA (Rubin *et al*., 2001).

➔ In sheeps

Sheep is a good model for examining this critical issue related to reproduction because their ovarian cycle, steroid, and GnRH secretion patterns are very similar to that of women and because the distribution of ERα and ERβ, the activation of GnRH neurons relative to the estradiol-induced LH surge and the critical period of sexual differentiation in sheep have been well characterized.

Prenatal treatment of Suffolk ewes with subcutaneous injections of BPA at 5 mg/kg/d from day 30 to 90 of gestation caused early postnatal hypergonadotropism characterized an increase in LH concentration by 2-fold ($p < 0.05$) and prolonged the first breeding season of 1 month ($p < 0.05$) which is suggestive of a reduced sensitivity to estradiol negative feedback. BPA treatment also reduces the magnitude of the LH surge after estrous cycle synchronization with prostaglandine F2α (Savabieasfahani et al., 2006). The same team tested the hypothesis that these effects of BPA on LH surge system involved changes in hypothalamic gonadotropin-releasing hormone (GnRH) and estrogen receptors ERα and ERβ mRNA expression in the medial preoptic area. They administrated the same treatment in pregnant ewe from GD30 through GD90 and found a decrease by a half in GnRH ($p < 0.05$) and ERβ ($p < 0.001$) mRNA expression whereas ERα expression was multiplied by 4 in the medial preoptic area (Mahoney et Padmanabhan, 2010).

Previous report of the same study suggest that the onset of the first cycle in sheep can be influenced by the photoperiodic environment which could have mitigated the effects of BPA in this study. However, the early increase by 2-fold in LH levels ($p < 0.05$) seen in prenatal BPA-treated females is consistent with advancement of neuroendocrine puberty. (Savabieasfahani *et al.*, 2006).

➔ In monkey

A recent study was realeased studying the effect of BPA on Rhesus Monkey. Initial studies using single oral doses of 400 $\mu g \cdot kg^{-1} \cdot d^{-1}$ administered to non pregnant females demonstrated rapid conjugation (inactivation) of BPA, with peak serum levels of 2–5 ng/mL attained 1–2 h after ingestion and a rapid decline thereafter. Because the dose was high (about 8 times the current FDA "safe" dose) but peak levels closely approximated levels observed in human studies (reviewed in refs. 22 and 23), it was concluded that human exposure must be significantly higher than assumed, not primarily restricted to oral routes, and nearly constant. Thus, in addition to using an oral dosing strategy, a protocol using the implantation of controlled-release silastic capsules to achieve sustained low-level exposure was developped.

The timing of exposure using both protocols was designed to mimic the developmental windows that elicited effects in the ovary in mice: an early exposure during the second trimester of pregnancy [gestational day (GD) 50–100], when germ cell differentiation and meiotic entry occur, and a late exposure during the third trimester (GD 100–term), when follicle formation takes place. This study demonstrates that the early stages of oocyte development in the rhesus monkey are vulnerable to disturbance by BPA. For animals receiving oral doses, mean levels of bioactive (unconjugated) BPA in maternal serum close to the time of ovarian tissue collection were 0.51 ± 0.20 ng/mL for second-trimester pregnancies and 0.31 ± 0.13 ng/mL for third-trimester pregnancies. These values were obtained 4 h after administration of the last maternal oral dose; in non pregnant females, this time point was closest to the average level during the 24 h following a single oral dose. For animals in the implant exposure group, mean levels of bioactive BPA in maternal blood at the time of ovary collection were 0.45 ± 0.23 ng/mL for second-trimester fetuses and 0.90 ± 0.13 ng/mL for third-trimester fetuses; these values represent the sustained levels achieved using the implant paradigm and are in close agreement with levels reported in human maternal serum. The analyses of second trimester fetuses exposed at the time of meiotic onset suggest that, as in mice, BPA induces subtle disturbances in the prophase events that set the stage for chromosome segregation at the first meiotic division. The analyses of thirdtrimester fetuses exposed to single daily oral doses during the time of follicle formation revealed an increase in multioocyte follicles analogous to that reported in rodents. However, two unique phenotypes were evident in continuously exposed animals: persistent unenclosed oocytes in the medullary region and small, nongrowing oocytes in secondary and antral follicles. (Hunt *et al.*, 2012)

4.11.1.1.2 Neonatal exposure

➔ Mice

As they found after a prenatal exposure, Newbold et al. found reproductive tract lesions in adult (18-months old) female CD-1 mice treated neonatally from PND1 to PND5 with subcutaneous injections of BPA at 10, 100 or 1000µg/kg bw/d (Newbold *et al.*, 2007). Ovarian pathologies (ovarian cysts, benign proliferative lesion, diminution of Corpora Lutea) and uterine pathologies (benign invasion, precursor lesion to adenocarcinoma and nepoplasic lesion) were more common in all treated groups as compared to the control (corn oil). As example, atypical hyperplasia of the uterus occurred in 4% and 5% of the females treated with 10µg/kg and 100µg/kg respectively, but not in the control group. However, only the increase in cystic ovaries ($p \leq 0.05$) and cystic endometrial hyperplasia ($p < 0.01$) in the 100 µg/kg bw/d treated group were statistically signigicant.

Neonatal CD-1 mice were injected subcutaneously with a 5 or 50 mg/kg bw/d of BPA on postnatal days (PND) 1–4 and ovaries analyzed on PND5. In the mouse, oocytes develop in germline cysts that undergo breakdown resulting in primordial follicles, consisting of a single oocyte surrounded by granulosa cells. During this process, approximately two-thirds of the oocytes die. Exposure of female mice to environmental estrogens can alter oocyte development, limiting the number of primordial follicles that can be used for reproduction. Cyst breakdown, oocyte survival and follicle development were altered by BPA. (Karavan *et al.*, 2012).

➔ Rats

Sprague Dawley

In the study of Fernandez *et al.* (Fernandez *et al*., 2010), female Sprague Dawley rats were injected subcutaneously with BPA dose ranged from 0.25 to 62.5 mg/kg bw/d from PND1 to PND10. Female offspring exposed neonatally to the highest dose (ranging from 25 to 62.5 mg/kg bw/d) had altered ovarian morphology, showing a large number of cysts ($p < 0.05$). They did not present any oocytes or deliver any pups being infertile. At lower dose (2.5 - 6.2 mg/kg bw/d), females delivered significantly fewer pups ($p < 0.05$) indicating subfertility, without impairment of ovulation. This was accompanied by accelerated GnRH pulse frequency in hypothalamic explants associated with increased serum testosterone and estradiol levels by approximately 30% ($p < 0.05$), reduced progesterone levels ($p < 0.05$) and a decrease of the fertility in adulthood.

Long Evans

Similarly, subcutaneous neonatal exposure, from PND1 to PND3, to BPA at 50µg/kg bw/d and 50 mg/kg bw/d in females Long Evans rats also induced at both dose ovarian malformations including cysts, degenerated, multinucleated and hemorrhagic follicles. The number of corpora lutea was also

significantly lower at 50 mg/kg of BPA ($p < 0.001$). These malformations were associated with accelerated pubertal timing and premature anestrus (Adewale *et al*., 2009).

Wistar rats

Rodriguez *et al.* who assessed the influence of a BPA exposure on the growth and maturation of follicles and demonstrated an effect at an earlier stage of oocytes development, at the onset of meiosis in the fetal ovary. They demonstrate that a subcutaneous exposure to BPA from PND1 to PND7 reduces the ovarian reserve of follicles in female Wistar rats at 20 mg/kg bw/d but not at 0.05 mg/kg bw/d. This reduction in the pool of primordial follicles was associated with an increase in the primordial to primary follicle transition. The results suggest that the follicle activation triggered by BPA exposure might be caused by an ERα and/or ERβ-mediated stimulatory effect on early folliculogenesis. Because all primordial follicles initiated to grow, are programmed to undergo apoptosis, menopause may result from this initial but not cyclic recruitment of follicles. The authors therefore hypothesized that BPA may lead to an early onset of menopause and consequently premature ovarian failure (Rodriguez *et al*., 2010).

Bosquiazzo *et al*. (2010) treated newborn female Wistar rats with subcutaneous injections of BPA at 20 or 0.05 mg/kg bw/d on PND 1, 3, 5 and 7. To evaluate the long term effect, rats were ovariectomized on PND80 and submitted to hormonal replacement. While treated with ovarian steroid, a decrease of the uterine endothelial proliferation of approximately 5% ($p < 0.05$) associated with a significant decrease ($p < 0.05$) in Vascular Endothelial Growth Factor (VGEF) mRNA is observed at both doses. The VEGF is likely the main factor responsible for the vascular permeability and angiogenesis during the pre-implantation period and is therefore important for the establishment and maintenance of pregnancy. These data therefore suggest that neonatal BPA exposure might have negative consequences on female fertility.

In 2011, the same team administrated again this treatment to female Wistar rats in order to assess the reproductive performance in adulthood, at the age of 80 days. On day 18 of pregnancy, the number of implantation sites was significantly lower (- 25%; $p < 0.05$) in rats treated post-natally as compared to the controls, suggesting an intrinsic uterine defect that preceded the embryo arrival. In addition, a tendency to have a higher number of resorptions, an indicator of post-implantation loss, was also observed (Varayoud *et al*., 2011). These findings were associated with a lower expression of Hoxa 10 gene on day 5 of pregnancy. The authors suggest that this early alteration in Hoxa 10 gene expression might affect functional differentiation of the uterus during pregnancy. Indeed, the Hoxa 10 gene is a member of the Hox genes family that are transcription factors essential both for development of the reproductive tract during the embryonic period (organogenic differentiation) and adult function (functional differentiation). Two members of this family, Hoxa10 and Hoxa11, are essential for female fertility. Gene expression profiling experiments reveal that Hoxa10 is an important regulator of implantation-associated events, such as uterine stromal cell proliferation and local immunosuppression.

➔ In sheeps

Evans *et al.* and Collet *et al*. examined the effect of long term exposure during the prepubertal period. In Evans's study, female sheep of 4 weeks of age were treated twice a week for 7 weeks with intramuscular injections of BPA at 3.5 mg/kg bw. Results obtained in the seventh week demonstrate that BPA can suppress LH secretion in ovariectomized sheep and can thus exert negative feedback effects on gonadotropin secretion. These changes were associated with modifications of the uterine morphology (Evans *et al*., 2004). The same treatment was administrated in female sheep of 4-5 months of age for 8 weeks (Collet *et al.,* 2010). The mean LH pulse frequency and basal concentrations, but not the amplitude, were slightly decreased from the sixth week of treatment.

Collet *et al*. also investigate the impact of acute BPA exposure by treating prebubertal female sheeps for 54h hours with IV infusion at 0.5, 1, 2.5, 5, 10, 20, 40 and 80 mg/kg bw/d. The inhibitory effect of BPA appeared to follow a dual mechanism of action. At the highest doses, i.e, 40 mg/kg bw and upward, BPA triggered an immediate (within 1 hour) inhibition of LH secretion suggesting a non-genomic pathway at the pituitary level. By contrast, at lower levels, BPA still inhibited the LH pulse frequency but only after a 48-h period of latency, a delay consistent with a genomic effect. Similar qualitative events were observed with the 17-β estradiol used as positive control but for lower plasma concentrations: the lowest plasma concentration of estradiol associated with inhibition of pulsatile secretion of LH is 2 pg/mL versus 38 ng/mL for BPA (Collet *et al.,* 2010).

4.11.1.1.3 Prepubertal exposure

Hunt *et al*., treated juvenile female mice (20 to 22-day-old) with oral doses of BPA at 20, 40 or 100 µg/kg bw/d for 6-8 days preceding oocytes collection and analysis. A dose-related increase in the level of abnormalities leading to aneuploidy, like non-disjunction at anaphase, was observed ($p < 0.05$). It appears that low dose BPA exposure during final stages of follicle growth is sufficient to cause meiotic abnormalities resulting in the production of chromosomally abnormal eggs (Hunt *et al*., 2003).

4.11.1.1.4 Exposure during adulthood

Al Hiyasat *et al*. demonstrated in female Swiss mice that BPA exposure during adulthood may impact the fertility. Exposure in 60 days old female Swiss mice to BPA at 25 and 100 µg/kg bw/d administrated intragastrically for 28 days induced no effect on the number of pregnancies, implantations or in the number of viable fetuses but resulted in a significant increase ($p < 0.01$ at 25 µg/kg and $p < 0.05$ at 100µg/kg) in the total number of resorptions out of the total number of implantations. Furthermore, there was an increase in the number of animals with resorptions: only

11.1% of the animals in the control group had resorptions, whereas in mice exposed to 5, 25 and 100 µg/kg, the percentage of animals with resorptions was 57.1, 62.5 and 66.1%, respectively (Al Hiyasat *et al.*, 2004).

Finally, a study performed by Aldad et alin ten oophorectomised adult female African green monkeys (Chlorocebus aethiops sabaeus) of reproductive age shows that exposure to the combination of estradiol and BPA resulted in decreased PR expression compared to estradiol exposure alone ($p<0.01$). Both BPA exposure and diminished progesterone action have been associated with pregnancy loss, endometriosis and endometrial hyperplasia/cancer (Aldad *et al.*, 2011).

4.11.1.1.5 Multigeneration exposure

➔ Mice

Continuous breeding study (Copy of the RAR-UK, Final report 2003, study considered as key study)
The effects of bisphenol-A on fertility and reproductive performance have been extensively studied in CD-1 mice (n= 20/ treated group/ sex (F0 generation), n= 40/ control group/ sex) using the test system known as the "Fertility Assessment by Continuous Breeding" (NTP, 1985b). This system involves four successive tasks (Task 1, dose-finding; Task 2, continuous breeding phase; Task 3, identification of the affected sex, and Task 4, offspring assessment.). Bisphenol-A was administered in the diet at concentrations of 0, 0.25, 0.5 or 1.0% (daily intakes of BPA 0, 300 or 325, 600 or 650 and 1,200 or 1300 mg/kg in males or females repspectively) during a one-week premating period and a 14-week mating trial (Task 2). After the premating period, males and females from each group were randomly paired and allowed to cohabit for 14 weeks. During the cohabiting period the reproductive performance was monitored by counting the number of F1 generation litters produced by each breeding pair and recording on the day of birth the litter size, proportion of live pups, litter size and sex ratio of the pups; all pups were then immediately removed and discarded. All litters produced after the cohabiting period remained with their mothers until weaning on day 21 post partum. The twenty F0 males and twenty F0 females from the top dose group (1.0% bisphenol-A) were then mated with twenty control females and twenty control males, respectively. Bisphenol-A was discontinued in the diet during this 7-day cohabitation period and then reinstated for 21 days upon separation of the breeding pairs. A control group of twenty untreated breeding pairs was also included (Task 3). The same reproductive assessment as described for the continuous breeding phase was conducted. Parental animals were sacrificed within 1 week of delivery. A maximum of twenty male and twenty female F1 generation offspring (from the final litters of the control and highdose groups in the continuous breeding phase) were retained after weaning for assessment of their reproductive capacity (Task 4). After rearing to sexual maturity, each F1 female was paired with a F1 male from the same dose group for 7 days. The resulting litters were evaluated and discarded on the day of birth as described for the litters produced during the F0 generation cohabitation phase. For all control and high-dose F0 and all reared F1 animals, liver, kidneys, adrenals and reproductive organs were weighed and subjected to histopathological examination. In

males, sperm analysis (concentration, motility and morphology) was undertaken, and effects on the oestrous cycle assessed in females. There were no clinical signs of toxicity among F0 generation animals. In the continuous breeding phase, a statistically significant decrease in maternal body weight was observed after each litter (between 6 and 9%), at the top dose, on postnatal day 0 compared to controls. No effect was observed on maternal postnatal (day 0) body weight following the cross-over mating phase. However, at study termination, a small but statistically significant decrease in body weight (4%) was observed in treated females compared to controls. No adverse effects on body weight gain were observed in treated males. An adverse effect on fertility was observed in the continuous breeding experiment and cross-over mating experiment. In the continuous breeding phase, a statistically significant decrease compared to controls was observed in the number of litters produced per pair (4.5 and 4.7 compared to 5.0 for controls), litter size (6.5 and 9.8 compared to 12.2 for controls) and the number of live pups per litter (6.3 and 9.7 compared to 12.1 for controls) in the high and mid-dose group. The litter size reductions occurred across all matings and the magnitude of all these decreases were dose-related. No effects on fertility were observed in the low-dose group. A statistically significant decrease in litter size (controls: 11.4, treated males: 9.1, treated females: 5.9) and number of live pups per litter (controls: 11.3, treated males: 8.4, treated females: 5.5) were observed in the cross-over mating. In the continuous breeding phase, a statistically significant decrease in live pup weight (6%) on postnatal day 0 was observed in females at the top dose after adjustment for litter size, including live and still births. In the continuous breeding phase a small but statistically significant decrease in body weight gain (4%) was only observed in treated females at study termination. No effect was observed on the sex ratio in the F1 generation. In the F1 litters used in the cross-over breeding experiment, post natal (day 0) pup weights were significantly increased in males (9-11%) and in females (8-10%) in the mid- and high-dose groups compared to controls. These increases were no longer evident in either sex at 21 or 74 days of age. Deaths among F1 generation were observed during lactation (day 0-21) and post weaning (day 21-74). At the top dose there were only 8 litters that had at least one male and one female for the mating phase, and therefore only 11 breeding pairs at the top dose compared to 19-20 breeding pairs in the control, low-dose and mid-dose groups. In those litters selected for mating deaths had been observed in 6%, 4%, 14% and 38% animals up to day 74 in the control, low-dose, mid-dose and high-dose groups, respectively. It is not known how many animals of this total died during lactation. However, it does raise the possibility that there may be potential effects on pups due to exposure to bisphenol-A via the milk. In the F1 generation, bisphenol-A treatment had no effect on the fertility index, litter size, number of live pups per litter, sex ratio or mean pup weights at birth. At necropsy of the F0 generation (controls and top dose group only), treatment-related effects were seen at the highest dose level; for both sexes relative liver weight was increased about 28% and relative combined kidney/adrenal weight increased 10-16% compared to controls. No histological changes were observed in female reproductive organs and no effect was observed on the oestrous cycle. Overall, the signs of general systemic toxicity were not marked in this study and therefore the effects on fertility are not considered to be a consequence of parental toxicity. At necropsy of the F1 generation, treatment-related effects of similar magnitude were generally observed in males and females; compared to controls, increased relative liver weights (6-29%) and kidney/adrenal weights (13-20%) were observed in all treated groups. No histological changes were observed in the female reproductive organs. In this study, adverse effects on fertility, namely a reduction in litter size and number of live pups per litter, were observed in each litter from the F0

generation in the continuous breeding experiment at approximately 600 mg/kg and above, and at the only dose level tested in the crossover breeding experiment, approximately 1,200 mg/kg. A treatment-related decrease in the number of litters produced was also observed at 1,200 mg/kg during the continuous breeding phase. These effects were observed in the absence of significant parental toxicity. No effect on fertility was observed at 300 mg/kg, though no histopathology was conducted on these animals. Histological examination was conducted on all F animals, and the only effects observed were toxicity to the liver and kidney at all doses. No adverse effect on fertility was observed in the F generation up to approximately 1,200 mg/kg, which might have been expected in view of the observed effects on fertility in the F generation. Nevertheless, the absence of effects following the single F mating does not detract from the reproducible results across the 4-5 litters produced by each F generation pair. Therefore, overall, an adverse effect on fertility has been observed with BPA at approximately 600mg/kg and above.

Tyl *et al*. performed two multigeneration studies in 2002 and 2008. The studies design and the systemic toxicy findings are reported here with the results on females' reproductive toxicity. The results for the males are described below in the paragraph 4.11.2.1.6. The latest study is presented at first because a lot of supplementary data were available allowing an in depth evaluation.

In 2008, a 2-generation study was performed according to the OECD guideline 416, in mice (Tyl *et al*., 2008). Mice were exposed by gavage to 0, 0.018, 0.18, 1.8, 30, 300 and 3500 ppm (equivalent to approximately 0.003, 0.03, 0.3, 5, 50 and 600 mg/kg bw/day) of BPA (purity 99.7%). The positive control group was exposed to the 17β-estradiol, and the negative control group received vehicle only. Each of the 8 groups was composed of 28 male and 28 female CD-1 mice (F0). Mice were exposed 8 weeks prior to mating, and then from conception to adulthood (chronic exposure). No toxicity was observed in the F0 or F1 generations and effects on the fertility were only observed at the highest dose (3500 ppm: 600 mg/kg bw/d). Although it has been described in the paper that systemic toxicity can be observed at this dose, a thorough observation of the data provided as supplementary tables with the paper did not allow validating this statement. Indeed, F0 male body weights (BW) were comparable all along the study between the various treatment groups and control. As shown in figure 1, F1 male (parental and retained (not presented here)) have the same growth curve as controls during the treatment period whatever the treatment group. The difference observed comes from PND14's BW. At this timepoint, 3500 ppm pups' BW is lower by 10% (compared to controls) and this difference persists along the entire treatment period (from day 0 on the Figure 1 to mating period). 3500 ppm F2 pups are also smaller by 5% compared to controls at PND14. The origin of this difference in BW is unclear as birth's BWs are similar among the different groups. This strongly suggests an impact of 3500 ppm-BPA exposure on lactation. Interestingly, this BW difference persists in males treated with 3500 ppm of BPA although they eat more than controls whatever the timepoint and the generation (food consumption of F1 parental sd 0 to 7 = control + 12.3%) but diseappears in females during direct treatment although treated females do not eat more than controls.

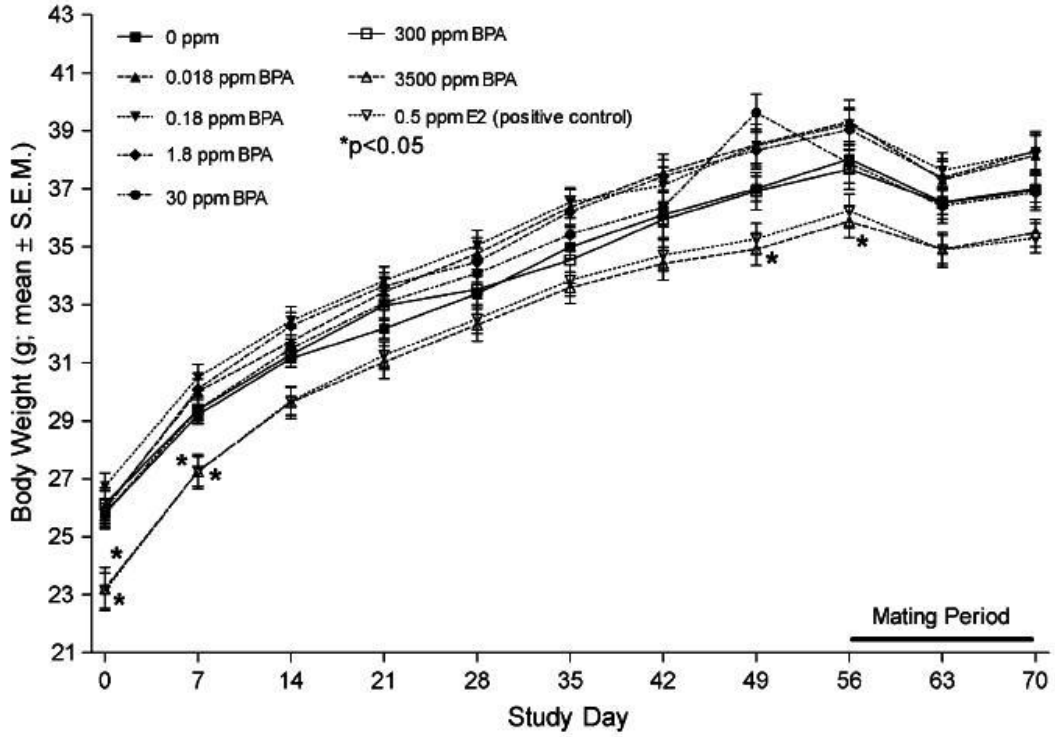


Figure 1 from (Tyl *et al.*, 2008): F1 parental body weights during the prebreed and mating periods.

Signs of toxicity were observed as increased kidney and liver weight from 300 ppm and onward for F0 males, from 0.018 ppm in F1 parental males, in F0 and F1 females and in F1 & F2 pups (male and females) at 3500 ppm. However, these results suggest rather a strong and direct effect of BPA on these organs than systemic toxicity.

Together with the effect of BPA on BW evolution depending on the sexe of the animals, another finding points out the potential endocrine effect of BPA: Pituitary relative weight is increased in F1 parental and retained male at all doses (significant at 300 ppm). Only F0 E_2-treated males have this finding. Detailed brain dissection was not performed and brain global was weighted in pups, so we cannot confirm this finding in the next generation. Therefore, BPA exposure impacts the pituitary gland after an *in utero* exposure that might affect fertility through sexual hormone modifications.

In females, most of the reproductive parameters (i.e. reproductive organ weights, ovarian primordial follicles count, histopathology of ovaries and uterus, mating and fertility indices, litter size at birth, sex ratio, percent of post-implantation loss) were unaffected by the treatment. Effect of BPA on reproduction and the offspring were only observed at 3500 ppm. At this dose, BPA exposure increased the length of the gestation by 0.3 days, reduced the body weight of the pups during lactation, and F0 treated females were twice more in estrus compared to controls as shown in the supplementary table 22 p. 6/7, line 5.

➔ Rats

The effect of BPA on fertility was evaluated in an extensive oral two generation reproduction toxicity study (Copy of the RAR-UK, Final report 2003, study considered as key study) in Crj;CD (SD) IGS rats (Ema *et al.*, 2001). The F0 generation consisted of groups of 25 rats per sex per group administered 0, 0.2, 2, 20 and 200 μg/kg/day BPA by gavage during a premating period of 10 weeks for males and 2 weeks for females and a 2-week mating period. Males and females from each group were randomly paired and co-habited for 2 weeks. Females were also administered the test material during gestation and lactation. F0 males and females were sacrificed after the mating period and weaning of F1 pups, respectively. Twenty-five male and female F1 generation offspring from each group were retained after weaning for assessment of their reproductive capacity. F1 animals were administered bisphenol-A for a 10-week premating period and a 3-week mating period (see below). Again, females received the test material during gestation and lactation, and male and female parental animals were sacrificed at the same times used for the F0 generation. Twenty-five male and female F2 generation offspring from each group were retained after weaning for assessment of sexual maturation. Males and females were administered the test material until they were sacrificed at the age of 7 and 14 weeks, respectively.
For all F0 and all reared F1 and F2 animals, observations and weighings were performed regularly. In addition to determining reproductive capacity, various other parameters were assessed. Learning tests were conducted using a water filled multiple T-maze with 6 male and 6 female F1 animals per dose group at 5-6 weeks of age. Several reflex assessments were determined in 1 rat per sex per litter until successfully completed. Sexual maturation (vaginal opening and preputial separation) was determined in F1 and F2 parent animals, along AGD. After sacrifice, all F0 and F1 parent animals were subjected to a thorough macroscopic and microscopic examination. In males, this included examination and weighing of the epididymis, prostate and seminal vesicles (including the coagulating gland). Serum testosterone, oestradiol, prolactin, LH, FSH, T3, T4 and TSH concentrations were also determined in 6 animals per sex per group from the F0 and F1 generations. Seminal vesicles and coagulating gland were weighed and subjected to histological examination. The motility and morphology of sperm in the epididymis was also determined in F0 and F1 males. All pups that were not selected for further assessment were sacrificed and also underwent histopathological examination.
In parental animals, no clinical signs of toxicity, nor any effects on body weight gain, food intake or treatment-related deaths were observed in any generation. No effect on behaviour (i.e. performance in learning tests) was observed in F1 animals. Oestrus cycle, fertility index and the number of implantations in F0 and F1 females were not affected by treatment with bisphenol-A. (The mating period for F1 animals was extended for a week, as at the end of the first week mating was confirmed in only 19/25 females administered 0.2 μg/kg/day, compared to 24/25,22/25, 23/25 and 21/24 at 0, 2, 20 and 200 μg/kg/day respectively. At the end of the 3-week mating period no significant effect on the fertility index was observed between treated and control animals). No significant differences were observed between BPA and control animals for the time to vaginal opening. Compared to controls, a statistically significant decrease (<5%) in AGD was seen F1 and F2 females at 20 and 200 μg/kg/day. These decreases were not statistically significant when the ratio of the AGD to body weight was determined (the AGD is correlated with body weight). No treatment related changes were observed in any of the serum hormone levels measured. BPA had no

effect on sexual maturation or the oestrus cycle in F2 animals and F2 females, respectively. At necropsy, no treatment-related macroscopic findings or organ weight changes were observed in F0 and F1 parental animals.
In the offspring (all live pups up to day 21), no clinical signs of toxicity or effects on body weight gain during lactation were observed in F1 and F2 pups. No treatment-related changes were seen in the litter size, survival, sex ratio, AGD and reflex ontogeny. At necropsy, no treatment related macroscopic findings were observed in F1 and F2 pups.

In the oldest study (Tyl *et al.*, 2002), exposure of males and females CD Sprague Dawley rats to BPA (purity at 99.5%) administrated in the diet at 0, 0.015, 0.3, 4.5, 75, 750 or 7500 ppm (this doses were equivalent in actual intake to 0.0007-0.003, 0.015-0.062, 0.22-0.73, 4.1-15.4, 37.6-167.2 and 434-1823 mg/kg bw/day in males and females respectively) for three generations was evaluated under Good Laboratory Practice using the U.S. EPA OPPTS test guidelines (U.S EPA OPPTS 837.3800, 1998). 30 rats per sex and per dose were exposed 10 weeks prior to mating, and then for males continued through a 2-week mating period and for an additional 3 weeks after mating. Females were exposed from conception trough gestation and lactation. Males and females from a same group were mate together, 3-generations of males and females were then studied. For each generation 30 weanlings animals per sex and per dose were selected in order to become the parents of the next generation, and 3 animals per sex and per litter were necropised and undergo further analysis. Adult systemic toxicity were limited to reduced body weight due to lactational effects together with smaller body weight gains (-22% of the F1 7500ppm treated males). However, feed consumption did not show clear treatment-related effects. Although the data available for this study are less detailed than for the study above, we can affirm from the previous study that the slight to mild renal tubular degeneration and chronic hepatic inflammation observed in females for the three generations at 750 and 7500 ppm is a strong and direct effect of BPA on these organs rather than systemic toxicity.

Results show that there was no effect of BPA on estrous cycle length, paired ovarian primordial follicle counts or in reproductive organs histology. Similarly, many reproductive parameters including mating, fertility, pregnancy, dead pups per litter or percent post-implantation loss remain unaffected in F0, F1, F2 females. However, at 7500 ppm, the number of implants, total pups and live pups/litter at birth and on PND4 were reduced ($p < 0.001$) and the absolute and relative organ paired ovary weights were decreased in F1, F2 and F3 offspring and adult ($p < 0.05$ and $p < 0.001$ respectively). In female offspring, AGD was significantly increased in the F2 generation at all dietary doses, with the exception of the 75 and 7500ppm groups. The absolute age at vaginal patency (days) was significantly delayed in the F1, F2 and F3 generations at 7500ppm (and at 75 ppm only for the F2 generation).

4.11.1.1.6 Transgeneration exposure

In a more recent study by Hiyama *et al*. (2011), the transgenerational effects of BPA exposure on the reproductive tract across generations were examined in F2 generation issued from F0 pregnant ICR mice treated during GD12 to GD16 with sub-cutaneous injections at doses 100, 200, 500 or 1,000 mg/kg bw/d leading to a dose dependant increase in body weight. In this experiment, the F2 mice were never exposed directly. Decrease in the relative ovary weight at 200 and 500 mg/kg ($p < 0.05$), abnormal morphology such as expansion or emphraxis of the uterine lumen and partial loss of the uterine epithelium were observed. Unmethylation of Hoxa 10 gene in the intron region was also observed in the uterus at 100 and 200 mg/kg. These results suggest that BPA exposure during the development *in utero* of the genital tract in F1 mice could alter the histology and methylation pattern in the reproductive organ of F2 mice that hadn't been exposed directly to BPA. DNA methylation might be one of the ways that BPA induces the endocrine disruption. This publication shows that BPA, through gene imprinting, could have transgenerationnal effects on the female reproductive tract. Those data have not been reproduced so far.

4.11.1.1.7 Evaluation by effects

- Effects on the reproductive tract and ovaries

Effects on the meiosis and on the oocyte development

In several studies, BPA has been shown to adversely affect chromosome segregation leading to meiotic disturbances, including aneuploidy.

A recent study in Rhesus Monkey show that BPA induces subtle disturbances in the prophase events that set the stage for chromosome segregation at the first meiotic division. The analyses of third trimester fetuses exposed to single daily oral doses during the time of follicle formation revealed an increase in multioocyte follicles analogous to that reported in rodents. (Hunt *et al.*, 2012)
Similarly, Susiarjo *et al*. showed that exposure to BPA from GD 11.5 to GD 18.5 (time of ovaries isolation from female fetuses) via implanted time-release BPA pellets (20 µg/kg bw/d) in pregnant C57BL/6 females displayed gross aberrations in meiotic prophase, including increased levels of recombination leading to increased aneuploid eggs and embryos significant proportion of oocytes prior to sexual maturation, reducing the pool of oocytes in the adult female. (Susiarjo *et al*., 2007).

Karavan *et al.* showed effect of subcutaneous injection (5 or 50 mg/kg bw/d) of BPA on neonatal CD-1 mice : percentage of single oocyte was reduced from 84% in controls to 75.4% with for the lower concentration of BPA (not significant) while the higher BPA concentration caused a

significant drop to 68.6%.. The oocyte number per section (oocyte survival) was increased from 8 to 13 (low dose) and 15.8 (high dose). Control mice injected with peanut oil alone had 62.3% primordial follicles, 27.9% primary follicles, and 9.7% secondary follicles. High and low concentrations of BPA significantly increased the percent of primordial follicles to 89.5% and 81%, respectively. While treatment with both concentrations of BPA did lower the percentage of primary follicles, this effect was significant only for the higher concentration of BPA, which had 10.5% primary follicles. Treatment with both concentrations of BPA also lowered the percentage of secondary follicles to 0.8% and 0%, but this effect was not significant in both instances. (Karavan *et al.*, 2012).

Similar results were found in rat by Rodriguez *et al.* who assessed the influence of a BPA exposure (subcutaneous/ postnatal) on the growth and maturation of follicles and demonstrated an effect at an earlier stage of oocytes development, at the onset of meiosis in the fetal ovary. (Rodriguez *et al*., 2010).

Treated juvenile female mice (20 to 22-day-old) with oral doses of BPA at 20, 40 or 100 µg/kg bw/d for 6-8 days preceding oocytes collection and analysis induced a dose-related increase in the level of abnormalities, leading to aneuploidy like non-disjunction at anaphase (Hunt *et al*., 2003).

In contrast, the guideline studies performed by Tyl on Sprague Dawley rats (Tyl *et al.*, 2002) or mice (Tyl *et al.*, 2008) found no effect of BPA on ovarian primordial follicles count. The 2 other guideline studies did not report this finding neither in mice (NTP, 1985b) nor in rat (Ema *et al*, 2001). The guideline studies contradict the other studies reported without straightforward explanation of this discrepancy.

Effects on the reproductive tract and ovarian morphology

Many studies demonstrate that BPA exposure to pregnant females or ealy in life affected the development of the genital tract of their female offspring at both the gross and cellular levels, long after the exposure had ended. These malformations are resumed in the table 9. In most of the studies, they are associated with ovarian morphology effects reported in table 8 thereafter. Ovarian cysts are one of the most common abnormalities found in ovaries in rodent (Fernandez *et al*., 2010; Adewale *et al*., 2009; Newbold *et al*., 2009; Newbold *et al.*, 2007; Signorile *et al*., 2010).

In Balbc-C mice, Signorile *et al*. (2010) found that subcutaneous injections with 100 or 1000 µg/kg bw/d of BPA *in utero* and during 7 days after birth (caused in 3-month old female offspring, endometriosis-like structures in the adipose tissue surrounding the genital tract and increased incidence of cystic ovaries, adenomatous hyperplasia with cystic endometrial hyperplasia, and atypical hyperplasia ($p = 0.085$). No cases of malignancy were found in this study, but animals were sacrified at 3 months of age and probably had no time to fully develop tumors (Signorile *et al*., 2010).

Prenatal (GD 9-16) exposure of females CD-1 mice to 1 µg/kg bw/day BPA led to benign ovarian cysts to more severe ovarian lesions at 10, 100 or 1000 µg/kg bw/d, including progressive proliferative lesions of the oviduct which occurrence seemed to increase following BPA exposure. (Newbold *et al*., 2009)

Bosquiazzo *et al*. (2010) showed that treatement of newborn (PND 1, 3, 5 and 7) female Wistar rats with subcutaneous injections of BPA (0, 0.05 mg/kg bw/d) decrease the uterine endothelial proliferation of approximately 5% ($p < 0.05$) associated with a significant decrease ($p < 0.05$) in Vascular Endothelial Growth Factor (VGEF) mRNA is observed at both doses while treating ovariectomized rats with hormonal replacement.

Fernandez *et al*. demonstrates that neonatal exposure to high dose of BPA in female rats could be linked with the development of polycystic ovarian syndrome in adulthood (Fernandez *et al*., 2010). These syndromes were accompanied by infertility. At lower dose (2.5 - 6.2 mg/kg bw/d), females delivered significantly fewer pups ($p < 0.05$) indicating subfertility, without impairment of ovulation.Similarly, subcutaneous neonatal exposure to 50µg/kg bw/d and 50 mg/kg bw/d in females Long Evans rats also induced at both dose ovarian malformations including cysts, degenerated, multinucleated and hemorrhagic follicles. The number of corpora lutea was also significantly lower at 50 mg/kg of BPA ($p < 0.001$). These malformations were associated with accelerated pubertal timing and premature anestrus (Adewale *et al*., 2009).

In the study by Newbold *et al.*, subcutaneous injections of 10, 100 or 1000µg/kg bw/d BPA of female CD-1 mice treated neonatally (PND1-5) (Newbold *et al.*, 2007) led to ovarian pathologies (ovarian cysts, benign proliferative lesion, diminution of Corpora Lutea). Uterine pathologies (benign invasion, precursor lesion to adenocarcinoma and nepoplasic lesion) were more common in all treated groups as compared to the control (corn oil). As example, atypical hyperplasia of the uterus occurred in 4% and 5% of the females treated with 10µg/kg and 100µg/kg respectively, but not in the control group. However, only the increase in cystic ovaries ($p \leq 0.05$) and cystic endometrial hyperplasia ($p < 0.01$) in the 100 µg/kg bw/d treated group were statistically signigicant.

No multigeneration study mentioned this type of effect.

Mechanisitic studies

A few studies have been conducted in order to pintpoint the mechanism of uterine effects of BPA. The long term effect of BPA on the tissue morphology reported previously such as cystadenomas, progressive proliferative lesions, atypical hyperplasia and stromal polyps may be due to a deregulation of the tissue homeostasis which is the result of a fine balance between cell proliferation, DNA synthesis, differentiation and cell death by apoptosis, processes that are highly regulated. Several publications have reported in reproductive tract tissue such an alteration in programmed cell death and in DNA synthesis, thus may contribute to carcinogenesis (Mendoza-Rodríguez *et al.*, 2011; Markey *et al*., 2005).

Mendoza-Rodríguez *et al.* showed that oral exposure of females Wistar rats to BPA during gestation and lactation induces in the 4 months old female offsprings a reduction in apoptotic cells in uterin epithelium ($p < 0.001$) which is associated with an increase thickness of both uterine epithelia and stroma ($p < 0.05$).

Administration of lower doses of BPA (25 and 250 ng/kg bw/d of BPA) via osmotic pumps implanted into pregnant CD-1 mice dams from GD9 until PND4 induces alterations in the genital tract of female offspring that are revealed during adulthood (3-months of age), including decreased weight of the vagina of 20 mg ($p < 0.01$), and decreased volume of the endometrial lamina propria of 11 mg ($p < 0.05$) observed at 250 ng/kg bw/d. These findings were assiocated at 250 ng/kg bw/d with an increase of the DNA synthesis in the uterine epithelium in the absence of any apoptotic change, suggesting modification of the balance proliferation/apopotosis leading to an increase number of new cells. In contrast to Mendoza-Rodriguez's results, the expression of ERα, revealed by immunolocalization within the luminal epithelial cells, was increased by approximately 4-fold at 25 ng/kg bw/d ($p < 0.05$) and by 5-fold at 250 ng/kg bw/d ($p < 0.01$). The expression of the progesterone receptor in the uterus was also observed increased by approximately 14-fold and 13-fold at 25 and 250 ng/kg bw/d respectively ($p < 0.01$) (Markey *et al*., 2005).

Schönfelder *et al.* (2004) studied more especially the influence of BPA treatment in the protein receptors ERα and ERβ expression and distribution in the uterus that may promote a uterine disruption. Indeed, most of the estrogenic effects on the uterus are mediated by ERα which regulates epithelial morphogenesis, cytodifferenciation, development and growth of the uterus. It has also been demonstrated that ERβ can modulate the ERα-mediated gene transcription leading to antiproliferative function in the uterus (Schönfelder *et al*., 2004). Gavage administration in gravid Sprague-Dawley dams of 0.1 or 50 mg BPA/kg from GD6 through GD21 induces in adult offspring an increase in ERα expression in the 50 mg/kg group ($p < 0.05$) whereas ERβ expression was significantly decreased at both dose ($p < 0.00001$). These results were revealed by immunohitochemistry and Western blot analysis. A decrease (-10%) of the thickness of the total uterin epithelium was also observed during estrus ($p < 0.05$).

Varayoud *et al.*, who later linked alteration of Hox gene expression with decreased implantation site revealed the involvement of Hox gene in the onset of the uterus malformations. They examined by RT-PCR in female Wistar rats whether early postnatal exposure to BPA at 20 or 0.05 mg/kg bw/d on PND 1, 3, 5 and 7, alters Hoxa10 and Hoxa11 mRNA uterine expression, shortly after treatment and in adulthood. The results show that BPA exposure at both doses induced a significant reduction of the level of mRNA encoding Hoxa10 and Hoxa11 (by approximately 80 and 75% respectively, $p < 0.001$) on PND8 and in adult females indicating that the Hoxa10 and Hoxa11 alteration persists until adulthood. These alterations were associated with a decrease in stromal cell proliferation (Varayoud *et al*., 2008).

Contradictory results were reported by Smith and Taylor who found a dose-response increase in uterine stromal cells Hoxa10 expression in 2 and 6 weeks old CD-1 mice exposed *in utero* from GD9-GD16, to BPA at 0.5, 1, 5, 50 or 200 mg/kg (Smith and Taylor, 2007).
However, both studies show that Hox genes are targets of endocrine disruption. Those results confirm that the expression of these genes is highly susceptible to exogenous parameters together with windows of development.

Recently, the unmethylation of Hoxa 10 gene in the intron region was proposed as a vector of the transgenerational effects of BPA exposure observed by Hiyama *et al*. (2011). Indeed, the ICR F2 mice were never exposed directly displayed a decreased relative ovary weight when issued from Fo mice treated with 200 and 500 mg/kg ($p < 0.05$), abnormal morphology such as expansion or emphraxis of the uterine lumen and partial loss of the uterine epithelium.

Table 8: Summary table of the BPA effects on the ovarian morphology in female animals

Species	Exposure period	Dose Route	Observations	References
Gestational exposure				
Mice CD-1 n = 5 females /dose	GD9 – GD16	0.1, 1, 10, 100, 1000 µg/kg bw/d Subcutaneous	Observations in adult females (18-months of age) - ↗ cystic ovaries at 1µg/kg - neoplasic lesions : cystadenomas at 10, 100 and 1000µg/kg - progressive proliferative lesion of the oviduct at all doses - No data on the GLP / OECD guideline compliance	Newbold *et al*., 2009
Mouse C57BL/6 No data on the number of animals /dose	GD11.5 – GD18.5	20µg/kg/d Implantation	Fetus oocytes analysis at GD18.5: ↗ in synaptic abnormalities ($p < 0.0001$) ↗ in the number of recombination aberrations Oocytes analysis in offspring at 4-5 months of age: ↗ I the average number of chiasmata per cell ($p < 0.01$) and in the frequency of univalents ($p < 0.05$) ↗ in the number of hyperploidy in eggs ($p <0.001$) and in embryos No data on the GLP / OECD guideline compliance	Susiarjo *et al*., 2007
rhesus monkey (cohort 1: n = 6 treated, 6 control; cohort 2: n = 6 treated, 2 control)	In utero second trimester of pregnancy [gestational day (GD) 50–100], and a late exposure during the third trimester	1st cohort: pieces of fruit containing 400 µg/kg bw of dBPA; continuous exposure via subdermal implant: 2.2 to 3.3 ng/mL of	disturbances in the prophase events that set the stage for chromosome segregation at the first meiotic division. Increase in multioocyte follicles Persistent unenclosed oocytes in the medullary region and small, nongrowing oocytes in secondary and antral follicles.	Hunt *et al.*, 2012

	(GD 100–term)	unconjuated dBPA		
Perinatal exposure				
Mice Balbc-C 6 females / dose	GD1 – PND7	100 – 1000 µg/kg bw/d Subcutaneous	Observations in adult females (3-months of age) - ↗ cystic ovaries at both doses (p = 0.008) - No data on the GLP / OECD guideline compliance	Signorile *et al.*, 2010
CD1 outbred mouse strain	(PND) 1–4	(1) 5 mg/kg/d or 10 µg per pup and (2) 50 mg/kg/d or 100 µg per pup. Subcutaneous	Cyst breakdown, oocyte survival and follicle development altered. Single oocyte ↘from 84% in controls to 50–75%. The oocyte number per section was increased from 8 to 12–16. Follicle activation was reduced with 62% primordial follicles in controls to over 80% in most cases.	Karavan *et al.*, 2012
Postnatal exposure				
Rat Long-Evans 10-12 females / group.	PND1- PND3	50µg/kg bw/d 50 mg/kg bw/d Subcutaneous	- ↗ cystic ovaries at both doses - ↘ in the number of corpora lutea at 50 mg/kg (p < 0.001) - Presence of hemorrhagic follicles at 50µg/kg - Presence of multinucleated and hemorrhagic follicles at 50 mg/kg - No data on the GLP / OECD guideline compliance	Adewale *et al.*, 2009
Mice CD-1 24 females pups / dose.	PND1- PND5	10 - 100 - 1000 µg/kg bw/d Subcutaneous	Observations in adult females (18-months of age) - ↗ cystic ovaries at 100µg/kg (p ≤ 0.05) - ↘ corpora lutea as the dose increased - para-ovarian cysts of mesonephric origin at all doses - progressive proliferative lesion of the oviduct at all doses - No data on the GLP/ OECD guideline compliance	Newbold *et al.*, 2007
Rat Sprague Dawley No data on the number of dams/dose. 7 to 8 pups per litter	PND1-PND10	2,5, 62.5 mg/kg bw/d Subcutaneous	Observations in adult females (4, 5-months of age) - ↘ of the ovarian weight at all doses - ↗ cystic ovaries at at doses ranging from 25 to 62.5 mg/kg - ↘ in the number of corpora lutea at doses ranging from 25 to 62.5 mg/kg - ↗ in the number of atretic follicles at doses ranging from 25 to 62.5 mg/kg - ↘ in the number of antral follciles at all doses - No data on the GLP /OECD guideline compliance	Fernandez *et al.*, 2010
Rat Wistar The number of animals per treatment group was at least 8.	Subcutaneous	0.05 or 20 mg/kg/d PND1, 3, 5 and 7	Observations in females on PND8: - ↘ in the percentage of primordial follicles at 20 mg/kg (p < 0.001) - ↗ in the percentage of recruited follicles at 20 mg/kg (p < 0.001) - ↗ in p27 protein expression in primordial and recruited follicles - ↗ in ERβ expression in recruited follicles at	Rodriguez *et al.*, 2010

			20 mg/kg ($p < 0.01$) - ↗ in ERα expression in primary follicles at 20 mg/kg ($p < 0.05$) - No effect on multioocyte follicles incidence, oocytes survival, apoptosis and on PR expression - No data on the GLP /OECD guideline compliance	
Prepubertal exposure				
Mouse C57BL/6 No data on the nb of animals per dose	Mice of 20-22-days of age were treated for 6-8days	20, 40 or 100 µg/kg/d Oral (gavage)	<u>Oocytes analysis at the end of the treatment:</u> ↗ in congression failure ($p < 0.05$) at all doses Dose-related increase in the level of meiotic abnormalities No data on the GLP /OECD guideline compliance	Hunt *et al.*, 2003
Multigenerationnal exposure				
Rat **IGS (SD) rats** 25 rats /sex /group administered	2 generation study similar to OECD 416 (Deviations: *Female treated for 2 weeks only before mating. *Low doses used)	0, 0.2, 2, 20 and 200 µg/kg/day Oral by gavage	Effect not reported.	Ema et al., 2001
Rat Sprague-Dawley 30 males/dose, 30 females/dose	Exposure from 10 weeks before mating until PND21 (3 generations)	0.001, 0.02, 0.3, 5, 50 or 500 mg/kg/d Oral	↘ number of total and live pups per litter at birth at 7500ppm for F1, F2 and F3. According to EPA OPPTS 837.38000, 1998 GLP compliant study	Tyl et al., 2002
Mice CD-1 (n= 20/ treated group/ sex, n= 40/ control group/ sex)	Continuous breeding study (Task 1, dose-finding; Task 2, continuous breeding phase; Task 3, identification of the affected sex, and Task 4, offspring assessment.)	0, 0.25, 0.5 or 1.0% (daily intakes BPA estimated 0, 300 or 325, 600 or 650 and 1,200 or 1300 mg/kg for males or females resp. In diet	Effect not reported.	NTP, 1985b

Mice CD-1 N=28 animal/dose	Exposure from 8 weeks before mating until PND21 (2 generations)	0.003, 0.03, 0.3, 5, 50 or 600 mg/kg/d Oral	No effect on fertility, litter size Follow OECD guideline 416 (two generation reproduction toxicity study), TG 416 enhanced GLP compliant study	Tyl et al., 2008
Trans-generational exposure				
Mice ICR No data on the nb of animals / dose	Subcutaneous	100, 200, 500 or 1000 mg/kg bw/d GD12-GD16 in the F0 generation	<u>Observations in the F2 generation at 8 weeks of age:</u> ↘ in the relative ovary weight at 200 and 500 mg/kg ($p < 0.05$) No data on the GLP/OECD guideline compliance	Hiyama *et al.*, 2011

Table 9: Summary table of the BPA effects on the uterus morphology in female animals

Species	Exposure period	Dose Route	Observations	References
Gestational exposure				
Mice CD-1 n = 5 females/dose	GD9 – GD16	0.1, 1, 10, 100, 1000 µg/kg bw/d Subcutaneous	<u>Observations in adult females (18-months of age)</u> • Benign lesions: - Adenomatous hyperplasia with Cystic Endometrial Hyperplasia (CEH) at 1 and 100µg/kg • Precursor lesions to adenocarcinoma: - Atypical hyperplasia at 0.1µg/kg (21%), 1µk/kg (15%), 1000µg/kg (8%) • Neoplasic lesions: - Squamous metaplasia at 1 and 100µg/kg - Endometrial polyps at 0.1, 1 and 10 µg/kg - No data on GLP/OECD guideline compliance	Newbold *et al*., 2009
Perinatal exposure				
Mouse CD-1 6-10 females / dose.	GD9 – PND4	25, 250 ng/kg bw/d Implant	<u>Observations in adult females (3-months of age)</u> - ↘ of the volume of the endometrial lamina propria at 250 ng/kg - ↗ DNA synthesis in the uterine epithelium at 250 ng/kg - No data on GLP/OECD guideline compliance	Markey *et al*., 2005
Balbc-C Mice 6 females/dose	GD1 – PND7	100, 1000 µg/kg bw/d Subcutaneous	<u>Observations in adult females (3-months of age)</u> • Benign lesions: - 25% of Adenomatous hyperplasia with CEH at both doses • Precursor lesions to adenocarcinoma: - Atypical hyperplasia at both doses (p = 0.085) - No data on GLP/OECD guideline compliance	Signorile *et al*., 2010
Rat Wistar 5 females /dose.	GD6 – PND21	1.2 mg/kg bw/d Oral in drinking water	- ↗ thickness of uterine epithelia and stroma - ↘ apoptotic cells in the uterin epithelium - No data on GLP/OECD guideline compliance	Mendoza-Rodriguez *et al*., 2011
Post-natal exposure				
Mice CD-1 24 females pups / dose.	PND1-PND5	10, 100, 1000 µg/kg bw/d Subcutaneous	<u>Observations in adult females (18-months of age)</u> • Benign lesions: - CEH at 100 µg/kg ($p < 0.01$) - Adenomyosis at all doses - Uterine leiomyomas at all doses • Precursor lesions to adenocarcinoma: - Atypical hyperplasia at 10 and 100 µg/kg • Neoplasic lesions: - Stromal polyps at 100µg/kg (25%) - No data on GLP/OECD guideline compliance	Newbold *et al*., 2007
Multigenerationnal exposure				
None of the multigeneration study report this endpoint				
Trans-generational exposure				
Mice ICR No data on the nb of animals /	Subcutaneous	100, 200, 500 or 1000 mg/kg bw/d GD12-	<u>Observations in the F2 generation at 8 weeks of age:</u> abnormal morphology such as expansion or emphraxis of the uterine lumen and partial loss of the uterine epithelium	Hiyama *et al*., 2011

dose		GD16 in the F0 generation	No data on the GLP/OECD guideline compliance	

- Effets on the age at puberty

The vaginal opening time is conventionally considered as a reliable external sign of puberty. Another indicator is the first vaginal estrus which is highly correlated with first post-pubertal ovulation.

Studies with exposure limited to pregnancy reported effects consistent with an acceleration of puberty in female mice exposed *in utero* assessed by the age at vaginal opening and/or the age at first estrus (Honma *et al.*, 2002; Howdeshell *et al.*, 1999; Nikaido *et al.*, 2004).

Surprisingly, BPA treatment in rats during exposure period encompassing the second half of gestation and the lactation period until puberty, do not reveal any effect of BPA on the age at vaginal opening and/or at first estrus. (Kwon *et al.*, 2000; Ryan *et al.*, 2010; Takagi *et al.*, 2004; Yoshida *et al.*, 2004; Rubin *et al*., 2001). Other publication indicated more variable results in rats : An increase in the age of vaginal opening (VO) in the Alderley Park rats was observed at the highest dose evaluated (50 mg/kg bw/d) tested by gavage during gestation while no effect were detected in Sprague Dawley (Tinwell et al., 2002). Similarly to the study by Tinwell et al. Howdeshell's team did not demonstrate any changes in puberty age in male Long-Evans hooded rats exposed daily by oral route during pregnancy through lactation. However, the latest study showed a decreased duration of 2.5 days between vaginal opening and first vaginal estrus after BPA intrauterine exposure. (Howdeshell et al., 1999)
Early postanal exposure induces an advanced age at vaginal opening for a broad range of dose (50 µg/kg bw/d to 6 mg/kg bw/d) via subcutaneous injections both in rats and mice (Adewale *et al.*, 2009; Fernandez *et al.*, 2009; Nah *et al*., 2011).

No effect on the age at puberty was generated by a peripubertal exposure to BPA in mice (Nikaido *et al*., 2005) while multigenerational studies tend to show an effect in rats and mice (Tyl *et al.*, 2002& 2008)

In summary, experiments in rodents following an *in utero* exposure point out a possible effect of BPA on accelerating the puberty. This effect is also seen after an early postnatal exposure. However, when exposed *in utero* together during lactation, the age of puberty is not modified suggesting complex sensitivity windows regarding this endpoint. Results from rodent studies on that endpoint are not easily interpretable and difficult to reproduced pending on window and duration of exposure.

Table 10: Summary table of the BPA effects on the age at puberty in female animals

Species	Exposure period	Dose Route	Observations	Reference
Gestational exposure				
Mouse CF-1 21 females /dose.	GD11 – GD17	2.4 µg/kg bw/d Oral gavage	- No effect on the age at vaginal opening - ↘ number of days of 2.5 days between vaginal opening and first vaginal estrus ($p < 0.05$) - No data on GLP/OECD guideline compliance	Howdeshell *et al.*, 1999
Mouse ICR Jcl 10 females / dose.	GD11-GD17	2, 20 µg/kg bw/d Subcutaneous	- Advanced age at vaginal opening (ca. 1 day) at 20µg ($p < 0.01$) - Advanced age at first estrus (ca. 1 day) at 20µg ($p < 0.05$) - No data on GLP/OECD guideline compliance	Honma *et al*., 2002
Mouse CD-1 12 females /dose	GD15 – GD19	0.5, 10 mg/kg bw/d Subcutaneous	- Advanced age at vaginal opening (1 or 2days) at 10 mg ($p < 0.01$) - No data on GLP/OECD guideline compliance	Nikaido *et al*.,2004
Sheep Suffolk 10 pregnant females.	GD30 – GD90	5mg/kg bw/d Subcutaneous	- No effect on the age at first estrus determined with progesterone level - No data on GLP/OECD guideline compliance	Savabieasfahani *et al*., 2006
Perinatal exposure				
Rat Donryu 0.006 mg/kg/d group: 15 females 6 mg/kg/d group: 19 females	GD2 – PND21	0.006, 6 mg/kg bw/d Oral gavage	- No effect on the age at vaginal opening - No data on GLP/OECD guideline compliance	Yoshida *et al*., 2004
Rat Sprague Dawley 6 females / dose.	GD6 – end of lactation period	0.1, 1.2 mg/kg bw/d Oral: in drinking water	- No effect on the age at vaginal opening - No data on GLP/OECD guideline compliance	Rubin *et al*., 2001
Rat Long Evans 13 - 29 females / dose in block 1 6 - 14 females / dose in block 2	GD7 – PND18	2, 20, 200 µg/kg bw/d Oral	- No effect on the age at vaginal opening - No data on GLP/OECD guideline compliance	Ryan *et al*., 2010
Rat Sprague Dawley 8 pregnant females / dose	GD11 – PND20	3.2, 32, 320 mg/kg bw/d Oral gavage	- No effect on the age at vaginal opening - No effect on the age at first estrus - No data on GLP/OECD guideline compliance	Kwon *et al*., 2000

Rat Sprague Dawley 5-6 dams/group 6 pups for PND-10	GD15 – PND10	Ranging from 7 to 300 mg/kg bw/d Oral	- No effect on the age at vaginal opening - No data on GLP/OECD guideline compliance	Takagi *et al*., 2004
Post-natal exposure				
Rat Long-Evans 10-12 females / group.	PND1-PND3	50µg/kg bw/d 50 mg/kg bw/d Subcutaneous	- Advanced age at vaginal opening (2 days) at 50µg ($p < 0.01$) but not at 50mg No data on GLP/OECD guideline compliance	Adewale *et al*., 2009
Rat Sprague Dawley 11-15 females /group	PND1-PND10	Ranging from 2.5 to 62.5 mg/kg bw/d Subcutaneous	- Advanced age at vaginal opening of 2 days at dose ranging from 2.5 to 6.2 mg/kg and of 4 days at dose ranging from 25 to 62.5 mg/kg. ($p < 0.05$). - No data on GLP/OECD guideline compliance	Fernandez *et al*., 2009
Mice ICR 15 females per dose	PND8	0.1, 1, 10, 100 mg/kg bw Subcutaneous	- Advanced age at vaginal opening (1 or 2 days) at all doses ($p < 0.05$). No data on GLP/OECD guideline compliance	Nah *et al*., 2011
Peri-pubertal exposure				
Mice ICR No data on the nb of animal per dose	4 days from 15 to 18 days of age	10 mg/kg bw/d Subcutaneous	- No effect on the age at vaginal opening No data on GLP/OECD guideline compliance	Nikaido *et al*., 2005
Muligenerational exposure				
			-	
Rat Sprague-Dawley 30 males/dose 30 females /dose	Exposure from 10 weeks before mating until PND21 (3 generations)	0.001, 0.02, 0.3, 5, 50 or 500 mg/kg/d Oral	- The absolute age at puberty (evaluated by the age at vaginal patency) was delayed in the F2 generation at 50mg/kg and in the F1, F2 and F3 generations at 500 mg/kg. According to EPA OPPTS 837.38000, 1998 GLP compliant study	Tyl *et al*., 2002[1]
Mice CD-1 N=28 animals /dose	Exposure from 8 weeks before mating until PND21 (2 generations)	0.003, 0.03, 0.3, 5, 50 or 600 mg/kg/d Oral	- No effect on the absolute age at puberty at any dose (evaluated by the age at vaginal patency) at any dose. - Vaginal patency was accelerated when adjusted for the PND21 body weight at 600mg/kg Follow OECD guideline 416 (two generation reproduction toxicity study), TG 416 enhanced GLP compliant study -	Tyl *et al*., 2008

[1] This is the same study as Tyl 2000 named in the EU RAR from UK.

- Effects on the estrous cycle

Altered patterns of estrous cycle in the female offspring of dams exposed during gestation and/or lactation have been reported in several studies. In most cases, BPA treatment induces significantly longer estrous cycle. It has been demonstrated that not only extended periods of estrus were seen, but also extended period of diestrus (Rubin *et al*., 2001; Mendoza-Rodriguez *et al*., 2011; Nikaido *et al*., 2004; Honma *et al*., 2002; Tyl *et al*., 2008). Another study revealed also premature anestrus (Adewale *et al*., 2009).
Similarly, Mendoza-Rodriguez *et al*., observed in 79.2% of female rats exposed perinatally from GD6 through PND21 to 1.2 mg/kg bw/d of BPA irregular estrous cycles characterized by predominant persistent estrus or persistent diestrus (Mendoza-Rodriguez et al., 2011). Mendoza-Rodríguez *et al.* showed that oral exposure of female Wistar rats to BPA during gestation and lactation induces in the 4 months old female offsprings a modification of the estrous cyclicity and a down regulation of the protein Estrogen Receptor α (ERα) in uterin cells on estrus day were also described by immunohistochemistry (Mendoza-Rodríguez *et al.*, 2011).

Estrous cycle alteration characterized by an elongated cycle length of 3 days in average ($p < 0.01$) due to prolonged diestrus phase ($p < 0.01$) and reduced age at puberty was also reported by Nikaido *et al*. on CD-1 mice exposed *in utero* for four days from GD15 to GD 19 to 0.5 or 10 mg/kg/day of BPA via subcutaneous injections in pregnant dams (Nikaido *et al*., 2004).

In contrast, 2 studies failed to revealed significant differences in patterns of estrous cycle after perinatal exposure to high levels of BPA (Kwon *et al*., 2000), or after a prepubertal exposure (Nikaido *et al*., 2005). Kwon *et al*. hypothesize that this lack of effect may be due to insensitivity of Sprague-Dawley rats to endocrine-mediated toxicity. This is confirmed by the Tyl *et al.* study (Tyl *et al*., 2002). Similarly, ICR mice have been described as being insensitive to estrogen (Nagao *et al*., 2002). Therefore, the effect of BPA on estrous cycle of females form strains sensitive to estrogen is highly reproducible.

Table 8: Summary table of the BPA effects on the estrous cycle in female animals

Species	Exposure period	Dose route	Observations	Reference
Gestational exposure				
Mouse CD-1 12 females /dose	GD15 – GD19	0.5, 10 mg/kg bw/d Subcutaneous	↗ the length of the estrous cycle of 3 days by prolonging diestrus ($p < 0.01$). No data on the GLP/OECD guideline compliance	Nikaido *et al.*,2004
Mouse ICR Jcl 10 females / dose.	GD11- GD17	2, 20 µg/kg bw/d Subcutaneous	↗ the length of the estrous cycle of 1 day at both dose ($p \leq 0.05$). No data on the GLP/OECD guideline compliance	Honma *et al*., 2002

Perinatal exposure				
Rat Wistar 5 females/dose	GD6 – PND21	1.2 mg/kg bw/d Oral in drinking water	79.2% of female offspring had irregular cycles characterized by predominant persistent estrus and few persistent diestrus. No data on the GLP/OECD guideline compliance	Mendoza-Rodriguez *et al*., 2011
Rat Sprague Dawley 6 females / dose.	GD6 – end of lactation period	0.1, 1.2 mg/kg bw/d Oral: in drinking water	<u>At 1.2 mg/kg:</u> 79% of females at 4 months of age ($p < 0.0001$) and 77% of females at 6 months of age ($p < 0.005$) had irregular cycles characterized by extended diestrus, proestrus or estrus periods. No data on the GLP/OECD guideline compliance	Rubin *et al*., 2001
Rat Sprague Dawley 8 pregnant females / dose	GD11 – PND20	3.2, 32, 320 mg/kg bw/d Oral gavage	No effect on estrous cycle in 4-month old female at any dose. No data on the GLP/OECD guideline compliance	Kwon *et al*., 2000
Postnatal exposure				
Rat Long-Evans 10-12 females / group.	PND1- PND3	50µg/kg bw/d 50 mg/kg bw/d Subcutaneous	<u>At 50µg/kg:</u> 14% of females were not cycling anymore by 15 weeks after vaginal opening. <u>At 50mg/kg:</u> 67% of females were not cycling anymore by 15 weeks after vaginal opening. No data on the GLP/OECD guideline compliance	Adewale *et al*., 2009
Rat Sprague Dawley 11-15 females /group	PND1- PND10	Ranging from 2.5 to 62.5 mg/kg bw/d Subcutaneous	<u>At dose ranging from 25 to 62.5 mg/kg:</u> Irregular estrous cycle with extended estrus period by 72% ($p < 0.05$). No data on the GLP/OECD guideline compliance	Fernandez et *al*., 2009
Prebubetal exposure				
Mice ICR No data on the nb of animals/dose	4 days from 15 to 18 days of age	10 mg/kgbw/d Subcutaneous	No effect on estrous cycle. No data on the GLP/OECD guideline compliance	Nikaido *et al*., 2005
Muligenerational exposure				
Rat Sprague-Dawley 30 males/dose 30 females /dose	Exposure from 10 weeks before mating until PND21 (3 generations)	0.001, 0.02, 0.3, 5, 50 or 500 mg/kg/d Oral	No effect on estrous cycle lenght. According to EPA OPPTS 837.38000, 1998 GLP compliant study	Tyl *et al*., 2002
Mice CD-1 N=28 animals/dose	Exposure from 8 weeks before mating until PND21 (2 generations)	0.003, 0.03, 0.3, 5, 50 or 600 mg/kg/d Oral	F0 treated females were twice more in estrus as compared to controls at 600 mg/kg Follow OECD guideline 416 (two generation reproduction toxicity study), TG 416 enhanced GLP compliant study	Tyl *et al*., 2008

- Effects on the hyphotalamic-hypophyseal axis

Awakening of the gonadotropic system at puberty and its proper function later in life, critically depends on the adequate functional organization of the hypothalamic GnRH (Gonadotropin Releasing Hormone) network at early stages of development. Early BPA exposure during the period of brain sexual differentiation may exert indirect effects on reproductive tract tissue by altering the function of the hypothalamic-pituitary-gonadal axis, an effect that would become apparent after puberty, long after exposure to BPA have ceased (Navarro *et al.*, 2009) .

➔ In rodents

In rodents the period from late embryonic to early postnatal age, defines the critical window of brain sex differentiation. Exposure to BPA during this period causes modifications in the pattern of the hypothalamic-pituitary hormones secretion. These modifications may be responsible for long term disturbance on the reproductive function. (Fernandez *et al*., 2010; Rubin *et al*., 2001; Navarro *et al*., 2009; Patisaul *et al.*, 2009 ; Brannick *et al*., 2012).

Recently, Brannick *et al.* showed that prenatal exposure to BPA increases pituitary gonadotroph development in females: pregnant mice were administered 0.5 µg/kg/day BPA, 50 µg/kg/day BPA, or vehicle beginning on GD 10.5 trough 18.5. At parturition, pituitaries from female offspring exposed in utero to either dose of BPA had increased proliferation, as assessed by mKi67 mRNA levels and immunohistochemistry. Coincidently, gonadotroph number also increased in both treated females LHβ ($p=0.029$, $p=0.049$) and FSHβ ($p=0.020$, $p=0.002$) immunoreactive cells as the average percent of positive cells /to total cells in the anterior pituitary compared to vehicle controls. However, they observed a dichotomy between mRNA levels of Lhb and Fshb (gene encoding for the beta subunit of their respective hormone LHand FSH). Female mice exposed to 0.5µg/kg/day BPA had increased mRNA levels of gonadotropins and the GnRH receptor (Gnrhr), which mediates GnRH regulation of gonadotropin production and release. In contrast, mice treated with 50µg/kg/day BPA had decreased gonadotropin mRNA levels, Gnrhr and Nr5a1, a transcription factor required for gonadotroph differentiation. No other pituitary hormones were altered on the day of birth in response to in utero BPA exposure and male pituitaries showed no change in the parameters tested. These data show that it is difficult to describe precise mode of action of BPA, which effect is implicated in multiple pathways regulated by numerous key players.

As presented before, Rubin *et al*. exposed Sprague Dawley dams via drinking water from day 6 of gestation through the period of lactation to 0.1 or 1.2 mg BPA/kg bw/day. Offspring perinatally exposed to the high dose of BPA exhibited altered patterns of estrous cycle and decreased levels of plasma luteinizing hormone (LH) of 19% ($p < 0.001$) after ovariectomization in adulthood (Rubin *et al*., 2001).

As presented before, Fernandez *et al*. reported that female Sprague Dawley rats exposed via subcutaneous injections for 10 days after birth to doses ranging from 2.5 to 62.5 mg BPA/kg/day, accelerated GnRH pulse frequency in hypothalamic explants associated with increased serum testosterone and estradiol levels by approximately 30% ($p < 0.05$), reduced progesterone levels ($p < 0.05$) and a decrease of the fertility in adulthood (Fernandez *et al*., 2010).

In contrast, Adewale *et al*., suggest that BPA disrupts ovarian development but not the ability of GnRH neurons to respond to steroid-positive feedback which potentiates the surge in GnRH and, subsequently, luteinizing hormone (LH) that precedes ovulation. Indeed, FOS protein induction in hypothalamic gonadotropic (GnRH) neurons (an indicator of GnRH activation) after hormone priming was not significantly reduced in the BPA-treated groups whereas it was decreased in the positive control group (estradiol benzoate). In this study, females Long Evans rats were neonatally exposed from PND1 to 3 by subcutaneous injections of BPA at 50µg/kg or 50mg/kg. This study, however, assumes the same mode of action for estradiol benzoate and BPA and shows the complexity of hormonal homeostasis in the scope of sexual differenciation (Adewale *et al.*, 2009 described pg 21).

Mechanistic data

Several authors studied deeply the mechanism by which BPA exposure impairs the pubertal maturation and the gonadotropic function, and in particular the implication of Kisspeptins (KISS) a family of neuropeptides encoded by the KiSS-1 gene. Growing evidence suggests that kisspeptin neurons play a significant role in the regulation of pubertal onset, ovulation and the release of pre-ovulatory GnRH. In rodents, KISS neurons reside in both the anterior ventral periventricular (AVPV) and the arcuate (ARC) nuclei. These two populations are thought to have distinctly different functional roles: the AVPV population is thought to be critical for the regulation of steroid positive feedback and the initiation of the pre-ovulatory surge of GnRH in females, while the ARC population is thought to be important for steroid negative feedback in both sexes.

In Wistar rats, subcutaneous injections of boluses of BPA at 100 and 500µg/rat from day 1 through day 5 after birth was shown to diminish significantly by approximately 64% ($p < 0.01$) the relative expression levels of KiSS-1 mRNA in the hypothalamus of pre-pubertal male and females animals of 30 days of age. These effects persist into adulthood in males of 75 days of age (Navarro *et al*., 2009).

Similarly, Patisaul *et al*. studied in adult female Long Evans rats the effects of neonatal subcutaneous exposure to BPA at 50 µg/kg bw or 50 mg/kg bw on KISS immunoreactivity (-ir) in AVPV and the ARC using estradiol benzoate (EB) and an ERα selective agonist, the propyl-pyrazoletriol (PPT) as positive controls. ARC KISS-ir levels following ovariectomy and hormone priming, were significantly diminished in the high dose of BPA and in the EB groups ($p < 0.03$)

whereas, AVPV KISS-ir was significantly lower in the EB and PPT groups but not in the BPA groups (Patisaul *et al.*, 2009).

These studies show that influences of xenobiotics are as wide as they are different and highlight the fact that BPA can exert a neuroendocrine effect on the hypothalamo-pituitary axis. However, subtle effect of BPA on the hypothalamus is difficult to demonstrate and interpret as it is interspersed in complex feedback loop.

➔ In sheeps

Sheep is a good model for examining this critical issue related to reproduction because their ovarian cycle, steroid, and GnRH secretion patterns are very similar to that of women and because the distribution of ERα and ERβ, the activation of GnRH neurons relative to the estradiol-induced LH surge and the critical period of sexual differentiation in sheep have been well characterized.

Prenatal treatment of Suffolk ewes with subcutaneous injections of BPA (5 mg/kg/d GD 30 to 90) caused an increase in LH concentration by 2-fold ($p < 0.05$) and prolonged the first breeding season of 1 month ($p < 0.05$) which is suggestive of a reduced sensitivity to estradiol negative feedback, it also reduces the magnitude of the LH surge after estrous cycle synchronization with prostaglandine F2α (Savabieasfahani et al., 2006). Similarly, long term exposure during the prepubertal period demonstrate that BPA can suppress LH secretion in ovariectomized sheep and can thus exert negative feedback effects on gonadotropin secretion (Evans *et al*., 2004). The same treatment was administrated in female sheep of 4-5 months of age for 8 weeks. The mean LH pulse frequency and basal concentrations, but not the amplitude, were slightly decreased from the sixth week of treatment. (Collet *et al.*, 2010).

Collet *et al*. also investigate the impact of acute BPA exposure by treating prebubertal female sheeps for 54h hours with IV infusion at 0.5, 1, 2.5, 5, 10, 20, 40 and 80 mg/kg bw/d. The inhibitory effect of BPA appeared to follow a dual mechanism of action. At the highest doses, i.e, 40 mg/kg bw and upward, BPA triggered an immediate (within 1 hour) inhibition of LH secretion suggesting a non-genomic pathway at the pituitary level. By contrast, at lower levels, BPA still inhibited the LH pulse frequency but only after a 48-h period of latency, a delay consistent with a genomic effect. Similar qualitative events were observed with the 17-β estradiol used as positive control but for lower plasma concentrations: the lowest plasma concentration of estradiol associated with inhibition of pulsatile secretion of LH is 2 pg/mL versus 38 ng/mL for BPA (Collet *et al.*, 2010).

Table 9: Summary table of the BPA effects on the hyphotalamic-hypophyseal axis in female animals

Species	Exposure period	Dose Route	Observations	References
Gestational exposure				

Sheep N = 10 pregnant females	GD30 – GD90	5 mg/kg/d Subcutaneous	- ↗ in mean LH concentration at 1 month of age ($p < 0.05$) →reduced sensitivity to estradiol negative feedback - Prolonged first breeding season. of 1 month ($p < 0.05$). - ↘ of the LH pulse amplitude after estrus synchronization with $PGF_{2\alpha}$ at 10 months of age - No data on GLP/OECD guideline compliance	Savabieasfahani *et al.*, 2006
Sheep No data on the nb of animals /dose	GD30 – GD90	5 mg/kg/d Subcutaneous	<u>Observation in adult offspring (21 months) after estrus synchronization with $PGF_{2\alpha}$</u> - ↘ in GnRH and ERβ mRNA expression in the medial preoptic area ($p < 0.05$ and $p < 0.001$) - ↗ in ERα expression in the medial preoptic area ($p < 0.05$) - No data on GLP/OECD guideline compliance	Mahoney et Padmanabhan, 2010
Perinatal exposure				
Rat Sprague Dawley 6 females/ dose	GD6 – end of lactation period	0.1 or 1.2 mg/kg/d via drinking water	<u>Observation in ovariectomized females 3 months after the ovariectomization:</u> - ↘ in plasma LH level of ca 19% at 1.2 mg/kg ($p < 0.001$) - No data on the GLP/OECD guideline compliance	Rubin *et al.*, 2001
Post natal exposure				
Rat Long Evans 10-12 females / group.	PND1-PND3	50µg/kg/d or 50mg/kg/d Subcutaneous	<u>Observations in adult female ovariectomized on PND148 and after hormone replacement:</u> - No effect on FOS-induction in hypothalamic gonadotropic neurons - No data on the GLP/OECD guideline compliance	Adewale *et al.*, 2009
Rat Long-Evans N= 8-11 females / dose 6-11 males /dose	PND0-PND3	50µg/kg/d or 50 mg/kg/d Subcutaneous	<u>Observations in adult female ovariectomized on PND148 and after hormone replacement:</u> - ↘ of the KISS-ir levels in the ARC at 50 mg/kg ($p < 0.03$) - No effects on the KISS-ir in the AVPV at any dose - No data on the GLP/OECD guideline compliance	Patisaul *et al.*, 2009
Rat Wistar 10 animals/ sex / dose	PND1 – PND5	100 – 500 µg/rat Subcutaneous	<u>Observations in prepubertal females of 30d of age:</u> - ↘of ca 64% the relative expression levels of KiSS-1 mRNA in the hypothalamus at both doses ($p < 0.01$) - No data on GLP/OECD guideline compliance	Navarro *et al.*, 2009
Rat Sprague Dawley No data on the nb of animals/ dose	PND0 – PND10	2.5 to 62.5 mg/kg/d Subcutaneous	<u>Observations in hypothalamic explants from females at 4-5 months of age:</u> - ↗ in GnRH pulse frequency - No data on GLP/OECD guideline compliance	Fernandez *et al.*, 2010
Sheep Poll Dorset 6 females / group.	7 weeks from the 4^{th} week of life	3.5 mg/kg biweekly Intramuscular	<u>Observations in ovariectomized sheep during the 7^{th} week of treatment:</u> - ↘ of the mean basal LH concentration ($p < 0.05$) and of the LH pulse amplitude and frequency($p < 0.005$) → negative feedback effects of BPA on	Evans *et al.*, 2004

			gonadotropin secretion. - No data on GLP/OECD guideline compliance	
Prepubertal exposure				
Sheep Lacaune 3 females/ dose	8 weeks from the 5th month of life	3.5 mg/kg biweekly Intramuscular	<u>Observations during the treatment:</u> - ↘ of the mean LH pulse frequency and basal concentrations, but not the amplitude after 6 weeks of treatment ($p < 0.05$) - No effect after 1 and 5 weeks of treatment - No data on GLP/OECD guideline compliance	Collet *et al.*, 2010
Sheep Lacaune 5 females/ dose	54h from the 3th or 4th month of life	0.5, 1, 2.5, 5, 10, 20, 40 or 80 mg/kg bw/d IV infusion	<u>Observations during the infusion:</u> - Abolition of the LH pulse at 40 and 80 mg/kg immediately after the beginning of the infusion. - ↘ of the LH pulse frequency only after a 48-h period of latency at 20, 5 and 2.5 mg/kg - No effect at 0.5 and 1 mg/kg - No data on GLP/OECD guideline compliance	

o <u>Effets on the female reproductive capacities</u>

These multiple effects mentioned above after an exposure to BPA during early development might be expected to compromise overall reproductive success. Several authors tested the hypothesis that these BPA-associated alterations may contribute to diminish the reproductive capacity of females.

Adult female Wistar rats treated with subcutaneous injections of BPA (20 or 0.05 mg/kg bw/d) while newborn (PND 1, 3, 5 and 7) displayed a significantly lower (- 25%; $p < 0.05$) number of implantation sites (day 18 of pregnancy) compared to controls. In addition, a tendency to have a higher number of resorptions, an indicator of post-implantation loss, was also observed (Varayoud *et al*., 2011).

Fernandez *et al*., investigated whether neonatal exposure to subcutaneous injections of BPA impairs ovulation and/or fertility. At dose ranging from 2.5 to 6.2 mg/kg, animals delivered significantly fewer pups, (3 pups in less as compared to the control, $p < 0.05$), indicating subfertility and at the highest doses (25 to 62.5 mg/kg) animals were infertile (Fernandez *et al.*, 2010).

In contrast to Cabaton *et al.* study's results, females Long Evans rats whom mother were orally exposed to doses of BPA ranging from 2 to 200 µg/kg bw/day during pregnancy from GD7 through PND18, did not show any alteration in age at puberty, sexual behavior or in fertility and fecundity in a 4 months breeding protocol. However, the authors emphasized that the sample sizes were very small and thus the result are not conclusive (Ryan *et al*., 2010).

In the two studies by Tyl (Tyl *et al*., 2002 & 2008), described in the paragraph 4.11.1.1.3, no effect of BPA on fecundity was shown exept at the highest dose in the 3rd generation study (Tyl *et al*.,

2002). Indeed, at 7500ppm (500 mg/kg/d), reduced total and live litter sizes were observed on PND0 for F1, F2 and F3. This might be due to preimplantation loss and/ or fewer eggs ovulated at this dose. The relatively marginal data obtained from the 2 key multigeneration studies (Tyl et al. 2002 et ema et al., 2001) could be explained by the low sensitivity of SD rats to estrogenic coumpounds. (Kwon et al., 2000).

Berger *et al.* investigated the impact of BPA exposure during the first 5 days of gestation, timing that coincides with the period of implantationupon pregnancy in CF-1 mice. High subcutaneous injection (3,375 mg/day and 10,125 mg/day) resulted in a significant decrease in the average number of pups ($p < 0.05$ and $p < 0.01$, respectively) and there was a significant reduction by approximately 70% ($p < 0.001$) in the number of females that gave birth at 10,125 mg/animal/day only. Then they treated mice via oral route with diet contaminated with either 3 or 6% of BPA. None of the animals treated with the 6% contaminated food (corresponding to a dose level of 68 mg BPA/animal/day) was parturient although in controls animals, 11 of 12 were parturient ($p < 0.001$) (Berger *et al*., 2007). In 2008, Berger assessed the impact of acute and repeated subcutaneous administration of BPA (6,75 and 10,125 mg/animal/day (approximately 200 mg/kg/d and 300 mg/kg/d, respectively)) upon intrauterine implantation of fertilized ova (GD1-4) and urinary levels of 17β-estradiol and progesterone in inseminated female mice. At 6.25 mg no animals showed any implantation sites, while only one animal did at the 10.125 mg dose ($p < 0.0001$). This disruption in implantation coincided with a decrease in urinary progesterone levels seen from day 2 to 5 of pregnancy at 10.125 mg ($p < 0.0005$). A single injection of 10,125 mg/animal when given on day 0 or 1, and a single injection of 6.75 mg/animal on day 0 were sufficient to disrupt implantation, indicating effects of just one exposure. However, lower environmentally relevant doses did not affect pregnancy, implantation, or hormonal output. These results indicate that previously observed reductions in litter size in response to BPA may be mediated by a disruption of intrauterine blastocyst implantation rather than post-implantation effects (Berger *et al*., 2008). These results were confirmed in a third study (Berger *et al.*, 2010)

Al Hiyasat *et al*. demonstrated in female Swiss mice that BPA exposure during adulthood induced no effect on the number of pregnancies, implantations or in the number of viable fetuses but resulted in a significant increase ($p < 0.01$ at 25 µg/kg and $p < 0.05$ at 100µg/kg) in the total number of resorptions out of the total number of implantations.

The differences observed between these studies are difficult to explain based on strain sensitivity to estradiol as Sprague Dawley rats are found responders (Fernandez *et al.*, 2010; Tyl *et al;*, 2002) whereas results on mice CD-1 are contradictory depending on the studies (Cabaton *et al;*, 2010; Tyl *et al;*, 2008). It is not surprinsing that fecundity is the result of complex mechanism that are difficult to modulate experimentally.

Table 10: Summary table of the BPA effects on the fertility in female animals

Species	Exposure period	Dose Route	Observations	Reference
Gestational exposure				
Mouse ICR Jcl 10 females/dose	GD11- GD17	2, 20 µg/kg bw/d Subcutaneous	- No effect on the total number of pups per mother and on sex ratio - No data on the GLP/OECD guideline compliance	Honma *et al.*, 2002
Perinatal exposure				
Rat Long Evans 13 - 29 females / dose in block 1 6 - 14 females / dose in block 2	GD7 – PND18	2 to 200 µg/kg bw/d Oral gavage	- No effect on the total number of pups per mother - No data on the GLP/OECD guideline compliance	Ryan *et al.*, 2010
Mouse CD-1 18-20 females /dose	GD8 – PND16	0.025, 0.25, 25 µg/kg bw/d Subcutaneous implant	- Alteration in fertility: ↘ the number of pregnancies at 25µg/kg ($p = 0.024$) - Alteration in fecundity: ↘ of the number of pups born per litter at 0.25 and 25µg/kg - No data on the GLP/OECD guideline compliance	Cabaton *et al.*, 2010
Postnatal exposure				
Rat Wistar Treatment: 28-33 female pups per dose. Prebubertal study: 20-25 females per dose. Adult study: 8 females per dose.	Subcutaneous	0.05 or 20 mg/kg/d PND1, 3, 5 and 7	<u>Observations in prebubertal females (on PND8)</u> ↘ in Hoxa10 and Hoxa11 mRNA uterine expression at both doses ($p < 0.001$) <u>Observations in adult ovariectomized females (90-d old)</u> ↘ in stromal cell proliferation at both doses ($p < 0.001$) ↘ in ERα expression at 0.05 mg/kg ($p < 0.05$) ↘ in Hoxa10 mRNA uterine expression at both doses ($p < 0.001$) ↘ in Hoxa11 mRNA uterin expression at 0.05 mg/kg ($p < 0.05$) No effect on the methylation pattern of Hoxa10 promoter No data on GLP/OECD guideline compliance	Varayoud *et al.*, 2008
Rat Wistar BPA at 0.05mg/kg: n=17 BPA at 20	Subcutaneous	0.05 or 20 mg/kg/d PND1, 3, 5 and 7	<u>Observations in adult females (80-d old)</u> ↘ in the number of implantation sites on day 18 of pregnancy at 20 mg/kg ($p < 0.05$) ↗ of the number of resorptions site on day 18 of pregnancy at both doses	Varayoud *et al.*, 2011

mg/kg: n=20			↘ in the ERα and PR mRNA levels on day 5 of pregnancy at both doses ($p < 0.05$) ↘ in Hoxa10 uterine expression on day 5 of pregnancy at both doses ($p < 0.05$) No effect on the number of Corpora Lutea and on E2 and P serum levels No data on GLP/OECD guideline compliance	
Rat Sprague Dawley No data on the nb of animals/ dose	PND1- PND10	Ranging from 0.25 to 62.5 mg/kg bw/d Subcutaneous	- Infertility at doses ranging from 25 to 62.5 mg - Subfertility at doses ranging from 2.5 to 6.2 mg - No effect at doses ranging from 0.25 to 0.62 mg - No data on GLP/OECD guideline compliance	Fernandez *et al*., 2010
Multigenerationnal exposure				
Rat **IGS (SD) rats** 25 rats /sex /group administered	2 generation study similar to OECD 416 (Deviations: *Female treated for 2 weeks only before mating. *Low doses used)	0, 0.2, 2, 20 and 200 µg/kg/day Oral by gavage	No effect on behaviour (i.e. performance in learning tests) , oestrus cycle, fertility index and the number of implantations in F0 and F1 females were not affected by treatment with BPA. Absolute AGD decreased but no more relevant when correlated with BW (decreased). - Overall no effect.	Ema et al., 2001
Rat Sprague-Dawley 30 males/dose, 30 females/dose	Exposure from 10 weeks before mating until PND21 (3 generations)	0.001, 0.02, 0.3, 5, 50 or 500 mg/kg/d Oral	- Reduced number of total and live pups per litter at birth at 7500ppm for F1, F2 and F3. According to EPA OPPTS 837.38000, 1998 GLP compliant study -	Tyl *et al*., 2002
Mice CD-1 N=28 animal/dose	Exposure from 8 weeks before mating until PND21 (2 generations)	0.003, 0.03, 0.3, 5, 50 or 600 mg/kg/d Oral	- No effect on fertility, litter size Follow OECD guideline 416 (two generation reproduction toxicity study), TG 416 enhanced GLP compliant study -	Tyl *et al*., 2008
Mice CD-1 (n= 20/ treated group/ sex, n= 40/ control group/ sex)	Continuous breeding study (Task 1, dose-finding; Task 2, continuous breeding phase; Task 3, identification of the affected sex, and Task 4, offspring assessment.)	0, 0.25, 0.5 or 1.0% (daily intakes BPA estimated 0, 300 or 325, 600 or 650 and 1,200 or 1300 mg/kg for males or females resp. In diet	Adverse effect on fertility : statistically significant ↘/ ctrl in the number of litters produced / pair (4.5 and 4.7 compared to 5.0 for ctrls), litter size (6.5 and 9.8 compared to 12.2 for controls) and the number of live pups per litter (6.3 and 9.7 compared to 12.1 for controls) in the high and mid-dose group. The litter size reductions occurred across all matings and =f(dose-related). No effects on fertility were observed in the low-dose group. A statistically significant ↘ in litter size (controls:	NTP, 1985b

			11.4, treated males: 9.1, treated females: 5.9) and number of live pups per litter (controls: 11.3, treated males: 8.4, treated females: 5.5) were observed in the cross-over mating. In the continuous breeding phase, a statistically significant ↘ in live pup weight (6%) on postnatal day 0 was observed in females at the top dose after adjustment for litter size, including live and still births. In the continuous breeding phase a small but statistically significant ↘ in body weight gain (4%) was only observed in treated females at study termination. No effect on the sex ratio in the F1 generation. Possibility that there may be potential effects on pups due to exposure to BPA via the milk. In the F1 generation, BPA treatment had no effect on the fertility index, litter size, number of live pups per litter, sex ratio or mean pup weights at birth.	

Conclusion on female reproductive system in animals:

CCl RAR UK 2003

The effects of bisphenol-A on fertility and reproductive performance have been investigated in three good quality studies: two generation and multigeneration studies in the rat, and a continuous breeding study in the mouse. Although no effect on fertility was seen in the rat twogeneration study, low-dose levels were employed (0.2-200 μg/kg/day). In the multigeneration study, an effect on fertility (reduction in litter size) was seen in all three generations at the top dose of 500 mg/kg. Although this effect was seen only at a dose level causing parental toxicity (a reduction in body weight gain (>13%) in both sexes and renal tubule degeneration in females only), it is not clear whether or not the finding could be a secondary consequence of parental toxicity, or a direct effect of bisphenol-A. In the light of this uncertainty, and given that an adverse effect on fertility has been seen in the mouse, it is prudent to assume that bisphenol-A may be having a direct effect on fertility in this study. No effects on fertility were seen at 50 mg/kg. The continuous breeding study in the mouse provides some evidence that bisphenol-A can cause adverse effects on fertility. In the F0 generation, no effects on fertility were seen at 300 mg/kg/day, but at dose levels of approximately 600 mg/kg/day and above, reductions in the numbers of litters produced, litter size and numbers of live pups per litter were observed in each of the 4-5 litters produced. These effects were observed in the absence of significant parental toxicity. In contrast, no adverse effects on fertility were observed in the single litter tested at each dose level from the F1 generation. A statistically significant and dose-related decrease in epididymal weight was seen at all doses in the F1 generation. However, the significance of this finding is uncertain given that there was no effect on fertility in this generation,

and where an adverse effect on fertility was seen (in the F0 generation), there was no effect on epididymal weight. In spite of the uncertainty, the epididymis is associated with sperm transport and storage, and any reduction in the weight of this organ would be of concern.

Conclusion CLH report

In animals various effects have been observed on female reproductive tract morphology and function and on fertility. Most of the studies were performed in rodents, and sometimes in sheeps, during a prenatal, neonatal, or postnatal period. A very few were realized in order to assess the impact of BPA exposure in adulthood.

Many ovarian and uterine malformations following an *in utero* and/or postnatal exposure to BPA were described. Concerning the ovarian abnormalities, the increase occurrence of ovarian cysts was found in all studies investigating the influence of BPA on ovarian morphology and can thus be considered as a proven effect (Fernandez *et al*., 2010; Adewale *et al.*, 2009; Newbold *et al*., 2009; Newbold *et al*., 2007; Signorile *et al*., 2010). More over, in some studies this increase in ovarian cyst was very important. As example, 67% of females treated *in utero* with 1 µg/kg bw/d showed cysts (Newbold *et al*., 2009). More severe lesions were also found in ovaries including progressive proliferative lesions of the oviduct but this effect was seen in only 2 studies (Newbold *et al*., 2007; Newbold *et al*., 2009). Concerning the oocytes development, meiotic abnormalities leading to aneuploidy were demonstrated in three studies with different exposure period (prenatal, postnatal or prepubertal exposure) (Hunt *et al.*, 2003; Susiarjo *et al*., 2007; Rodriguez *et al*., 2010). However, too few studies on this subject are available and it is thus difficult to draw conclusions.

BPA also induces changes in the uterus morphology in several studies. Benign lesion like endometrial hyperplasia, or atypical hyperplasia which is a precursor lesion to adenocarcinoma were revealed in three studies following *in utero* exposure (Newbold *et al*., 2009), perinatal exposure (Signorile *et al*., 2010) or postnatal exposure (Newbold *et al*., 2007). Malignant invasions (squamous metaplasia or polyps) were also described in the two studies performed by Newbold (Newbold *et al*., 2007; Newbold *et al*., 2009). Although these effects were significant, it is again difficult to conclude due to the lack of studies.

Concerning the age at puberty, a large discrepancy exists between the studies. A significant advanced age at puberty was reported for a broad range of dose (2µg/kg to 100mg/kg) following an *in utero* (Howdeshell *et al*., 1999; Honma *et al*., 2002; Nikaido *et al*., 2004) or an early postanal exposure period (Adewale *et al*., 2009; Fernandez *et al*., 2009; Nah *et al*., 2011). In contrast, and without being able to explain, oral treatment during exposure period encompassing the second half of gestation and the lactation period do not reveal any effect of BPA on the puberty timing (Yoshida *et al*., 2004; Rubin *et al*., 2001; Ryan *et al*., 2010; Kwon *et al*., 2000). The delayed puberty observed in the high dose group of the multigeneration study was proposed to be due to reduced body weight (Tyl et al., 2002).

Consistent results were reported in 6 studies for an adverse effect of BPA on the estrous cycle, including irregular and prolonged cycles, following either prenatal (Nikaido *et al*., 2004; Honma *et al*., 2002), perinatal (Mendoza-Rodriguez *et al*., 2011; Rubin *et al*., 200) or postnatal exposure (Adewale *et al*., 2009; Fernandez *et al*., 2009). 2 studies, however, failed to reveal an effect of BPA on the estrous cycle (Kwon *et al*., 2000 and Nikaido *et al*., 2005in species shown to be insensitive to estrogen-mediated toxicity. Concerning the study by Nikaido, performed just before the puberty, the window of exposure different from the others studies could explain the absence of effect.

All the studies investigating effect on the hypothalamic-hypophyseal axis on rodents and on sheeps show that BPA can influence the pattern of GnRH or LH secretion but a subtle effect of BPA is difficult to demonstrate as it is interspersed in the feedback loop and it depends on both the exposure and observation periods. However, a decrease in LH concentration and in LH pulse amplitude, suggesting a negative feedback exerts by BPA, was found in several studies in sheeps and in rats after a gestational, perinatal postnatal and prepubertal exposure (Savabieasfahani *et al*., 2006; Rubin *et al*., 2001; Evans *et al*., 2004; Colet *et al*., 2010) and can thus be considered as a proven effect.

A decline in reproductive capacity, ie a decrease in the number of pregnancies and a decrease in the number of pups born, was observed when exposure occurs *in utero* and during the lactation period (Cabaton *et al*., 2010) or during the first days of life only (Fernandez *et al*., 2010). By contrast, in the study performed by Honma, *in utero* exposure to BPA didn't affect the female reproductive ability assessed by the total number of pups born (Honma *et al*., 2002). However, the observations in this study were limited to the first pregnancy of the offspring exposed during gestation and both Honma and Cabaton highlighted the importance to include repetitive breeding protocol and to not limit the observations to the first pregnancy in order to observe an effect on fertility and/or fecundity. Another study, Ryan *et al*., 2010, failed to demonstrate an effect of BPA on the fecundity. However, the authors emphasized that the sample sizes were very small leading to results not very conclusive. Finally, Tyl *et al*. did demonstrate this effect in all 3 generations tested but only at high doses (Tyl *et al*., 2002 see below).

When exposure occurs during adulthood, BPA induces an increase in total number of resorption after intragastrically administration (Al Hiyasat *et al*., 2004) or induces a decrease of the number of pregnancies and implantations following subcutenaous injections (Berger et al., 2007, 2008 and 2010). This effect was also found after a postnatal exposure (Bosquiazzo *et al*., 2010 and Varayoud *et al*., 2011) and can thus be considered as proven for these two windows of exposure. In the 3-generation study performed by Tyl, although the percent of post-implantation loss was unaffected, the number of implants, total pups, and live pups per litter were significantly reduced at 7500 ppm for all generations but not in the 2nd study up to 3500ppm (Tyl *et al*., 2008). The authors do not explain this difference. The decrease observed is probably due to pre-implantation loss that could not be evaluated in this study, by the time the parental females were scarified. However, as demonstrated in the studies performed by Berger *et al*., decrase in litter size seems to be mediated

by a disruption of intrauterine blastocyst implantation rather than by a post-implantation effect. Therefore it can't be excluded that the decrease in implants and the reduced live litter size at birth observed in this study may result from a pre-implantation disruption.

Table 11: Summary table of the BPA effects on the female reproductive tract in animals

Species	Routes	Dose Exposure Period	Effects	References
Gestational exposure				
Rat Sprague-Dawley N= 6 female offspring per dose.	Oral (gavage)	0.1 or 50 mg/kg/d GD6- GD21	<u>Observations in F1 offsprings at 4 months of age</u> ↘ the thickness of the total uterine epithelium at 50 mg/kg ($p < 0.05$) ↗ ERα expression at 50 mg/kg ↘ ERβ expression at both doses No data on GLP/OECD guideline compliance	Schönfelder *et al*., 2004
Mouse CD-1 n = 5 females/ dose	Subcutaneous	0.1 - 1 - 10 - 100 and 1000 µg /kg bw/d GD9 - GD16	<u>Observations in F1 offsprings of 16 or 18-months of age</u> Ovarian modifications: - ↗ cysts at 1µg/kg, - ↗ neoplasic lesions - ↗progressive proliferative lesion in the oviduct Uterine modifications : - ↗cystic endometrial hyperplasia at 1, 10, 100 and 1000 µg/kg - ↗ adenomatous hyperplasia at 1 and 100 µg/kg - ↗ atypical hyperplasia at 0.1, 1 and 1000 µg/kg - ↗ endometrial polyps at 0.1, 1 and 10 µg/kg Mammary mass were seen at 1 and 1000 µg/kg No data on GLP/OECD guideline compliance	Newbold *et al.*, 2009
Mouce CD-1 At least three pregnant females received each dose of BPA.	Intra-peritoneally	0.5, 1, 5, 50 or 200 mg/kg/d GD9 – GD16	<u>Observations in females of 2- or 6-weeks of age</u> ↗ in uterine stromal Hoxa 10 expression at 0.5, 1 and 5 mg/kg ($p < 0.01$) No data on GLP/OECD guideline compliance	Smith and Taylor, 2007
Mouse CF-1 21 females /dose.	Oral	2.4 µg/kg/d (n = 21) GD11 – GD17	↗ in body weight on PND22 ↘ of the number of days between VO and first estrus in females that was positioned in utero between two females or between one female and one male ($p < 0.01$) No data on GLP/OECD guideline compliance	Howdeshell e*t al.*, 1999
Mouse ICR 10 females/ dose	Subcutaneous	2 or 20 µg/kg/d (n = 10/dose) GD11- GD17	<u>Observations in F1 females:</u> ↘ in body weight on PND22 at both doses ($p < 0.05$) ↘ in body weight on PND60 at 20µg/kg only ($p < 0.05$)	Honma *et al.*, 2002

			↗ in AGD on PND22 at 2 µg/kg Advanced age at VO ($p < 0.01$) and at first estrus ($p < 0.05$) at 20µg/kg ↗ in estrous cycle length (1 day) at both doses ($p < 0.05$) No effect on the total number of pups and sex ratio in a F1 reproductive capacity study No data on the GLP/OECD guideline compliance	
Mouse C57BL/6 No data on the nb of animals / dose	Implantation	20µg/kg/d GD11.5 – GD18.5	<u>Fetus oocytes analysis at GD18.5:</u> ↗ in synaptic abnormalities ($p < 0.0001$) ↗ in the number of recombination aberrations <u>Oocytes analysis in offspring at 4-5 months of age:</u> ↗ I the average number of chiasmata per cell ($p < 0.01$) and in the frequency of univalents ($p < 0.05$) ↗ in the number of hyperploidy in eggs ($p <0.001$) and in embryos No data on the GLP compliance/ OECD guideline	Susiarjo *et al.*, 2007
Mouse CD-1 n = 12 females / dose	Subcutaneous	0.5 - 10 mg/kg bw/d GD15-GD18	<u>Observations in F1 offsprings</u> ↗ body weight at 16 weeks of age at both doses Advanced pubertal onset at 10mg/kg ↗ cycle length due tu elongated diestrus phase at both doses ↘ corpora lutea in mice at 4 weeks old only at both doses ↗ vaginal cornification in mice without corpora lutea Accelerated mammarian gland diferenciation at weeks of age only at both doses No modification in uterus morphology No data on the GLP compliance/ OECD guideline	Nikaido *et al.*, 2004
Ewe Suffolk N = 10 pregnant females	Subcutaneous	5 mg/kg bw/d GD30 - GD90	<u>Observations in F1 offsprings</u> ↘ body weight at birth ↗LH concentration in prebubertal females Modification of LH preovulatory surge No effect on the age at puberty No data on GLP/OECD guideline compliance	Savabieasfahani *et al.*, 2006
Sheep No data on the nb of animals/ dose	Subcutaneous	5 mg/kg bw/d GD30 - GD90	<u>Observations in F1 euthanized adults</u> ↗ ERα and ↘ ERβ expression in the medial preoptic area ↘ GnRH expression in the medial preoptic area No data on GLP/OECD guideline compliance	Mahoney et Padmanabhan, 2010
Perinatal exposure				

Mouse Balb-C 6 females / dose	Subcutaneous	100 - 1 000 µg/kg bw/d GD1 - PND7	<u>Observations in F1 offspring (3 months of age)</u> Ovarian malformations : - ↗ cystic ovaries at both doses - No modification in the number of the corpora lutea Uterine malformations : - ↗adenomatous hyperplasia with cystic endometrial - ↗hyperplasia at both doses - ↗ atypical hyperplasia ↗ endometriosis-like structure in the adipose tissue surrounding the genital tracts at both doses BPA was found in the liver of treated mice and female offsprings No data on the GLP/OECD guideline compliance	Signorile *et al.*, 2010
Rat Donryu	Oral (gavage)	0.006 or 6 mg/kg/d GD2 – PND20	<u>Observations in female offspring</u> No effect on: - The age at VO, - estrous cycle, - uterine cell proliferation - uterine ERα expression - serum FSH and LH levels	Yoshida *et al.*, 2004
Rat Wistar 5 females/ dose	Oral (drinking water)	10 mg/L in drinking water Aproximate dose : 1.2 mg/kg bw/d GD6 - PND21	<u>Observations in F1 offsprings</u> irregular estrous cycle ↗ thickness of the uterine epithelia and stroma ↘ of apoptotic cells in the uterine epithelium ↘ ER-α receptor expression in uterine epithelium on estrus day No modification in estradiol and progesterone serum levels No data on the GLP/OECD guideline compliance	Mendoza-Rodríguez *et al.*, 2011
Rat Sprague-Dawley 6 females/ dose	Oral (drinking water)	1 or 10 mg/l in drinking water Approximate dose: 0.1 or 1.2 mg/kg bw/d GD6 - End of lactation period	<u>Observations in F1 offsprings</u> ↗ body weight in newborn and adult animals at both doses Irregular estrous cycle at 1.2mg/kg ↘ LH plasma levels after ovariectomy at 1.2 mg/kg No modification in pubertal timing, anogenital distance, or in macroscopic morphology in genital tract tissue No uterotropic response after ovariectomy was observed in adult females No data on the GLP/OECD guideline compliance	Rubin *et al.*, 2001
Mouse CD-1 6-10 females /	Subcutaneous (implantation)	0.025 – 0.25 µg/kg bw/d GD9 - PND4	<u>Observations in F1 offsprings</u> Reproductive tract abnormalities (↘vagina weight, ↘ volume of the endometrial lamina propia at 0.25µg)	Markey *et al.*, 2005

dose.			Alteration in DNA synthesis in the uterine epithelium at 0.25µg ↗ expression of ERα and PR receptors in uterine epithelium at both doses No data on the GLP/OECD guideline compliance	
Mouse CD-1 18-20 females/ dose	Subcutaneous (implantation)	0.025 - 0.25 - 25 µg/kg bw/d GD8 - PND16	↘ cumulative number of pups at 0.025 and 25 µg/kg (nonmonotonic dose-response effect) ↘ fertility (total number of litters per dam) at 25 µg/kg ↘ fecundity (number of pups born at each delivery) at 0.25 and 25 µg/kg No data on the GLP/OECD guideline compliance	Cabaton *et al.*, 2010
Rat Long-Evans 13 - 29 females / dose in block 1 6 - 14 females / dose in block 2	Oral (gavage)	2 - 20 - 200 µg/kg bw/d GD7 - PND18	No effect in any parameters observed (F0 and F1 weight, anogenital distance, age at puberty, reproductive tract morphology, fertility, fecundity, or sexual dimorphic behaviors) No data on the GLP/OECD guideline compliance	Ryan *et al.*, 2010
Rat Sprague-Dawley 8 pregnant females/ dose	Oral (gavage)	3.2, 32 or 320 mg/kg/d (n = 8/dose) GD11 – PND20	<u>Observations in F1 females:</u> No effect on : - the number of live pups per litter and body weights of live pups on PND1 or 7. - the age at vaginal opening and the age at first estrus. - the estrous cycle - the lordosis behavior - the volume of the SDN-POA - the morphology of ovaries and uteri. No data on the GLP/OECD guideline compliance	Kwon *et al.*, 2000
Postnatal exposure				
Rat Long-Evans 10-12 females / group.	Subcutaneous	50 - 50 000 µg/kg bw/d PND0-PND3	<u>Observation in females at adulthood</u> advanced pubertal onset (at 50µg/kg bw/d only) ↗ number of acyclic animals ↗ ovarian malformations (↗ cysts, ↘ corpora lutea, follicle hemorrhage and degeneration) No modification of the sexual behavior (lordosis quotient) No modification in FOS expression within GnRH neurons No data on GLP/OECD guideline compliance	(Adewale *et al.*, 2009

Mouse CD-1 24 females pups / dose.	Subcutaneous	10 - 100 - 1000 µg/kg bw/d PND1- PND5	<u>Observations in females of 18-months of age</u> Ovarian modifications: - ↗ cysts at 100µg/kg - decreasing trend in corpora lutea as the dose increased - ↗progressive proliferative lesion in the oviduct Uterine modifications: - ↗cystic endometrial hyperplasia at 100µg/kg - Increasing trend in number of adenomyosis, atypical hyperplasia, leiomyomas, stromal polyp and enlarged mesonephric duct remnants No modification in body weight No data on the GLP/OECD guideline compliance	(Newbold *et al.*, 2007)
Rat Sprague-Dawley 11 – 15 females/ group	Subcutaneous	50 µg/50µL (2.5 – 6.2 mg/kg), 500 µg/50µL (25 – 62.5 mg/kg) PND1 – PND10	↘ in GnRH-induced LH secretion at 500µg ($p < 0.05$) No effect on GnRH-induced FSH secretion ↗ GnRH pulsatility in *ex-vivo* study at both doses Advanced age at VO at both doses ($p < 0.05$) Irregular estrous cycle at 500µg ($p < 0.05$) No data on the GLP/OECD guideline compliance	Fernandez *et al.*, 2009
Rat Sprague-Dawley No data on the nb of animals/ dose	Subcutaneous	5 µg/50µL (0.25 – 0.62mg/kg) 50 µg/50µL (2.5 – 6.2 mg/kg), 500 µg/50µL (25 – 62.5 mg/kg) PND1 - PND10	reduced fertility at 50µg and total infertility at 500µg ↗ ovarian malformations (↘ ovarian weight in all doses, ↗ cysts, ↘ corpora lutea, and ↗ atretic follicle) at 500µg ↗ testosterone and estradiol serum levels and ↘ progesterone level in all doses alteration in GnRH secretion in vitro at 50 and 500µg No data on GLP/OECD guideline compliance	(Fernandez *et al.*, 2010)
Rat Long Evans 8-11 females / dose 6-11 males /dose	Subcutaneous	50 µg/kg bw/d or 50 mg/kg bw/d PND1-PND5	<u>Observations in adult females</u> ↘ in KISS immunoreactivity in the ARC nuclei at 50 mg/kg bw/d No modification in the KISS-ir in the AVPV at both doses. <u>Observations in adult males</u> No effects on the KISS-ir in both AVPV and ARC nuclei No data on GLP/OECD guideline compliance	(Patisaul *et al.*, 2009)
Rat Wistar 10 animals / sex /	Subcutaneous	100 - 500 µg/animal PND1 - 5	<u>Observations in prepubertal male and females</u> ↘ in relative expression of KISS-1 m-RNA in the hypothalamus at both doses. No data on GLP/OECD guideline compliance	(Navarro *et al.*, 2009)

dose.				
Rat Wistar 8 females per dose.	Subcutaneous	0.05 or 20 mg/kg/d PND1, 3, 5 and 7	<u>Observations in adult ovariectomized females (90-d old)</u> ↘ in the endothelial proliferative response to ovarian steroid treatment at both doses ($p < 0.05$) ↘ in VGEF mRNA levels at both doses ($p < 0.05$) ↘ of the ERα uterine subepithelial expression at 0.05 mg/kg ($p < 0.05$) ↗ of the NCOR1 subepithelial expression at both doses ($p < 0.05$) No effect on the PR subepithelial expression No data on GLP/OECD guideline compliance	Bosquiazzo *et al*., 2010
Rat Wistar Treatment: 28-33 female pups per dose. Prebubertal study: 20-25 females per dose. Adult study: 8 females per dose.	Subcutaneous	0.05 or 20 mg/kg/d PND1, 3, 5 and 7	<u>Observations in prebubertal females (on PND8)</u> ↘ in Hoxa10 and Hoxa11 mRNA uterine expression at both doses ($p < 0.001$) <u>Observations in adult ovariectomized females (90-d old)</u> ↘ in stromal cell proliferation at both doses ($p < 0.001$) ↘ in ERα expression at 0.05 mg/kg ($p < 0.05$) ↘ in Hoxa10 mRNA uterine expression at both doses ($p < 0.001$) ↘ in Hoxa11 mRNA uterin expression at 0.05 mg/kg ($p < 0.05$) No effect on the methylation pattern of Hoxa10 promoter No data on GLP/OECD guideline compliance	Varayoud *et al*., 2008
Rat Wistar BPA at 0.05mg/kg: n=17 BPA at 20 mg/kg: n=20	Subcutaneous	0.05 or 20 mg/kg/d PND1, 3, 5 and 7	<u>Observations in adult females (80-d old)</u> ↘ in the number of implantation sites on day 18 of pregnancy at 20 mg/kg ($p < 0.05$) ↗ of the number of resorptions site on day 18 of pregnancy at both doses ↘ in the ERα and PR mRNA levels on day 5 of pregnancy at both doses ($p < 0.05$) ↘ in Hoxa10 uterine expression on day 5 of pregnancy at both doses ($p < 0.05$) No effect on the number of Corpora Lutea and on E2 and P serum levels No data on GLP/OECD guideline compliance	Varayoud *et al*., 2011
Rat Wistar The number of animals per treatment group was at	Subcutaneous	0.05 or 20 mg/kg/d PND1, 3, 5 and 7	<u>Observations in females on PND8:</u> ↘ in the percentage of primordial follicles at 20 mg/kg ($p < 0.001$) ↗ in the percentage of recruited follicles at 20 mg/kg ($p < 0.001$) ↗ in p27 protein expression in primordial and recruited follicles ↗ in ERβ expression in recruited follicles at 20 mg/kg ($p < 0.01$) ↗ in ERα expression in primary follicles at 20	Rodriguez *et al*., 2010

least 8.			mg/kg ($p < 0.05$) No effect on multioocyte follicles incidence, oocytes survival, apoptosis and on PR expression No data on GLP/OECD guideline compliance	
Mouce ICR 15 females per dose	Subcutaneous	0.1, 1, 10 or 100 mg/kg (n = 15/dose) PND8	↘ in body weight from PND18 to 30 at 10 and 100 mg/kg ($p < 0.05$) Advanced age at VO at all doses ($p < 0.05$) ↘ in the number of estrus days at 100mg/kg ($p < 0.05$) ↘ in ovarian weight at PND25 at all doses and at 1, 10 and 100 mg/kg at PND30 ↘ in uterine weight at PND30 at 100 mg/kg No effect on the uterine weight at PND25 No effect on the uterine and ovarian weights at PND70 No data on GLP/OECD guideline compliance	Nah *et al.*, 2011
Prebubertal exposure				
Sheep Poll Dorset 6 females/ group	Intramuscular	3,5 mg/kg bw twice a week Lambs of 4 weeks of age were treated for 7 weeks	<u>Observation in prebubertal females</u> ↘ in LH pulse amplitude and frequency Uterine malformations No modification on mean body, ovarian, kydney and adrenal weights No data on GLP/OECD guideline compliance	Evans *et al.*, 2004
Sheep Lacaune	IV infusion 3 females/dose	0.5 - 1 - 2.5 - 5 - 10 - 20 - 40, and 80 mg/kg bw/d Lambs of 3-4 months of age were treated for 54h	<u>Observation in prebubertal females:</u> Abolition of the LH pulse at 80 and 40 mg/kg bw/d ↘ in LH pulse frequency at 20, 2.5 and 5 mg/kg bw/d No effect detected at 10, 1 and 0.5 mg/kg bw No data on GLP/OECD guideline compliance	Collet *et al.*, 2010
	Intramuscular 5 females/dose	3.5 mg/kg bw twice a week Lambs of 4-5 months of age were treated for 8 weeks	<u>Observation in prebubertal females</u> ↘ mean LH pulse frequency and basal concentration after 6 weeks of treatment. No effect on the mean LH pulse amplitude No data on GLP/OECD guideline compliance	
Mouse CD-1 No data on the nb of animals/ dose	Subcutaneous	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 No data on the GLP/OECD guideline compliance	Nikaido *et al.*, 2005

Mouse C57BL/6 No data on the nb of animals/ dose	Oral (gavage)	20, 40 or 100 µg/kg/d Mice of 20-22-days of age were treated for 6-8days	<u>Oocytes analysis:</u> ↗ in congression failure ($p < 0.05$) at all doses Dose-related increase in the level of meiotic abnormalities No data on the GLP/OECD guideline compliance	Hunt *et al*., 2003
Adult exposure				
Mice Swiss 15 female mice per dose.	Intra-gastrically	5, 25 or 100 µg/kg bw/d 28 days from 60 days of age	↗ in the number of resorptions out of the total number of implantations at 25 ($p < 0.01$) and 100 µg/kg ($p < 0.05$) ↗ in the number of animals with resorptions at all doses No effect on the number of pregnancies, implantations or in the number of viable fetuses No data on the GLP/OECD guideline compliance	Al-Hiyasat *et al*., 2004
Mice CF-1 No data on the nb of animals / dose	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	The dose of 68.84 mg of BPA/d/animal (corresponding to a BPA supplementation at 6%) causes the abortion of all gestations No modification of litter size, percentage of pups surviving after birth, or in sex ratio of pups No data on the GLP/OECD guideline compliance	Berger *et al*., 2007
	Subcutaneous	0.0005, 0.0015, 0.0046, 0.0143, 0.0416, 0.125, 0.375, 1.125, 3.375 or 10.125 mg/animal/day GD1- GD4	↘ in number of pups born at 3.375 ($p < 0.05$) and 10.125 mg/d ($p < 0.01$) ↘ in the percent of females that gave birth at 10.125 mg/d ($p < 0.001$) ↘ in the number of implantation sites at 10.125 mg/d No data on the GLP/OECD guideline compliance	
Mice CF-1 No data on the nb of animals / dose	Subcutaneous	0.0005, 0.0045, 0.05, 0.125, 1.125, 3.375, 6.75, or 10.125 mg/animal/d GD1- GD4	↘ in implantation sites a 6.75 and 10.125 mg/d ($p < 0.01$) on GD6 ↘ in Progesterone urine concentration on day 2-5 of pregnancy at 10.125 mg/d ($p < 0.0005$) No effect on estradiol urine concentration on day 2-5 of pregnancy No data on the GLP/OECD guideline compliance	Berger *et al*., 2008
Mice CF-1 (n = 4-9)	Subcutaneous	100 - 200 -300 mg/kg bw/d GD1 - GD4	<u>Observations on GD6:</u> ↘ in implantation sites at 200 and 300 mg/kg ($p < 0.001$) Histological modifications of the wall of the uterine cavity at 200 and 300 mg/kg	Berger *et al*., 2010

			ERα and PR expression were affected according to a non monotone relationship No data on the GLP/OECD guideline compliance	
ICR Mice No data on the nb of animals / dose	Subcutaneous	10mg/kg bw/d GD0 - GD7	↘ in the embryo number ↘ in the weight of the uterus and marked modifications of placental structure No data on the GLP/OECD guideline compliance	(Tachibana *et al.*, 2007)
10 adult female African green monkeys (Chlorocebus aethiops sabaeus) of reproductive age	All monkeys were anesthetized (20 mg/kg ketamine, i.m, followed by 20 mg/kg pentobarbital i.v.), intubated, and ovariectomized through a median laparatomy under sterile conditions.	BPA (50µg/kg/day), estradiol, both or vehicle control (at reproductive age)	- exposure to the combination of estradiol and BPA resulted in decreased PR expression compared to estradiol exposure alone ($p<0.01$). - Both BPA exposure and diminished progesterone action have been associated with pregnancy loss, endometriosis and endometrial hyperplasia/cancer.	Aldad et al. 2011
Multigeneration exposure				
Rat **IGS (SD) rats** 25 rats /sex /group administered	2 generation study similar to OECD 416 (Deviations: *Female treated for 2 weeks only before mating. *Low doses used)	0, 0.2, 2, 20 and 200 µg/kg/day Oral by gavage	No effect on behaviour (i.e. performance in learning tests) , oestrus cycle, fertility index and the number of implantations in F0 and F1 females were not affected by treatment with BPA. Absolute AGD decreased but no more relevant when correlated with BW (decreased). Overall no effect.	Ema et al., 2001
Rat Sprague-Dawley 30 males/ dose 30 females/ dose	Exposure from 10 weeks before mating until PND21 (3 generations)	0.001, 0.02, 0.3, 5, 50 or 500 mg/kg/d Oral	The absolute age at puberty (evaluated by the age at vaginal patency) was delayed in the F2 generation at 50mg/kg and in the F1, F2 and F3 generations at 500 mg/kg. Reduced number of total and live pups per litter at birth and on PND4 at 500 mg/kg for F1, F2 and F3 ($p < 0.001$) The absolute and relative organ paired ovary weights were decreased in F1, F2 and F3 offspring and adult ($p < 0.05$ and $p < 0.001$ respectively) No effect on estrous cycle length, paired ovarian primordial follicle counts, reproductive organs histology, mating, fertility, pregnancy, dead pups per litter or percent post-implantation loss.	Tyl *et al.*, 2002

			According to EPA OPPTS 837.38000, 1998 GLP compliant study	
Mice CD-1 (n= 20/ treated group/ sex, n= 40/ control group/ sex)	Continuous breeding study (Task 1, dose-finding; Task 2, continuous breeding phase; Task 3, identification of the affected sex, and Task 4, offspring assessment.)	0, 0.25, 0.5 or 1.0% (daily intakes BPA estimated 0, 300 or 325, 600 or 650 and 1,200 or 1300 mg/kg for males or females resp. In diet	Adverse effect on fertility : statistically significant ↘/ ctrl in the number of litters produced / pair (4.5 and 4.7 compared to 5.0 for ctrls), litter size (6.5 and 9.8 compared to 12.2 for controls) and the number of live pups per litter (6.3 and 9.7 compared to 12.1 for controls) in the high and mid-dose group. The litter size reductions occurred across all matings and =f(dose-related). No effects on fertility were observed in the low-dose group. A statistically significant ↘ in litter size (controls: 11.4, treated males: 9.1, treated females: 5.9) and number of live pups per litter (controls: 11.3, treated males: 8.4, treated females: 5.5) were observed in the cross-over mating. In the continuous breeding phase, a statistically significant ↘ in live pup weight (6%) on postnatal day 0 was observed in females at the top dose after adjustment for litter size, including live and still births. In the continuous breeding phase a small but statistically significant ↘ in body weight gain (4%) was only observed in treated females at study termination. No effect on the sex ratio in the F1 generation. Possibility that there may be potential effects on pups due to exposure to BPA via the milk. In the F1 generation, BPA treatment had no effect on the fertility index, litter size, number of live pups per litter, sex ratio or mean pup weights at birth.	NTP, 1985b
Mice CD-1 N=28 animals/ dose	Exposure from 8 weeks before mating until PND21 (2 generations)	0.003, 0.03, 0.3, 5, 50 or 600 mg/kg/d Oral	No effect on the absolute age at puberty at any dose (evaluated by the age at vaginal patency) at any dose.Vaginal patency was accelerated when adjusted for the PND21 body weight at 600mg/kg. F0 treated females were twice more in estrus as compared to controls at 600 mg/kg. ↗ the length of the gestation by 0.3 days at 600mg/kg ↘ the body weight of the pups during lactation at 600mg/kg No effect on reproductive organ weights, ovarian primordial follicles count, histopathology of ovaries and uterus, mating and fertility indices, litter size at birth, sex ratio, percent of post-implantation loss Follow OECD guideline 416 (two generation	Tyl *et al.*, 2008

			reproduction toxicity study), TG 416 enhanced GLP compliant study	
Trans-generational exposure				
Mice ICR No data on the nb of animals / dose	Subcutaneous	100, 200, 500 or 1000 mg/kg bw/d GD12-GD16 in the F0 generation	<u>Observations in the F2 generation at 8 weeks of age:</u> ↗ of the body weight at 200, 500 and 1000 mg/kg Uterus histological abnormalities at 200, 500 and 1000 mg/kg Unmethylation in theHoxa10 intron region at 100 and 200 mg/kg No data on the GLP/OECD guideline compliance	Hiyama *et al*., 2011

4.11.1.2 *Human information*

4.11.1.2.1 Effects on the uterus

- <u>Endometriosis</u>

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). The women were recruited when consulting in a gynaecological-obstetric service. 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). It should be noted that 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 was adapted; however the impact of deconjugation during the extraction was not evaluated.

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 the stage of endometriosis and the authors found no significant association. The urinary levels of conjugated BPA measured appear consistent with levels found in Japan in several studies of the general population. Nevertheless, there were two main limitations weigh in the interpretation of this study: first, the determination of urinary BPA does not reflect the long-term exposure, but only the most recent. The second one is that there was no real control group.

- Endometrial hyperplasia

An *a priori* prospective cross-sectional study (since no inclusion criteria and no dates were specified) suggests that circulating levels of BPA would be lower in women with complex uterine hyperplasia (1.4 ± 0.4 ng/mL; n=9) and/or uterine adenocarcinoma (1.4 ± 0.5 ng/mL; n=7) than in women with normal endometrial histology (2.5 ± 1.5 ng/mL; n=11) or moderate endometrial hyperplasia (2.9 ± 2.0; n=10). Authors suggest that since BPA may mimic estrogen action in the endometrium in the uterus it is conceivable that elevated levels of BPA may cause endometrial hyperplasia. However, there was no difference between normal and simple endometrial hyperplasia groups. On the contrary, BPA levels in complex endometrial hyperplasia were significantly lower than those with normal women and patients with simple endometrial hyperplasia. The fact that BPA levels in patients with endometrial cancer is also lower than those with normal women and patients with simple endometrial hyperplasia suggests that there may be some relationship between BPA concentrations and malignant changes in endometrium. It is, however, difficult to explain the mechanism in which lower BPA levels causes the changes in the endometrium. One possible explanation for decreased BPA levels may be metabolic capability for BPA is increased in patients with complex endometrial hyperplasia and endometrial cancer although the precise mechanism is yet unknown. Alternatively, it is plausible that BPA exerts anti-estrogneic roles in the human endometrium, and that lower BPA levels are a favorable environment to grow complex endometrial hyperplasia and endometrial cancer.
Nevertheless, the analytical method (ELISA) used to measure the level of BPA is questionable and moreover 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, then the results of this study which observed more important concentrations of BPA in controls than in women with endometrial hyperplasia, should not be taken carefully (Hiroi *et al.*, 2004).

4.11.1.2.2 Effects during pregnancy

A very recent study was performed by Ehrlich *et al*. (2012) in women undergoing *in vitro* fertilization (IVF) in the United States of America. 137 women, female partners of couple seeking infertility evaluation and treatement were included in this study. These women undergo 180 IVF, which 42% (n=75) resultated in an implantation failure. The authors found that in adjusted models, there was an increased odd of implantation failure with higher quartile of urinary BPA concentrations. Women in the fourth quartile of exposure (specific gravity-adhusted urinary BPA, 3.80-26.48 µ/L) had almost twice the odds of implantation failure than women in the first quartile of exposure (≤ 1.96 µg/L). The urinary concentration of free and conjugated BPA species was measured, the geometric mean was 1.53 µg/L and was comparable to the BPA concentrations found in the general US population (NHANES study 2007-2008) which is 1.97 µg/L.

A case-control study evaluated the association between BPA exposure and the incidence of spontaneous miscarriages (Sugiura-Ogasawara *et al.*, 2005). 45 patients with a history of three or

more (3-11) consecutive first-semester miscarriages were studied. The control group was composed of 32 non-pregnant women with no history of live birth, infertility and miscarriage. The authors report a higher (more than 3 times as high) serum level of BPA in women with a history of three miscarriages (2.59 ± 5.23 ng/mL) compared to the control women (0.77 ± 0.38 ng/mL). Additionally, among 35 women that then became pregnant, there was some evidence of lower BPA among the women who subsequently had a successful pregnancy as compared to those that miscarried again. However, this study remains very controversial, especially in terms of the protocol for measuring BPA (the ELISA method was used), the comparability of groups, because of other confounding miscarriage factors, in terms of analyzing the results (median serum levels identical in both groups), and the statistical tools chosen (Berkowitz, 2006). Moreover the size of the population was small. The results from this study could not then be considered as totally proven.

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 pregnant women in Mexico City (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). Nevertheless this study is not really reliable since after an analysis of these data it appaeraed that 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.).

4.11.1.2.3 Effects on ovaries

In a prospective study by Mok-Lin *et al.* (2010) which included women (n=84) following an ovarian stimulation protocol as part of an *in vitro* fertilization, the authors indicated that there was a negative correlation between urinary levels of BPA (n=203 urine samples; 2 samples during 91 cycles and one sample during 21 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 estradiol 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. The BPA levels found were compared to urinary BPA concentrations observed in the general population in the NHANES 2003-2008 cohort. The concentration of urinary BPA found reflects exposure at the time of collection, but 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 undergoing an *in vitro* fertilisation to the general population. Nevertheless these results are consistent with those observed in a recent study, which shows that exposure to BPA is associated with a decreased likelihood of success of the *in vitro* fertilization

(fertilisation rate), which is attributed to an impaired oocyte quality (Fujimoto *et al.*, 2011). The urinary BPA level was measured in 58 women undergoing in vitro fertilization, and in 26 men partners of these women. The median in women was 2.53 ng/mL with a highest cocentration of 67.4 and 0.34 ng/mL in men with a highest concentration of 22.7. 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.

In a recent study by Bloom *et al.* (2011b) the association between the levels of serum BPA and follicular response to exogenous ovary stimulation. In their previous publication (Bloom *et al*., 2011a) an inverse association between male serum unconjugated BPA concentrations and oocytes fertilization among infertile couples undergoing IVF. The same cohort was studied in this study, and 44 women with data available for serum unconjugated BPA concentrations were included. Multivariable linear regression models suggest an inverse association between serum BPAconcentration and peak E_2, adjusted for race/ethnicity, cigarette smoking, and baseline antral follicle count. In contrast, no association is indicated for serum BPA concentrations and the number oocytes retrieved. This result is in line with results reported by Mok-Lin *et al*. (2010). Nevertheless, the size of the population is very limited, and then do not allow firm conclusions.

A cross-sectional study was conducted in Japan in women with polycystic ovary syndrome (PCOS) (Takeuchi *et al.*, 2004). The women with PCOS were either 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 using a non-validated immunoassay method. BPA was detected 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 because of the imprecision of the sampling, the very small size of the sampling, the lack of information on inclusion criteria, and the lack of accounting in the results of disorders in the controls. Then the results from this study could not be considered as totally proven.

However, the results of the Takeushi *et al.*'s study are consistent with the recent study by Kandaraki *et al.* (2011) who found that serum concentrations of BPA significantly higher (1.05±0.56 ng/mL) in women with Polycystic Ovary Syndrome (PCOS ; n=71) (obese or not) when compared to normal control women (n=100) 0.72 ± 0.37ng/mL ($P < 0.0001$) (Kandaraki *et al.*, 2011). In addition, BPA concentrations were significantly correlated with testosterone

concentrations and insulin resistance. In this study, 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 same obversations are made in the sub-groups: lean or owerweight women suffering of PCOS have significant higher BPA levels (1.13 ± 0.63 and 0.96 ± 0.46 ng/mL respectively) than control lean or overweight women (0.70±0.36 ng/ml ($P < 0.001$) and 0.72±0.39 ng/mL ($P < 0.05$) respectively).
The main limitation of this study is the analytical method (ELISA) which does not discriminate between the different forms of BPA. However, the concentrations obtained can be considered as a global indicator of the exposure to BPA.

4.11.1.2.4 Effects on puberty

Two studies of good quality investigated the effects of BPA on the puberty.
The first one, conducted by Wolff *et al.* demonstrated that there was no relationship between the urinary BPA concentration and the onset of puberty (Wolff *et al*., 2008). 186 nine-year old girls took part to this cross-sectional study, and adjustments for numerous factors have been made including for the urinary creatinine. The result of this study was confirmed in a cohort of 1151 girls between the ages of 6 to 8 years (Wolff *et al*., 2010), for whom there were no changes in the age of the puberty onset. Nevertheless, when compared with the first quintile of urinary BPA concentrations, the odds of earlier pubic hair development (stage 2+) were weakly, but non-significantly, elevated in the second (OR 1.03, 95% CI 0.96–1.09) and third (OR 1.06, 95% CI 0.99–1.13) quintiles of urinary BPA concentrations and null in the fourth (OR 0.99, 95% CI 0.92–1.05) and fifth (OR 1.00, 95% CI 0.94–1.07) quintiles. 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 mean ranged from 1.6 to 2.4 μg/L.

Conclusions on effects in humans (women)

In a study by Cobellis *et al*. (2009) endometriosis were reported in women in a gynaecological-obstetric clinic. BPA was not detected in control women, whereas serum levels of BPA were above the detection limit in women with endometriosis. However another study by Itoh *et al*. (2007) did not see any significant association between urinary BPA levels and the stage of the endometriosis. Since these studies were performed in specific population (infertile women or with gynecological problems) it is difficult to conclude. A lower exposure to BPA was also associated with endometrium hyperplasia (Hiroi *et al*., 2004). In a limited sample size (16 cases versus 21 controls) circulating levels of BPA appeared to be lesser in women with more severe endometrium hyperplasia.

Implantation failures were reported in women undergoing a medical-assisted procreation with higher urinary levels of BPA than control women (Ehrlich *et al*., 2012). A BPA exposure could also lead to spontaneous miscarriages (Sugiura-Ogasawara *et al.*, 2005). This study shows that patients with a history of 3 or more consecutive miscarriages had higher serum level of BPA compared to the control women.

Finally effects on the ovaries were associated with higher exposure levels to BPA. In a study by Mok-Lin *et al.* (2010) conducted with women undergoing an ovarian stimulation protocol as part of an in vitro fertilization (IVF), the ovarian response seemed to be diminished by the BPA exposure. This was confirmed in another study (Fujimoto *et al*., 2011) in which a significant association between the serum BPA concentrations of the women and decreased oocyte fertilization was reported. Additionnaly, higher incidence of polycystic ovaries was positively associated with higher serum BPA concentrations (Kandaraki *et al*., 2011 and Takeuchi *et al*., 2004).

Two recent studies performed in little girls (Wolff *et al.*, 2008 and 2010) demonstrated in an important number of little girls that there was no relationship between the urinary BPA concentration and the onset of their puberty.

Table 12: Summary of the studies on BPA effects on the human female reproductive system performed in women

Population and N	Results	BPA levels	Reference
Fertile women consulting a gynaecological-obstetric service for chronic pelvic pain, dysmenorrhea or ovarian cysts N=58 cases vs 11 controls	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%)		Cobellis *et al*., 2009
Female patients primarily complaining of infertility (endometriosis, 24-43 years) N=140	No significant association between urinary BPA levels (not adjusted and adjusted for creatinine) and the stage of endometriosis		Itoh *et al*., 2007
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	The correlation was the opposite of what was expected: the controls had more BPA than the cases (non-significant). Same inverse relationship observed in women with an endometrial carcinoma	In serum: 2.9 ng/mL in women with simple hyperplasia vs 1.4 ng/mL in women with complex hyperplasia	Hiroi *et al*., 2004

Female partners of couples seeking infertility evaluation	increased odd of implantation failure with higher quartile of urinary BPA concentrations	Urinary levels: Fourth quartile 3.80-26.48 µ/L First quartile ≤ 1.96 µg/L	Ehrlich *et al*., 2012
Women having had at least 3 first-trimester miscarriages N=45 cases vs 32 controls	Higher serum level of BPA in women with a history of three miscarriages compared to the control women.	In serum: 2.59 ± 5.23 ng/mL vs 0.77 ± 0.38 ng/mL in control women	Sugiura-Ogasawara *et al.*, 2005
Pregnant women N = 30 vs 30 controls	the 'premature' group (delivery < 37 weeks of pregnancy, N=12) had about twice as much BPA as the controls		Cantowine *et al.*, 2010
Women undergoing an ovarian stimulation protocol in the framework of an IVF N=84 (112 IVF cycles)	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	Urinary median level: 2.53 ng/mL	Mok-Lin *et al.*, 2010
Women undergoing an ovarian stimulation protocol in the framework of an IVF N=44	Bisphenol A is inversely associated with a reduced estradiol response during IVF. E_2 (β=−0.16; 95% confidence interval (CI) −0.32, 0.01), as well as with E_2 normalized to the number of mature-sized follicles at the hCG trigger (β=−0.14; 95%CI −0.24, −0.03). No association is observed for BPA and the number of oocytes retrieved (adjusted risk ratio=0.95; 95%CI 0.82, 1.10).	median unconjugated serum BPA concentration is 2.53 ng/ml (range 0.3–67.36 ng/ml).	Bloom *et al.* (2011b)
Women from the general population 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)	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.		Takeuchi *et al.*, 2004
Women with PCOS N =71 cases vs 100 control woemn	- 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.		Kandaraki et al;, 2011

	- BPA concentrations were significantly correlated with testosterone and androstenedione concentrations and insulin resistance. - BPA concentrations were significantly correlated with the existence of PCOS.		
9 year old girls N=186	No changes in the puberty onset in the girls		Wolff *et al*., 2008
Girls between 6- and 8-year old N=1151	No changes in the age of puberty onset.		Wolff et al., 2010

4.11.2 Effects on male reproductive tract

4.11.2.1 *Non-human information*

The data are presented by window of exposure and by type of effect.

4.11.2.1.1 *In utero* and lactation exposure

Several reseachers have studied BPA effects following an exposure during gestation and/or lactation period. These studies have been realized either in mice or rats with various routes of exposure. Six of these studies reported effects (Kabuto *et al.*, 2004; Okada et Kai, 2008; Watanabe *et al.*, 2003; Iida *et al*., 2002; Akingbemi *et al*., 2004; Timms *at al.,* 2005), two reported limited effects (Kobayashi *et al.*, 2002; Tinwell *et al.*, 2002) and two no effects at all (Howdeshell *et al.*, 2008, when control received phytoestrogens; LaRocca *et al*., 2011).

In the study by Iida *et al*. the effects of a prenatal exposure on the spermatogenesis in mice were investigated. BPA (unknown purity) dissolved in corn oil was orally administrated to pregnant ddY female mice from GD 10 to 17 at doses of 0, 1, 10 or 100 mg/kg bw/day. The mice in the control group received the vehicle only (i.e. corn oil). There were 5 dams per group except in the highest dose group in which there were only 4 dams. Offspring of three dams were examined in each group. The pups were terminated at adult age (60 days) and testes were removed except for 3 prenatally exposed mice from the 10 mg/kg and the control group who were terminated at 120 days after birth. Abnormalities were rarely found in the seminiferous tubules of the control group. In contrast, histological abnormalities were observed in all treatement groups of the prenatally BPA treated

male mice, such as loss of the luminal space in the tubules, reduction in the number of maturating elongate spermatids or reduction of the tubular diameter. In abnormal seminiferous tubules there was an aberrant distribution of spermatogenic cells within the epithelium, accumulation of amorphous material within the tubules and deposits of dense cells in the center of the tubules compared to normal seminiferous tubules which showed a highly organized distribution of the cells (Iida *et al*., 2002).

Okada *et al*. have exposed females ICR mice through a sub-cutaneous implant to BPA (purity unknown) at doses of 100 or 5000 µg (equivalent to doses of 1.2 or 60 µg/day) throughout pregnancy and lactation. The control group was implanted with a tube filled with 50 µL of sesame oil. At age of 4 weeks, all mice were killed, and after blood collection, the testes, epididymides and accessory reproductive glands (seminal vesicles with coagulating glands) were dissected out from the male pups and weighed. It appeared that the percentage of seminiferous tubules in the testes with mature spermatids was significantly lower in the groups given 5000 µg of BPA compared to the control group (Okada et Kai, 2008).

Akingbemi *et al*. (2004) exposed pregnant Long-Evans rats (n=7) to low dose of BPA (dissolved in corn oil; purity not known) of 2.4 µg/kg bw/day or vehicule only from GD 12 to PND 21. Subsequently, male offspring (n=12-14 per group) did not received any further BPA treatement and were terminated at age of 90-day. Although body weights were similar at birth (8.7 ± 0.1 vs. 8.5 ± 0.2 g; $p < 0.05$), male rats maternally exposed to 2.4 µg/kg/d BPA exhibited increased body weights but reduced testis weights at 90-day of age ($p < 0.01$). Serum LH and testosterone levels were equivalent in control and BPA-treated rats at 90 days, but the rate of testosterone production by Leydig cells was decreased by BPA treatment. Consistent with reduced Leydig cell steroidogenic capacity, testosterone concentrations in the testicular interstitial fluid of BPA-treated rats were significantly lower than control values. The weights of the seminal vesicles in BPA-treated rats were reduced by 18% compared with control values ($p < 0.01$), but prostate weights were unchanged. These findings show that perinatal exposure of rats to low dose of BPA decreased androgen biosynthesis by adult Leydig cells, as measured in the testicular interstitium and after incubation of Leydig cells *ex vivo*, and reduced seminal vesicle weight in adulthood.

In the study by Watanabe *et al.*, pregnant Crj:CD (SD) rats were exposed to BPA (purity > 99.8%) at 0 (control with corn oil only), 4, 40 and 400 mg/kg bw/d from GD 6 through PND 20 by gavage, then the pups were also exposed. Testis and blood of the male offspring were collected at the age of 9 and 36 weeks. No effect was observed on testes weight in BPA-exposed offspring. There was no remarkable change in plasma concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH) or estradiol**.** In contrast, plasma testosterone concentrations in offspring at 9 weeks old were significantly ($p < 0.05$) higher in BPA groups when compared with those of the control, even for those exposed to 4 mg/kg bw/d. At the age of 36 weeks the hormone concentrations showed an increase in a dose-dependent manner, although without statistical significance. The results therefore indicate that exposure to BPA during the perinatal period has a significant effect on

testosterone homeostasis ($p < 0.05$) in male offspring of rats, with possibly an effect on the aromatase activity or an increase of the enzymatic activity of the cytochrome P450 SCC (for side-chain cleavage or cholesterol side-chain cleavage enzyme) or a decrease of the 5 α-reductase level. At the age of 36 weeks the hormone concentrations showed an increase in a dose-dependent manner, although without statistical significance. (Watanabe *et al., 2003).*

In 2004, Kabuto *et al.* investigated the modifications in endogenous antioxidant capacity and oxidative damage in the brain, liver, kidney and testis in ICR mice exposed to BPA. Mice (n=6/group) were exposed throughout embryonic/fetal life and during lactation by feeding their pregnant/lactating mothers with BPA (purity unknown) at 5 or 10 µg per milliliter of drinking water. A control group was provided with drinking water (1% ethanol solution). Male mice were sacrified at the age of 4 weeks. It was demonstatred in this study that exposure to BPA increased the activity of catalase and glutathione peroxidase in the liver and kidney, respectively. It also increased thiobarbituric acid-reactive substances in the brain, kidney and testis at 10 µg/mL ($p < 0.05$), and significantly decreased the wet weight of the brain at 5µg/mL ($p < 0.05$), kidney at 10 µg/mL ($p < 0.05$) and testis at both doses ($p < 0.05$). These results suggest that this kind of exposure to BPA could induce tissue oxidative stress and peroxidation, ultimately leading to underdevelopment of testis for instance.

Timms *et al.* exposed from GD14 through GD18 CF-1 mice to BPA at 10µg/kg bw/d (n=6), vehicule only (oil; n=5) or 0.1 µg/kg/d of ethinylestradiol as a positive control (n=5). When the male mouse fetuses were examined, the authors found out that there was an epithelial proliferation of the primitive prostate gland ducts 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. This effect was observed in mice exposed to ethinylestradiol or BPA (Timms *et al*., 2005).

Salian *et al*. shows numerous transgenerational effects such as: post implantation loss, decrease in sperm count and motility, and a significant decrease of sexual hormone concentrations of FSH, LH, testosterone and oestradiol (Salian *et al*., 2009c). This study is developed below under paragraph 4.11.2.1.7.

In contrast to these previous studies, several authors found only limited effect (Tinwell *et al*., 2002 and Kobayashi *et al*., 2010 and 2012) and other failed to demonstrate significant effects of BPA exposure on the male reproductive tract, especially at low doses (Howdeshell *et al*., 2008; LaRocca *et al*., 2011).

In 2002, no significant effects were seen on the sexual development (litter size and weight, sex-ratio, weight of organs and reproductive organs, ano-genital distance (AGD) at birth and daily sperm production…) following an exposure of Sprague Dawley and Alderley Park rats by gavage to 20 µg, 100 µg or 50 mg /kg bw/d of BPA (purity of 99%) during gestations days (GD) 6-21 (n =

7/group). The control group was receiving the vehicule only (arachis oil). The only statistically significant effects observed were a decrease in daily sperm production and an increase in the age of vaginal opening (VO) in the Alderley Park strain at the highest dose evaluated (50 mg/kg bw/d) (Tinwell *et al.*, 2002).

Similarly to the study by Tinwell *et al.* in which only limited effects were seen, Howdeshell's team did not demonstrate any changes in various reproduction parameters (organ weights, ano-genital distance, sperm production, puberty age and hormone levels) in male Long-Evans hooded rats exposed daily by oral route during pregnancy through lactation, from GD 7 to postnatal day (PND) 18, to quite low doses (2, 20 and 200 µg/kg bw/d) of BPA pure at 99%. The control group received the vehicule only (corn oil). It should be noted that in this study the rats' food contained some phytoestrogens in the contrary of most of the other cited studies (Howdeshell *et al*., 2008).

In 2011, LaRocca *et al*. exposed C57/Bl6 mice via oral gavage to either vehicule (sesame oil; n=12), 50 µg (n=11) or 1000 µg /kg (n=14) of BPA (purity > 99%) from GD 10 through 16. The positive control group (n=14) received 2µg/kg of DES. No significant differences in male body weight or on male reproductive organs were found in treated animals, even in the positive control group (animals treated with DES). In animals treated with 1000 µg of BPA or DES a reduction in the litter size at birth was noted with also a decrease in the litter size at weaning (PND21) but was significant in DES animals only. Viability and diminution in sperm production (sperm head count) was observed in 1000 µg BPA and DES groups, but was not significant. Serum testosterone level was not affected neither by BPA nor by DES, AGD increased by DES only (LaRocca *et al*., 2011).

A very recent study by Kobayashi *et al.* (2012) observed a lack of effects on reproductive development in rats' offspring after a dietary exposure during *in utero* and lactational periods. Female Sprague-Dawley rats (9 weeks of age) were given 0 (control), 0.33, 3.3 or 33 ppm (corresponding to approximately 0.05, 0.5 and 5 mg/kg/d) of BPA (purity > 99.6%) in the diet from GD 6 trough PND 21 (n=12). The pups were weaned at 3-weeks of age. Twenty males and twenty female weanling pups from each group were selected and given normal diet. Concerning these pups, there were no BPA-related changes in F1 males in AGD, AGD index, absolute or relative epidydimis weight, absolute or relative testis weight in 5-week or 3-month old rats except for the epididymis weight in the 33 ppm group which was significantly decreased when compared to controls in the 3-month old rats ($p < 0.05$). Quality of sperm was considered as unaffected since there were no significant differences in the percentage of motile or progressive sperm in F1 males in the treated groups when compared to the control one. No difference was observed concerning the hormonal status (testosterone, dihydro-testosterone). However, in 5-week old females, there was a significant reduction in AGD and AGD index with exposure to 3.3 and 33 ppm of BPA.

It is difficult to find any specific reason explaining those contradictory results with the state of actual knowledge.

Differentiation of masculine and feminine behavior in mammals depends on perinatal sex steroids. As bisphenol-A (BPA) can be estrogenic and anti-androgenic, we examined impacts of perinatal exposure upon adult sexual behavior and morphology of male mice. In Experiment 1, dams were fed either a high- or low-phytoestrogen diet and received daily oral doses of 0, 0.175, 1.75, or 17.5 lg BPA from gestation day 10 through post-partum day 9. Male offspring from the high-phytoestrogen plus 17.5 lg BPA condition showed reduced mass of vesicular-coagulating but not other male glands, and showed increased latency to insemination when paired with females. In Experiment 2, these procedures were replicated but with all animals fed the high-phytoestrogen diet and perinatal BPA doses of 0, 17.5, 175, or 1750 lg/day. Adult masses of testes and male-accessory glands and levels of urinary steroids were not significantly affected. When males each encountered a sexually receptive female, there were fewer intromissions among those given 17.5 or 175 lg and fewer ejaculations among those given 17.5 lg, but the 1750 lg dose had no effect. Perinatal BPA dosages thus influenced male sexual behavior nonmonotonically, with impairment evident in a discrete dose range among males on a high-phytoestrogen diet. (Decatanzaro et al., 2013).

4.11.2.1.2 Neonatal exposure

The studies described in this paragraph reported effects on the sperm parameters or sexual function (Salian *et al*., 2009b; Aikawa *et al*., 2004), or histological abnormalities (Toyama and Yuasa, 2004b) following a neonatal exposure to BPA.

Salian *et al*., exposed, using sub-cutaneous injections, Holzman male rats (n=32/dose/group) to various doses of BPA pure at 99.8% (0.6, 1.2, 2.4, 5 and 10µg/30µl corresponding to approximately 100, 200, 400, 800, and 1600 µg/kg bw/drespectively) from PND1 to PND5. DES was used as the positive control and the negative control groups received sesame oil only. Male fertility was assessed during adulthood and the lowest dose of BPA that was most effective at impairing fertility was determined. Untreated females (n=24) mated with male rats that were neonatally exposed showed a significant increase in post-implantation loss (26.50%, $p < 0.001$ at 2.4µg; 32.01%, $p < 0.0001$ at 5µg and 24.61%, $p < 0.001$ at 10µg) and a decrease in litter size ($p < 0.001$ at 1.2µg and $p < 0.0001$ for the other doses). There were also significant changes in sperm count (0.6µg: 189.7 $x10^6$ cells, $p < 0.001$; 1.2µg: 184.8 $x10^6$; 2.4µg: 177.5 $x10^6$; 5µg: 169.1 $x10^6$; 10µg: 159.5 $x10^6$ with $p < 0.0001$ compared to vehicle control: 200.6$x10^6$), and sperm motility (%) (1.2µg: 83.47±0.90; 2.4µg: 77.8±1.71; 5µg: 74.72±1.70; 10µg: 72.41±1.98 with $p < 0.0001$ (except for the 1.2µg group where $p < 0.005$) compared to control: 90.38±0.45, except in the 0.6µg BPA group), along with hormonal imbalances in the rats exposed neonatally to BPA. The 400µg/kg bw/d dose of BPA was determined as the lowest dose that was capable of impairing male fertility (Salian *et al*., 2009b).

In the study by Aikawa *et al*. SHN mice (n=10-20/group) were exposed to 5 or 50 mg (equivalent to approximately 175 and 17,500 µg/kg bw/day) of BPA (purity unknown) diluted in sesame oil via subcutaneous injections for the first 5 days after birth. The animals were weaned at 3 weeks of age and kept with their littermates until 8 weeks of age. Then, all male mice were individually housed in

order to exclude the possibility of an influence from a social environment, such as social status, on sperm motility. The mice were sacrificed at 10 weeks of age.These exposures resulted in a significant decrease in the percentage of moving sperm in the 50 µg ($p < 0.05$), and an increase in the percent of malformed sperm in the epididymides at 10 weeks of age in both 5µg (approximatively 45%; $p < 0.05$); and 50 µg (78.2%; $p < 0.001$) groups compared to the vehicule control group (6.8%). Nevertheless no marked changes were found in the testicular histology in treated animals compared to the controls (Aikawa *et al*., 2004)

In a study by Toyama and Yuasa (2004b) new born Wistar rats and ICR (CD-1) mice (n=5) were subcutaneously injected with 4 different doses of BPA ranging from 0.1 to 10 µg BPA/animal/injection for mice and 1 to 600 µg BPA/animal/injection for rats. The BPA (purity not known) was dissolved in DMSO and then diluted in olive oil. Animals were treated on PND1, 3, 5, 7, 9 and 11, and were then terminated weekly at age 2-10 weeks. Control animals received the same volume of vehicle only. There were then four doses levels, 11 tests intervals and five replicates for each. They were 3-4 control mice and rats for these different experiments. It should be noted that all animals were healthy except the rats that received the highest BPA dose, (600µg/animal/injection) that died before 20 days of age. All animals from this group were then excluded from further analysis. These different exposures result in abnormalities in the acrosomal granule and nucleus of step 2-3 spermatids observed by microscopy, but no clear dose-response relationship was evident. Despite those abnormalities on spermatogenesis, the fertility was not affected since the same number of pups was obtained from females. Few abnormal spermatids were observed in F1 males indicating that adverse effects were transitory nevertheless they all displayed normal histology of their testes.

In the sudy performed by Kato *et al*., (2006), male Sprague-Dawley rats were given subcutaneously from birth to PND9 BPA (purity unknown) at doses of 2, 11, 56, 277 or 97000 µg/kg bw/d or 0.9 mg/kg of 17β-estradiol as a positive control, or the vehicle ethanol only. All BPA groups in which analysis were performed at PND10, 35 and 150 showed normal reproductive parameters (for instance preputial separation, sperm analisys, serum testosterone levels, copulatory and fertility rate, sexual organ weight…). The estrogen-mediated gene responses were not affected either.

4.11.2.1.3 Prepubertal exposure

Few studies investigated the effets of an exposure to BPA on male reproductive functions at doses comparable to human exposure.

In 2004, Akingbemi *et al*. exposed weanling Long-Evans rats (n=10-12/ group) by gavage with low (2.4 or 10 µg/kg bw/d) and high doses (100 or 200 mg/kg bw/day) of BPA (dissolved in corn oil; purity unknown) for 15 days, from PND21 to 35. The rats in the control group received vehicule only. This period of time was chosen because the prepubertal period is a time of active reproductive

tract development and hormonally active chemicals are known to exhibit greater potency during sexual differenciation. Exposure to 2.4µg/ kg bw/day of BPA decreased both serum LH and T levels by 15% and 20% respectively compared to control. The authors showed that the decrease in serum LH is the result of a BPA-induced declines in pituitary LH synthesis and secretion and the subsequent effect on T levels. BPA at milligram doses did not affect serum LH and T levels (Akingbemi *et al*., 2004).

In the study by Della Seta *et al*., the authors treated male Sprague- Dawley rats (n=12/group) orally from PND 23 to 30 with a dose of 40 µg/kg bw/d of BPA (purity unknown) and another group was exposed to 0.4 µg/kg bw/d of Ethinylestradiol (EE). The control group was treated with the vehicle only (peanut oil). Hormonal levels analysis and behavior were done at PND 37 and 105. It appeared that the levels of circulating testosterone were reduced in the juveniles at PND37 by one-third ($p < 0.002$) with BPA and by approximately 20% with EE. The decrement persisted in the adult animals (PND105) but reached significance only in the BPA group ($p < 0.03$). Exposure to BPA also modified the male sexual behavior (frequency and duration), but this effect was more marked with EE than with BPA exposure. No change was observed in the levels of estradiol in any of the treated group (Della Seta *et al*., 2006).

In another study Wistar/ST immature male rats (4 weeks old) were subcutaneously injected 4 days a week during 6 weeks with 0, 20, 100 or 200 mg BPA (purity > 99%)/kg/day (Nakamura *et al*., 2010). These dosages corresponded to 11.4, 57.1 and 114.2 mg BPA/kg bw/day. There were 2 control groups: one was sacrified at 4 weeks old (i.e. at the start of the treatment in order to investigate the reproductive system). Another was given the vehicle (corn oil) the same way than BPA. The rats were terminated 16 hours after the last dosage. Blood, epididymis, testes, seminal vesicles and prostate were collected. Low dose of BPA (20 mg/kg) did not influence the body weight but from the middle-dose (100 and 200 mg/kg) there was a dose-dependent decrease in the body weight compared to the control rats. BPA also affected the reproductive organs since the testis weight was decreased by 10% ($p < 0.05$) at the highest dose (200 mg/kg) and both middle and high dose exposure reduce the epidiymis by 10 to 18%, the seminal vesicles by 35 to 48% and the prostate weights by 30% compared to controls ($p < 0.05$). Prostate and seminal vesicles seemed to be the most vulnerable reproductive organs to a BPA exposure in this study. The plasma testosterone levels were also affected: BPA treatements at 100 and 200 mg/kg decreased the levels to one-third of the controls. The testicular testosterone levels were also decreased at these two treatement doses ($p <0.05$). Plasma FSH levels were not affected by the exposure to BPA, conversely to plasma LH levels which was affected after a 200 mg/kg exposure. Then this study demonstrated that 6-week exposure via subcutaneous injections of BPA to prepubertal male rats had adverse effects on their reproductive system. The reproductive organs were affected; the plasma and the testicular testosterone levels were diminished in a dose-dependent manner. And finally morphological abnormalities were observed since for instance there was a decrease in the number of Leydig cells per seminiferous tubule by 20% ($p < 0.05$) (Nakamura *et al*., 2010).

Another study was conducted by Tan *et al*. on juvenile rats. They exposed rats from PND 23 to PND53 by gavage at 0 or 100 mg/kg bw/d of BPA (purity not known) in diluted Tween-80 (in distilled water 1:9 v/v). The control group was gavaged with Tween 80 in corn oil. There was no significant effect on weight gain in rats treated with BPA. Only 66.7% of the treated rats reached a complete preputial separation compared to 100% in control rats, therefore an exposure to BPA seemed to be able to delay the onset of puberty in males. No significant effects were seen on the testis, epididymis or adrenal weight, nevertheless most of the rats in the treatment groups did show some evidence of morphological changes or differences in the testicular histology when compared with the control group. Four out of the 12 rats in the BPA treated group did not show any form of spermatogenic cycle and multinucleated giant cells were present in some of the lumen of the seminiferous tubules. The other eight rats from this group showed spermatogenic cycle in some of the seminiferous tubules; however giant cells were also present in these tubules (Tan *et al*., 2003).

Another study was performed in juvenile rats by Takahashi and Oishi in 2003. When Wistar rats (4 weeks old) were exposed trough injection (i.p.) of BPA (purity > 99%) at a dose of 0, 2 or 20 mg/kg bw/day dissolved in propylene glycol for 4 days a week during one month. A decrease of the prostate and seminal vesicle weights was observed but this decrease was significant for the ventral prostate weight of the 20 mg/kg group only ($p < 0.05$). There was no effect either on the testis or on epididymides weights. There was also a decrease of serum testosterone levels which was significant for the 20 mg/kg only (decrease by almost 70%; $p < 0.05$). Successive subcutaneous administration of BPA at a dose of 200 mg/kg/day for 4 weeks significantly decreased the testis, epididymis, prostate and seminal vesicle weights and the testicular daily sperm production ($p < 0.05$) in Jcl:Wistar rats (Takahashi and Oishi, 2003). When administrated to the same strain of rats but via an oral exposure to 0.25 % for 2 months, no effects were seen on the weights of the reproductive organs or on the daily sperm production or its efficiency. A previous study by the same authors (2001) showed that BPA surely induced reproductive toxicity when administered to male F344 rats in the diet at relatively high levels of 0.25–1.00%. The toxicity was characterized as seminiferous tubule degeneration and loss of late spermatids and the weight reduction of some sex accessory organs, while no serum testosterone concentrations were decreased.

In 2004, Akingbemi *et al*. exposed weanling Long-Evans rats (n=10-12 rats/group) to BPA (unknown purity) at low dose (2.4µg/kg bw/day) by gavage from PND21 to 90. The control received the vehicule only, i.e. corn oil. Within 24 hours after the last administration rats were killed. This chronic exposure to BPA at 2.4 µg/kg bw/day from PND 21 to 90 did not affect body weight or paired testes weight. Although serum testosterone levels were equivalent in control and BPA-treated rats, BPA treatment caused elevations in serum LH levels by more than 50% ($p < 0.01$). Leydig cell testosterone production, measured *ex vivo*, was reduced ($p < 0.05$) by BPA treatment as found in the other kind of treatement rapported in this study, and testosterone concentrations in the testicular interstitium of BPA-treated rats were lower than control values. Seminal vesicle weight, but not that of the prostate, was reduced by 15% ($p < 0.05$) in BPA-treated rats when compared to control group. According to the authors, these observations indicate that

chronic and direct exposures to low BPA concentrations, during this specific period of time, suppressed the androgen biosynthesis by adult Leydig cells and decreased seminal vesicle weights.

In the study of Norazit *et al.* (2012), juvenile male Sprague-Dawley rats (PND 22) were fed with soya extract, bisphenol A, and 17β-estradiol, respectively by oral gavage to determine the potential effect on the morphology of their reproductive organs and their hormonal levels. After three weeks of treatment (PND 43), all animals were sacrificed and the blood and testes were collected. All the three treatment groups showed histological differences in testes morphology compared to the control. Animals treated with soya extract and bisphenol A showed a decrease in circulating estradiol levels while animals treated with 17β-estradiol showed elevated circulating levels of estradiol. Only the animals treated with soya extract showed elevated levels of circulating testosterone. The results of the present study showed that, soya extract, bisphenol A, and 17β-estradiol can alter the histological structure of the testes and influence circulating steroidal hormone levels.

4.11.2.1.4 Adult exposure

All the studies published since 2002 describing effect of BPA exposure during adulthood on male fertility are positives.

In order to evaluate the effect of BPA on the antioxidant system of rat epididymal sperm, Chitra *et al.* (2003) exposed by gavage Wistar adult rats (45d old) to BPA (with a purity of 97%) during 45 days to doses of 0, 0.2, 2 and 20 µg/kg bw/day (n= 6 per group). The control group received vehicle only (olive oil). This study demonstrates a statistically significant dose-dependant decrease of the testes weight by approximately 6% ($p < 0.05$) and of the epididymides weight by 12-25% ($p < 0.05$). A very important increase of the ventral prostate weight (by 12-30%) and a decrease of the epididymal sperm motility and sperm count in a dose-dependent manner ($p < 0.05$). No change was observed for the seminal vesicules weight. It should be also noted that the body weight of the treated animals was not significantly different from the control one. The activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase were decreased while the levels of H_2O_2 and lipid peroxidation increased significantly in the treated rats as compared with the corresponding group of control animals. According to the authors, these results suggested that graded doses of BPA elicit depletion of antioxidant defence system and induce oxidative stress in epididymal sperm of rats and that the adverse effect of BPA on male reproduction may be due to the induction of an oxidative stress in sperm.

Herath *et al*. (2004) have also examined the effects of the BPA on epididymal sperm counts and sperm motility, various sex hormones and accessory reproductive organs. Pubertal Winstar rats (50-day old) were exposed via sub-cutaneous injections during 5 weeks to doses of BPA (purity of

95%) of 3 mg/kg bw/d. Each group contained 10 to 11 animals and the control group received vehicule only (DMSO). A decrease of the sperm count ($p < 0.05$) with no change in the motility was observed, with a significantly lowered by more than 50% plasma testosterone level ($p < 0.01$) and an increased progesterone level ($p < 0.05$). An increase of the ventral prostate weight was also noted (enlargement by 28%; $p < 0.01$). The decrease of the sperm count could be directly related to the effect on testosterone level, since these rats were exposed directly after their pubertal age.

Even if the fertility was not directly assessed, in both studies by Chitra *et al*. and by Herath *et al*. the same kind of effects was observed on the sperm count and the ventral prostate on the same exposure period, although the way of exposure was different. Then an adult exposure appears to be a sensitive window for BPA to exert its effects.

Sakaue *et al*. (2001) have exposed 13-weeks old Sprague-Dawley male rats (n=5/group) orally (gavage) to doses of BPA (purity of 99.6%) ranging from 2 ng to 200 mg/kg bw/d. The control group was exposed to vehicle only (corn oil). The authors did not find any significant change in the sperm count of rats exposed to the lowest doses (2, 20, 200, 2000 ng/kg bw/d). It should be noted that in this study rats were exposed during 6 days only (and not during 45 days like in Chitra *et al*.'s study). Nevertheless, a significant decrease in the daily sperm production ($p < 0.05$) and fertility was observed in rats exposed to the highest doses (20 µg - 200 mg/kg bw/d) and maximum suppression of sperm production occurred at 20 µg/kg/day.

In a study by Toyama *et al*. (2004), ICR mice and Wistar rats (6/species/dose) were exposed subcutaneously during 6 consecutive days to BPA. ICR mice (3 months old) and Wistar rats (4 months old) were injected with BPA dissolved first in dimethyl sulphoxide (DMSO) and then in olive oil. Doses were 20 or 200 µg/kg bw/injection. Controls were exposed to olive oil containing DMSO. After termination abnormalities were observed in the spermatids: the acrosomal vesicles, acrosomal caps, acrosomes and nuclei of the spermatids were severely deformed in both treated groups. Sertoli cells were not affected except for the ectoplasmic specialization between them and around the spermatids: incomplete specialization, redundant ectopic specialization and aplasia were observed. Ectoplasmic specializations between adjoining Sertoli cells, or the blood-testis barrier, were not affected by BPA exposure. The adverse effects of BPA observed on rats and mice spermiogenesis were reversible since the fertility of the treated males was not affected when test were performed 2 months after the end of the treatement in remaining animals (2/species + 1 control/species), these effects were then transitory.

4.11.2.1.5 Multiple exposure

In 2002, Nagao *et al.* found that low doses of BPA given in diet did not affect reproductive organs in estrogen-sentitive mice (C57BL/6N), when they are exposed at the embryonic, juvenile or sexually mature stage.

In a first part of the study, the authors tested the susceptibility to 17β-estradiol of two different strains of mice: C57BL/6N and ICR male mice. They were treated subcutaneously with E_2 at 10 µg/kg from PND 27 to 48. The susceptibility to estrogen was measured by weighing the reproductive organs and subjected them to necropsy. It was confirmed that the C57BL/6N was estrogen-sensitive since there was a significant decrease in the absolute and relative weights of the reproductive organs in E_2-treated animals when compared to the control group, when ICR mice were found insensitive. Moreover, no hisptopahologic changes were either observed in the ICR mice between treated and control groups conversely to C57BL/6N mice.

In a second part of the study, groups of 10 C57BL/6N pregnant mice were exposed to BPA at 0, 2, 20 or 200 µg/kg bw/day by oral gavage from GD 11 through 17. Male pups (30 males from 10 litters/ group) were weaned on PND 21 and exposed to BPA at 0, 2, 20 or 200 µg/kg by oral gavage from PND 21 to 43. Effects of BPA on adult animals were also investigated by treating groups of twenty C57BL/6N male mice 10-week old by gavage to BPA (purity > 99.0%) at 0, 2, 20 or 200 µg/kg for 6 consecutive days. The controls were given 0.5% carboxymethyl cellulose (5 mL/kg). The mice were terminated six weeks after the final administration. In C57BL/6N mice exposed to BPA as embryos, during lactation and during their puberty, the only effect seen was a significant decrease in the absolute weight of seminal vesicles in the 2µg/kg group ($p < 0.05$) as compared to the controls, but the effect was not dose-dependent. According to the authors it suggests that the decrease was not related to the BPA exposure since no effects were seen in the other treated groups.

In male exposed to BPA during adult or immature stage, there were no significant differences between the BPA-treated groups and the controls in reproductive organ weights (testes, epididymides, seminal vesicles – the prostate was not examined due to the difficulty according to the authors to sample the prostate only in mice) or their relative weights. Similarly there were no significant differences in the density of sperm between the BPA-treated groups and the controls for any of the exposure stages. These negative results might be due to the length of treatment corresponding to an insensitive window of exposure, to the endpoints investigated: no hormones measured.

4.11.2.1.6 Multigeneration exposure

➔ Mice

Continuous breeding study (Copy of the RAR-UK, Final report 2003, study considered as key study)
The effects of BPA on fertility and reproductive performance have been extensively studied in CD-1 mice (n= 20/ treated group/ sex (F0 generation), n= 40/ control group/ sex) using the test system known as the "Fertility Assessment by Continuous Breeding" (NTP, 1985b). BPA was administered in the diet at concentrations of 0, 0.25, 0.5 or 1.0% (daily intakes of BPA 0, 300 or 325, 600 or 650 and 1,200 or 1300 mg/kg in males or females repspectively) during a one-week premating period

and a 14-week mating trial (Task 2). No effects on fertility were observed in the low-dose group. A statistically significant decrease in litter size (controls: 11.4, treated males: 9.1, treated females: 5.9) and number of live pups per litter (controls: 11.3, treated males: 8.4, treated females: 5.5) were observed in the cross-over mating.
At necropsy of the F0 generation (controls and top dose group only), treatment-related effects were seen at the highest dose level; for both sexes relative liver weight was increased about 28% and relative combined kidney/adrenal weight increased 10-16% compared to controls, and relative seminal vesicle weight and proportion of motile sperm were decreased 19% and 39% compared to controls, respectively. No histological changes were observed in male reproductive organs. Overall, the signs of general systemic toxicity were not marked in this study and therefore the effects on fertility are not considered to be a consequence of parental toxicity. At necropsy of the F1 generation, treatment-related effects of similar magnitude were generally observed in males and females; compared to controls, increased relative liver weights (6-29%) and kidney/adrenal weights (13-20%) were observed in all treated groups. In males, a statistically significant decrease in relative right epididymis weight (11%, 16% and 18%) was observed in all treated groups, compared to controls. Left testis/epididymis weights were significantly decreased by 10% at the mid dose and 9% at the high dose, and seminal vesicle weight was significantly decreased by 28% at the top dose. A statistically significant decrease in sperm motility in the mid-dose group only was not considered treatment-related, but a chance finding. No histological changes were observed in the male reproductive organs. In the F1 generation at 300 mg/kg the only effect observed was a statistically significant decrease in epididymis weight of 11%. Histological examination was conducted on all F animals, and the only effects observed were toxicity to the liver and kidney at all doses. At 300 mg/kg no adverse effects on fertility were observed, though a decrease was seen in F epididymis weight. This effect is considered treatment-related as the magnitude of the decrease was dose-related. Although this was the only effect observed on reproductive organs at 300 mg/kg, the health significance of this finding is not clear.

Two multigenerational studies were performed by Tyl *et al*. The protocols and the discussion about the systemic toxicity have already been described in paragraph 4.11.1.1.3. Therefore, only the results for male are exposed here.

In the 2-generation study performed in CD-1 mice by Tyl *et al*. (2008), no toxicity was observed in the F0 or F1 generations and effects on the fertility were only observed at the highest dose (3500 ppm: 600 mg/kg bw/d). BPA exposure led to a decreased adjusted anogenital distance in F1 pups. In male pups, exposure to BPA induced a slight increase of the cryptorchism incidence at the weaning age, hypoplasia of the seminiferous tubules in the offspring at the weaning age and retardation in the preputial separation. Exposure to 3500 ppm of BPA decreased the epididymal sperm concentration in F0 male. A decreased paired testis weight (-17% compared to control) was observed in 3500 ppm F1 male pups (also observed in F2 male pups as relative testis weight/brain) together with decreased paired epididymal weight in 3500 ppm F1 parental male (-7% compared to control). In F2 male pups, the seminal vesicle coagulating gland weight was also decreased at all doses and significantly at 3500ppm (Tyl *et al*., 2002).

Authors reported also an abnormally high prostate weight for control animals that exceeds by > 70% the prostate weights reported by other authors in studies performed in the same strain and at similar age (Myers *et al*., 2009). This suggests that the dissection procedures for the prostate in this laboratory may include other non-prostatic tissues in the weight measurements, rendering them unusable for studying weight changes in the prostate due to exposure to estrogenic compounds. Moreover, as reported by Myers *et al.* (2009), Tyl *et al.* (2008) used estradiol as a positive control in their study. As indicated in a previous section, female mice were given estradiol before and during pregnancy and lactation at 80–220 μg/kg/day; after weaning, estradiol was fed to offspring at doses of 80–100 μg/kg/day. Estradiol was used as a positive control because BPA is a man-made endocrine-disrupting estrogenic chemical. Many published findings reporting effects of very low doses of positive control estrogens and BPA in CD-1 mice (Myers *et al.*, 2009) demonstrate that the CD-1 mouse was somehow rendered insensitive in the test system used by Tyl *et al.* (2008). The fact that a dose of 100 – 200 μg/kg bw/day estradiol was necessary to show an effect of the positive control predicts that Tyl *et al.* (2008) should not detect effects of BPA < 10–100 mg/kg/day (far above the low-dose range relevant to human exposures that was supposedly of interest) corresponding to the dose where Tyl *et al*. described effects in these studies, validating the low sensitivity of the model used by Tyl *et al.*

➔ Rats

The effect of BPA on fertility was evaluated in an oral two generation reproduction toxicity study in Crj;CD (SD) IGS rats (Emma *et al.*, 2001). The F0 generation consisted of groups of 25 rats per sex per group administered 0, 0.2, 2, 20 and 200 μg/kg/day BPA by gavage during a premating period of 10 weeks for males and 2 weeks for females and a 2-week mating period. Males and females from each group were randomly paired and co-habited for 2 weeks. Females were also administered the test material during gestation and lactation. F0 males and females were sacrificed after the mating period and weaning of F1 pups, respectively. Twenty-five male and female F1 generation offspring from each group were retained after weaning for assessment of their reproductive capacity. F1 animals were administered bisphenol-A for a 10-week premating period and a 3-week mating period (see below). Again, females received the test material during gestation and lactation, and male and female parental animals were sacrificed at the same times used for the F0 generation. Twenty-five male and female F2 generation offspring from each group were retained after weaning for assessment of sexual maturation. Males and females were administered the test material until they were sacrificed at the age of 7 and 14 weeks, respectively.
No significant differences were observed between BPA and control animals for the time to preputial separation. Compared to controls, a statistically significant decrease (<5%) in AGD was seen in F1 males at 0.2, 20 and 200 μg/kg/day, and F2 males at 20 and 200 μg/kg/day. These decreases were not statistically significant when the ratio of the AGD to body weight was determined (the AGD is correlated with body weight). No changes in the motility and morphology of sperm were observed in F0 and F1 treated males.
Compared to controls, a statistically significant decrease in the absolute (17%) and relative (20%) weight of seminal vesicles (including the coagulating gland) was observed in F2 males only at 2

µg/kg/day but no statistically significant decrease was observed at 20 or 200 µg/kg/day. Histopathological examination revealed no morphological changes in the seminal vesicles and there were no weight changes in associated organs (prostate gland, testis and epididymis). Therefore, in this two-generation study, no parental toxicity or effect on fertility was observed at the low-dose levels employed.

In three generation study, most of the reproductive parameters (for instance mating, fertility and gestational indices, daily sperm production, offspring sex ratios, postnatal survival…) remain unaffected except at 7500 ppm where total pups and live pups/litter on postnatal day (PND 0) were decreased. This decrease was in line with what described by Fernandez *et al.* in 2010.The results on testes, epididymis, prostate and seminal vesicles weight are difficult to interpret due to decrease in body weight. Therefore, their absolute weight is reduced in most of the generations of the 7500 ppm group (the only exception was F0). Nevertheless when the relative weight of these organs (% of the sacrifice weight) is analysed, a significant increase of the paired testis and the paired epididymides is observed for all 3 generations. Another significant effect was on sperm endpoints. There was a decreased epididymal sperm concentration in F1 males and decreased daily sperm production in F3 males of the 7500 ppm dose group. There were no effects on sperm morphology or motility. In male offspring, the absolute age of preputial separation (days) was significantly delayed in the F1 generation at 750 and 7500ppm, in the F2 generation at 0.3, 75, 750 and 7500ppm, and in the F3 generation at 7500ppm. When the age of acquisition was adjusted for the body weight at acquisition, preputial separation was delayed in the F1 generation at 750 and 7500ppm and in the F2 and F3 generations at 7500ppm (Tyl *et al*., 2002).

4.11.2.1.7 Transgeneration exposure

More recently, in a study performed by Salian *et al*., (2009c) Holzman females rats (8 rats per group) were gavaged with either BPA pure at 99.8% (1.2 and 2.4 µg/kg bw), a vehicle control (sesame oil) or with a positive control, the Diethylstilbestrol (DES) (10 µg/kg bw) during the perinatal period (GD 12 to PND 21). The diet was total without phytoestrogens. Adult F1 males (75 days) were subjected to fertility assessment (n=24 animals/group) by mating with unexposed females. The reproductive functions of the subsequent F2 and F3 litters, that were never exposed to BPA, were investigated in a similar manner. No general toxicity was observed in any of the 3 generations. Hormonal analysis including testosterone, estradiol, LH and FSH serum levels, sperm count and motility, histological evaluation of testis and immunohistochemical localization of steroid receptors in the testes were carried out for the F1, F2 and F3 generation adult rats. An dose and generation dependant increase in post implantation loss was observed (3.93% in F1 at 1.2 µg/kg bw up to 18.7% in F3 at 2.4 µg/kg bw ($p<0.0001$)) and a decrease in sperm count (1.2µg: $181.6x10^6$ and 2.4µg: $162.8x10^6$ compared to 216.7 $x10^6$ in controls) and motility (1.2µg: 80.92% and 2.4µg: 84.42% compared to 92.13% in controls) were observed in the F1 male offspring, with a significant decrease of sexual hormone concentrations of FSH ($p < 0.001$ for the

1.2µg group and $p < 0.05$ in the 2.4µg group), LH ($p < 0.05$ for the 1.2µg group), testosterone ($p < 0.001$ for the 1.2µg group and $p < 0.05$ in the 2.4µg group) and oestradiol ($p < 0.001$ for both 1.2µg and 2.4µg groups). A reduction in the testicular expression profile of Estrogen Receptor β (ERβ) and Androgen Receptor (AR) was observed, whereas the ERα expression was increased. These effects were very prominent in the subsequent F2 and F3 generations, except for the ERα. For instance, the increase in post-implantation loss was significant in F3 (14.68% for 1.2µg; $p < 0.01$ and 18.73% for 2.4µ; $p < 0.0001$). These transgenerational effects let the authors think that they could be mediated via epigenetic mechanisms (Salian *et al*., 2009c).

Conclusion on male reproductive system in animals

In animals various effects have been observed on male fertility.

Concerning effects on the male reproductive system following a prenatal, neonatal or post-natal (during lactation) exposure, large discrepancy exist between the studies.
In the animals treated *in utero* and/or lactation, most of the studies performed in mice or rats found effects on sperm production or quality (Tinwell *et al.* (2002); Salian *et al.* (2009c)) or abnormalities in the seminiferous tubules (Iida *et al*., 2002; Okada and Kai, 2008). But some studies do not observed any effects or in one strain of rats only (Tinwell *et al*., 2001; Howdeshell *et al*., 2008 and to some extend La Rocca *et al*., 2011). Based on the complexicity of the fertility homeostasis, it is difficult to identify the cause explaining discrepancies between the different studies available. They are generally performed with different protocols, in different strains, which might demonstrate differences in susceptibility for this *in utero* exposure. Indeed, in has been demonstrated that the Sprague-Dawley rats may be insentitive to estrogenic compounds, and may then not be a reliable animal model to demonstrate effects with this kind of substance (NTP, 2001). Another parameter that could explain the absence of effects observed by Okada and Kai and by Kobayashi is the route of exposure. Actually the sub-cutaneous injections lead to no or limited effects while an oral exposure may elicit more effects when performed during this specific period of time.
When animals were exposed to BPA neonatally, this window of exposure generally induced effects on fertility (Salian *et al*., 2009b) with effect on the sperm parameters (Salian *et a.*;, 2009; Aikawa *et al*., 2004), on the reproductive organ weight, the histology of the seminiferous tubules or induce a decrease of plasmatic level of testosterone (Nakamura *et al*., 2010; Toyama and Yuasa, 2004b) in rats or mice, except in Kato *et al*.'s study (2006). In this latest publication, Sprague Dawley rats where exposed to BPA through sub-cutaneous injections leading to no effect on reproductive parameters. As for the neonatal exposure apparently Sprague-Dawley rat seems not to be a suitable strain due to his lack of sensitivity to estrogenic-like agents (NTP, 2001).

These findings are confirmed in all the juvenile studies when tested whatever the strain and the dosage used (Della Seta *et al.*, 2006 Takahashi and Oishi, 2003 Akingbemi *et al.*, 2004), or when

adult Wistar rats are exposed since two different ways of exposure (by oral route or using sub-cutaneous injections) a decrease in sperm count was observed, a decrease of the testis weight and an increase of the ventral prostate weight have been observed. (Chitra *et al*., 2003; Herath *et al*., 2004). Because of this latter effect, an effect on testosterone levels may be suspected, which has been demonstrated in Herath's study. The effect on testosterone was also observed when juvenile animals are exposed since a decrease in plasma testosterone levels was noted in the studies by Della Seta *et al*. (2006) and by Takahashi and Oishi (2003).

In 2 independent studies (Tan *et al*., 2003 and Toyama *et al*., 2004) histology of the testes was precisely investigated and morphological changes and abnormalities were reported. Other effects were observed following an exposure during the pubertal age, such as alteration of the sexual behavior but this result has to be confirmed.

In some studies in which rats were exposed *in utero* and/or during lactation no effects were observed. These studies are generally performed in different strains, so maybe some strains are more susceptible than others to this *in utero* exposure. The choice of the Sprague-Dawley rats could also explain the limited effects in the 3-generations study by Tyl *et al.* (2002). Effects were observed at the highest dose (7500 ppm) only. Despite the fact that this study was well-conducted, authors choose the SD rats, which are generally used in the reproductive study, but it has been demonstrated that this strain of rats have a very low sensitivity to estrogenic compounds, relatives to women this strain requires 100- to 400- higher doses to produce effects (Yamasaki *et al*., 2002). Then, in 2001, the NTP stated that 'because of clear species and strain differences in sensitivity, animal model selection should be based on responsiveness to endocrine-active agents of concern (i.e., responsive to positive controls) not on convenience and familiarity'. This statement of a low sensitivity of Sprague-Dawley rats to estrogenic compounds is supported by the study of Tinwell *et al*. (2002) in which effects are seen in male Alderley Park rats only (and not Sprague Dawley). This could also explain the limited results observed in an earlier study perfomed in IGS Sprague Dawley (Ema *et al*., 2001) at low dose of BPA. Only,statistically significant decrease in absolute AGD in F1 & F2 males (not statistically significant when the ratio of the AGD to body weight was determined) and statistically significant decrease in the absolute (17%) and relative (20%) weight of seminal vesicles in F2 males only at at the lowest dose.

Table 16: Summary table of the BPA effects on the male reproductive tract in animals

Method	Effets	Reference
Exposure during gestation/lactation		
Sprague Dawley and Alderley park (derived from Wistar) Rats Oral route 20 µg/kg, 100 µg/kg bw, 50 mg/kg GD6 - GD21	<u>Observations made in adults (90 days)</u> : Significant ↘ sperm production at 50 mg/kg only in AP rats	Tinwell *et al.*, 2002

ddY mice oral route by gavage 0, 1, 10 and 100 mg/kg bw/day GD10 - GD17	Observations made at 60 and 120 days (adults): Histological abnormalities in seminiferous tubules	Iida et al., 2002
Male Long Evans Rat Oral route 2 - 20 - 200 µg/kg bw/d GD7 - PND18	Observations made at adult age (4 months old): No significant effects observed on the organ weights of the reproductive tract and on the epidiymal sperm count but the controls received diet with phytoestrogens.	Howdeshell *et al.*, 2008
Crj: CD (SD) IGS strain Rats Oral route 4 - 40 and 400 mg/kg bw/d GD6 - PND20	Observations made at PND63 and PND252: significant ↗ of the plasma testosterone concentration at 9 weeks only, with no alterations of LH or FSH concentrations from 4mg/kg bw/d and onward.	Watanabe *et al.*, 2003
ICR mice Oral route 5 or 10 µg BPA/mL in drinking water throughout embryonic/fetal life and during lactation	Observations made in 4-weeks old pups: ↗ thiobarbituric acid-reactive substances in testis ↘ wet weight of testis	Kabuto *et al.*, 2004
Long-evans female rats Oral route – gavage 0 – 2.4 µg/kg bw/day GD12-PND21	Observations made at 90-day old: ↘ testis and seminal vesicles weight. Unchanged prostate weight. ↘ specific Leydig cells testosterone production	Akingbemi *et al.*, 2004
CF-1 mice Oral route 0 – 10µg/ kg bw/d GD14-18	Abnormal growth of the prostate since primitive prostate gland duct epithelial proliferation was found at birth	Timms *et al*., 2005
ICR mice Sub-cutaneous 100 µg and 5 mg for females 3d before mating + 1.2 or 60 µg/day throughout gestation and lactation	Observations made at age of 4 weeks: ↘ Percent of seminiferous tubules with mature spermatids in mice exposed to 5000 µg (60 µg/d) BPA. No changes for others parameters (weight of reproductive organs, testosterone, histological analysis).	Okada et Kai, 2008
Holtzman Rats Oral route 1.2 – 2.4 µg/kg bw/d GD12 - PND21	**F1, F2, F3** 1.2 et 2.4µg/kg bw/d : ↘ litter size significant ↗ post-implantation loss at both doses in F3. ↗ bodyweight (except F1 for 2.4µg/kg bw/d) ↘ sperm count and sperm motility at both doses. ↗ copulation delay. ↘ expression profile of testicular ER βe↘ expression profile testicular AR (except for F2 and F3 à 2.4µg/kg bw/d).	Salian *et al.*, 2009c

C57/BL6 mice Oral gavage 0 – 50 – 1000 µg/kg GD10-16	Observations made at PND56: No significant changes in male body weight in F1 exposed males. No differences detected in seminiferous tubules diameters. No effect on serum testosterone level. No changes in sperm production observed in adult testis after exposure to BPA.	LaRocca *et al.*, 2011
Male mice (Mus musculus) animals fed the high-phytoestrogen diet and perinatal BPA doses of 0, 17.5, 175, or 1750 lg/day.	When males each encountered a sexually receptive female, there were fewer intromissions among those given 17.5 or 175 lg and fewer ejaculations among those given 17.5 lg, but the 1750 lg dose had no effect. Perinatal BPA dosages thus influenced male sexual behavior nonmonotonically, with impairment evident in a discrete dose range among males on a high-phytoestrogen diet.	Decatanzaro D et al., 2012
Neonatal exposure		
Sprague-Dawley male rats Sub-cutaneous injections 0 – 2 – 11 – 56 – 277 – 97,000 µg BPA/kg bw/day Birth – PND9	Observations made at PND10, 35 and 150: Normal reproductive parameters.	Kato *et al*. 2006
Holtzman Rat Sub-cutaneous 100 - 200 - 400 - 800 - 1600 µg/kg bw/d PND1-5	Observations at adulthood : Effects on fertility parameters with a significant ↗ of the copulation delay and the post-implantation loss from the 200 µg/kg bw/d exposure dose. ↘ litter size from 200 µg/kg bw/d BPA. Significant ↘ of the sperm count and motility from 100 and 200 µg/kg bw/d respectively and onward. Significant reduction in the expression of Cx-43 (PND 45 and 90) and increase in N-cadherin (PND 45 et 90) and ZO-1 (PND 90) expression.	Salian *et al.*, 2009b
SHN newborn mice Sub-cutaneous injections 175 – 17,500 µg BPA/ kg bw/day Birth – PND5	Obervations made at 10 weeks: ↘ moving sperms at high dose; ↗ malformed sperms in epididymis at both doses. No marked histological changes in testes.	Aikawa *et al*. 2004
Long-evans weanling male rats Oral route – gavage 0 – 2.4 µg/kg bw/day PND21-35	Observations made at PND35: Decrease in the serum LH and T levels	Akingbemi *et al.*, 2004
ICD (CD-1) newborn mice or Wistar rats Sub-cutaneous injections from 0.1 to 10 µg BPA/animal/injection for mice from 1 to 600 µg BPA/injection/animal for rats At PND1, 3, 5, 7, 9 and 11	Observations made in juvenile-adult animals: Abnormalities in spermatids at all doses but not dose dependant. No effects on the fertility	Toyama and Yuasa (2004b)
Pubertal exposure		

Sprague Dawley rats Oral -gavage 40 µg/kg bw/d PND23 - PND30	Observations made at pubertal and adult age: ↘ Testosterone levels in juveniles, lasting until adult age. Decrease of sexual performances in adult animals.	Della Seta *et al.*, 2006
Wistar/ST 4-week old male rats Sub-cutaneous injections 0 - 11.4 - 57.1 - 114.2 mg BPA/kg bw/day 6 weeks	Observations made at 10 weeks: ↘ reproductive organ weights at 57.1 - 114.2 mg BPA/kg bw/day Effect of the plasma testosterone levels (↘)at 57.1 - 114.2 mg BPA/kg bw/day Morphological abnormalities in the seminiferous tubules.	Nakamura *et al.*, 2010
Sprague Dawley rats Oral route – gavage 100 mg/kg bw/d PND23-53	Observations made at PND53: Only 66.7% of the treated rats reached a complete preputial separation. No significant effects were seen on the testis, epididymis or adrenal weight but morphological changes or differences in testicular histology.	Tan *et al.*, 2003
4-week old Wistar rats i.p. injections 0 – 2 – 20mg BPA/kg bw/day 4days/week during 1 month	Observations made at pubertal age: ↘ prostate and seminal vesicles weight at 20mg BPA/kg bw/day. ↘ serum testosterone levels. No effect on testes or epididymis weight.	Takahashi and Oishi, 2003
Jcl:Wistar rats 4 weeks old Sub-cutaneous injections 200 mg BPA/ kg bw/day 4-week	Observations made at pubertal age: ↘ prostate, testis, epididymis and seminal vesicles weight. ↘testicular daily sperm production	
Long-Evans weanling male rats Oral route – gavage 0 – 2.4 µg/kg bw/day PND21-90	Observations made at 91days: No effect on the body weight or the testis weight. Decrease of the sex hormone levels, specifically the T levels produced by the Leydig cells.	Akingbemi *et al.*, 2004
juvenile male Sprague-Dawley rats, PND 22, Oral route – gavage. 3 weeks of treatment (until PND 43); 100 mg/kg bw	The results of the present study showed that, soya extract, bisphenol A, and 17β-estradiol can alter the histological structure of the testes and influence circulating steroidal hormone levels.	Norazit *et al.*, 2012
Exposure during adulthood		
Wistar Rat Sub-cutaneous 3000 µg/kg/d PND52 - PND87	Observations made at adult age: Significant ↘ of T plasma levels and epididymal sperms (motility not affected). Significant ↗ of ventral prostate weight with high IGF-1 level.	Herath *et al.*, 2004

Wistar Rat Oral route 0,2 - 2 - 20 µg/kg bw/d PND45 – PND90	Observations at adult age: Significant ↘ relative weights of testis and epididymis Significant ↗ of the relative weight of the ventral prostate. Significant ↘ epididymal sperm motility and sperm count. Effects on levels of enzymes related to oxydative stress.	Chitra *et al.*, 2003
Sprague-Dawley Rats Oral route 0.02 – 0.2 - 2 – 20- 200 mg/kg bw/d Exposure from Day 6 to adult age (11 weeks)	No significant effect on the sperm production.	Sakaue *et al.*, 2001
4-month old Wistar rats or 3-month old ICR mice Subcutaneous route 0, 20 or 200 µg/kg bw/injection Exposure during 6 consecutive days	Effects on the spermiogenesis (abnormalities in spermatids) at both doses. No effects on fertility when tested 2 months after the end of the treatement	Toyama *et al.*, 2004
Multiple exposure		
C57BL/6N female mice Oral route –gavage 0 – 2 – 20 – 200 µg/kg bw/day GD11-17	Decrease in absolute seminal vesicle weight at 2 µg/ kg/day only. No effects when treated at juvenile or adult stage.	Nagao *et al.*, 2002
Multi-generations exposure		
Rat **IGS (SD) rats** 25 rats /sex /group administered 0, 0.2, 2, 20 and 200 µg/kg/day Oral by gavage	statistically significant ↘ in absolute AGD in F1 & F2 males (not statistically significant when the ratio of the AGD to body weight was determined) and statistically significant ↘ in the absolute (17%) and relative (20%) weight of seminal vesicles in F2 males only at at the lowest dose. 2 generation study similar to OECD 416 (Deviations: *Female treated for 2 weeks only before mating. *Low doses used)	Ema et al., 2001
Mice Oral route 0 – 0.015 – 0.3 – 4.5 – 75 – 750 – 7500 ppm corresponding to 0.0007-0.003, 0.015-0.062, 0.22-0.73, 4.1-15.4, 37.6-167.2 and 434-1823 mg/kg bw/day Exposure from 10 weeks before mating until adult age. N = 28 animals/dose	No effect on reproduction has been seen except at the highest dose (7500 ppm): Effect on reproductive organ weights, on DSP and epididymal sperm concentration. Follow OECD guideline 416 (two generation reproduction toxicity study), TG 416 enhanced GLP compliant study	Tyl *et al.*, 2008

Rat Oral route 0 – 0.018 – 0.18 – 1.8 – 30 – 300 and 3500 ppm Exposure from 10 weeks before mating until adult age. 30 males/dose 30 females/dose	No effect on reproduction has been seen except at the highest dose (3500 ppm): According to EPA OPPTS 837.38000, 1998 GLP compliant study	Tyl *et al.*, 2002
Mice CD-1 (n= 20/ treated group/ sex, n= 40/ control group/ sex) 0, 0.25, 0.5 or 1.0% (daily intakes BPA estimated 0, 300 or 325, 600 or 650 and 1,200 or 1300 mg/kg for males or females resp. In diet	relative seminal vesicle weight and proportion of motile sperm ↘19% and 39% / ctrls. No histological changes were observed in male reproductive organs. Left testis/epididymis weights were significantly ↘ by 10% at the mid dose and 9% at the high dose, and seminal vesicle weight was significantly ↘ by 28% at the top dose. Continuous breeding study (Task 1, dose-finding; Task 2, continuous breeding phase; Task 3, identification of the affected sex, and Task 4, offspring assessment.)	NTP, 1985b

Additional studies:

Additional supportive studies exist, exploring the mechanisms that could explain the effects of the BPA on the male reproductive system in animals.

Salian *et al*. (2009b) exposed, using sub-cutaneous injections Holzman males rats to various doses of BPA pure at 99.8% (100, 200, 400, 800, 1600 µg/kg bw/d) from birth to PND5. DES was used as the positive control and the negative control groups received sesame oil. Male fertility was assessed during adulthood and the lowest dose of BPA that was most effective at impairing fertility was determined. Immunohistochemical localization for Connexin 43 (Cx-43, gap junctional), Zona Occludin-1 (ZO-1, tight junctions) and N-cadherin (adherens junction) was carried out on testicular tissue sections obtained from PNDs 15, 30, 45 and 90 of rats exposed to lowest dose of BPA that impaired fertility. These proteins are implicated in the junctions of the Sertoli cells, and are important for maintaining the spermatogenesis. Females mated with male rats that were exposed neonatally to various concentrations of BPA showed a significant increase in post-implantation loss and a decrease in litter size. There were significant changes in sperm count along with hormonal imbalances in the rats exposed neonatally to BPA. The 400µg/kg bw/d dose of BPA was determined as the lowest dose that was capable of impairing male fertility. A significant reduction in the expression of Cx-43 (PND 45 and 90) and increases in the expression of N-cadherin (PND 45 and 90) and ZO-1 (PND 90) were observed in the testes of rats exposed neonatally to effective dose of BPA.

This could be linked to what it has been demonstrated in a recent study (Li *et al.*, 2009) : adult rats treated with acute doses of BPA displayed signs of germ cells loss and the treatment was also able

to disrupt the blood-testis barrier when administered to immature 20-day-old rats. They were treated with BPA (purity not known), which was dissolved in absolute ethanol and then diluted in corn oil. Rats were treated with multiple doses of BPA. In this study, the exposure to BPA covered a range of 0.02 to 50 mg/kg bw/day due to its non-monotonic dose response curve observed for some endpoints according to the authors. It was shown using an immunofluorescence analysis that the reversible disruption of this barrier was associated with declining levels of several junction's proteins such as occluding, Connexin-43 (Cx-43) or N-Cadherin. The effect of the level of expression of these proteins could be one of the mechanisms leading to the alterations of the spermatogenesis. Since the Sertoli cells junction's proteins (SCJPs) are necessary to maintain the hemato-testicular barrier, the altered expression of all these 3 SCJPs in response to BPA suggests that BPA is a potential Sertoli cells toxicant.

These results are consistent with the previous results found *in vivo* in immature rats in the studies by Toyama *et al.* (2004), Chitra *et al*. (2003) or Herath (2004). The exposure used by Li *et al*. (2009) is supposed to mimick an acute short-term or accidental human exposure to BPA. Therefore, the doses used *in vitro* correspond to high level of BPA *in vivo.* Only an untreated control group was used to avoid the unnecessary slaughter of animals. However, a vehicle control group was included in the study to examine the effects of BPA on the blood-testicular barrier integrity in immature rats when a significant effect was detected based on pilot experiments.

The observations made *in vivo*, were confirmed using primary Sertoli cells cultured *in vitro* with established tight junction-permeability barrier that mimicked the blood-testis barrier *in vivo*. Cells were treated with either vehicule control (0.1% ethanol) or BPA at 40μM or 200μM on day 3–5 after the functional TJ-barrier was formed in cultured Sertoli cells. These concentrations of BPA were selected for *in vitro* experiments since BPA at 40μM was reported to induce junction disruption in the SerW3 Sertoli cell line (Fiorini *et al*., 2004), and 200μM of BPA is equivalent to ~45 mg/kg b.w. in the *in vivo* animal study.

The reversible disruption of Sertoli cell tight junction barrier by BPA was associated with an activation of ERK, and a decline in the levels of selected proteins at the tight junction, basal ectoplasmic specialization, and gap junction at the blood-testis barrier. Studies by dual-labeled immunofluorescence analysis and biotinylation techniques also illustrated declining levels of occludin, connexin 43, and N-cadherin at the cell–cell interface following BPA treatment. In summary, BPA reversibly perturbs the integrity of the blood-testis barrier in Sertoli cells *in vitro.*

These results are consistent with those previously reported in 2004 by Fiorini et al. (2004), who have already demonstrated that BPA was able to affect the intercellular junctions between Sertoli cells. SerW3 Sertoli cell line was used and treated with BPA at 45μM diluted in DMSO. The effects on junctional protein expression were investigated for doses that did not exert any effect on cell viability (as assessed with MTT). BPA reduced the level of occludin (tight junction protein), N-cadherin (adherens junctions protein) and connexin-43 (gap junctions protein), with the key effect on Cx-43. Zonula-occludens 1 (ZO-1) expression was not altered. Therefore, since the expression of proteins implicated in the junctions which form the blood-testis barrier was affected, it can be

concluded that it has been demonstrated *in vitro* that bisphenol A could affect the integrity of the barrier, and this could be an explanation for its effects on the male reproductive tract.

The results observed in vitro support the *in vivo* findings.

4.11.2.2 *Human information*

Several epidemiological studies have been conducted, each of them with methological limitations.

The following three studies considered of acceptable quality (Meeker, Mendiola and Galloway) address the issues of male sex hormones.

In the studies by Meeker *et al*. (2010a) and Mendiola *et al.* (2010), two populations were studied: men in infertile couples and fertile men. In both populations the concentrations of various proteins and hormones (FSH, LH, testosterone, oestradiol, Inhibin B…) and urinary concentrations of BPA were measured. Hormonal and protein changes were observed in both studies.

In Meeker *et al.* study, the relationship between urinary BPA levels and seric testosterone and other reproduction hormones levels was investigated. BPA was identified in 89% of the subjects with a median level of 1.3 ng/mL. BPA level has been shown to be inversely related to the Inhibin B level and to the E_2/T ratio. Since estradiol is produced through the aromatization of testosterone, a reduction in the E2:T ratio is considered a marker for declined aromatase activity. The BPA level was also directly correlated with FSH level and with the FSH/Inhibin B ratio. Furthemore BPA levels and free androgens index are negatively related.

It should be noted 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). Nevertheless this study has several limitations. First, the present study was cross-sectional with a single hormone measure on the same day as a single urine sample from over half the men. Thus, reverse causation cannot be rule out to explain the findings. Another limitation is the likelihood of exposure measurement error due to the high within-individual temporal variability in BPA exposure and the availability of multiple BPA measures from only a subset of participants. Finally, because the present study was conducted among men recruited through an infertility clinic the ability to generalize the results to the general population may be limited. However, because to date no evidence exists that men from an infertility clinic are differentially affected by BPA exposure; authors of the study expect their results to be generalizable.

In the study by Mendiola *et al.* (2010), various parameters were assessed in fertile men. No significant association was observed between semen parameter and urinary BPA concentration. But a significant inverse association was found between the latter and the Free Androgen Index (FAI,

which has been calculated as the total testosterone level x 100/SHBG level) and the FAI/LH ratio. A significant positive correlation between BPA concentration and SHBG (Sex hormone binding globuline) was also observed.

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, estradiol as well as SHBG (sex hormone-binding globulin) (Galloway *et al.*, 2010). In this study performed in the general population sex hormone levels were measured in 715 adult men (20-74 years), the daily BPA excretion was measured in urine samples. A higly significant association was reported between the daily BPA excretion and total testosterone concentrations. It should be noted that the number of men in this study with age between 66-74 years is abnormally high.

Several studies address the question of the quality of gametes in men.
The cross-sectional study of Li *et al.* (2010) recruited male workers exposed to BPA and estimated by questionnaire the frequency of certain sexual dysfunctions (reduced sexual desire, erectile difficulty, ejaculation difficulty and reduced satisfaction in sex life) declared during an interview (Li *et al.*, 2010). The questions were based on the International Index of Erectile Function and the Brief Male Sexual Function Inventory. The frequency of these disorders was compared with what was observed using a similar approach in a control population composed of men not exposed to BPA. The participation rate was 62% for those exposed versus 55% for those not exposed. The authors of the study concluded that there was a dose-response association between high levels of cumulative exposure to BPA and an increased risk of impaired sexual function. To remove the potential interference of exposure to other chemicals, the authors conducted additional analyses after excluding those who had a history of exposure to other chemicals or heavy metals. But the observed associations between a BPA exposure and the risk of sexual dysfunction remained. However some questions remain concerning the interviews, during which it was necessary to answer a questionnaire, were they blinded? And how were threshold values regarding sexual activity chosen? Moreover there are no clinical data to support these results obtained by self-reporting.

In a second article by Li *et al.*, which completed the first study, the level of BPA in urine was obtained in workers exposed to BPA because they were working in factories where BPA or epoxy resin was manufactured (Li *et al.*, 2010b). Workers from factories with no occupational exposure to BPA in the work environment were also recruited in the same regions. 514 (58% of the identified workers) agreed to participate. At the end some participants were excluded because they did not provide urine samples or because they did not answer to the sexual function questions. 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). These results further support the previously reported findings (Li *et al*., 2010a)

In 2011, Li *et al.* demonstrated a link between sperm quality and urinary BPA concentration in the population of workers exposed to BPA like this was demonstrated in their 2 previous studies. High BPA concentration level was associated with decreased sperm count, mobility and vitality. Specifically an increasing urine BPA level was associated with lower semen concentration, lower total sperm count, lower sperm vitality and lower sperm motility (forward movement). On the other hand no linear correlation between urine BPA levels and semen volume or sperm morphology has been observed. The participation rate was 58% (n= 514 of 888 eligible) but the analysis was done on only 218 men for whom the semen specimen meet WHO's collection guidelines or did in fact provide a semen sample (Li *et al.*, 2011). To determine if the observed association was still valid if the people exposed at their workplace are excluded (since the environmental BPA exposure level is lower compared to the occupational one), the authors examined the men exposed to BPA only because of an environmental exposure. Similar significant associations were observed in this reduced sample (n = 87-88).

In a population of fertile men (partners of pregnant women; N = 375), no correlation was found between sperm parameters and urinary concentrations of BPA (Mendiola *et al.*, 2010). The parameters analysed were the seminal volume, the sperm concentration, the percent of motile sperm and morphologically normal sperm, total motile count and total sperm count. Only an inverse relationship was observed between urinary BPA concentrations and the free testosterone index (FTI) and the FTI/LH ratio (as described previously, for both adjusted and unadjusted for creatinine).

A similar study was carried out by Meeker *et al.* in a population of men consulting in an infertility clinic (n=190). As described previously, the urinary concentration of BPA 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). But a link between BPA exposure and impaired sperm quality (only for urine samples obtained the same day as the semen collection) was also established. An increase in urinary BPA concentration was associated with declines of 23% in sperm concentration, of 7.5% in motility, and 13% in morphology, along with a 10% increase in sperm DNA damage measured as the percentage of DNA in comet tail. Nevertheless these data remain to be confirmed (Meeker *et al.*, 2010b) especially because some of the semen analysis parameters were not performed according to the WHO recommandations (WHO, 1999).

A more recent study (Bloom *et al*., 2011a) is only a preliminary prospective cohort study due to the small number of subjects (n= 27 couples). This dataset includes couples for whom embryos were generated during an In Vitro Fertilization (IVF). Embryo cell number (ECN) was assessed on the day of embryo transfer as the number of blastomeres present. Embryo fragmentation score (EFS) was assessed on the day of transfer (usually 48 h post-fertilization) as: Grade 1, 0% fragmentation;

Grade 2, 1–10%; Grade 3, 11–25%; Grade 4, 26–50%; and Grade 5, ≥51%. There was no association for BPA measured in women with ECN (n = 184) or with EFS (n=186) adjusted for male BPA as well as for the age and race of each partner, and day of transfer for ECN. However the authors found a 30% decrease in the adjusted odds for a higher ECN is suggested, and a 46% decrease in the adjusted odds for higher EFS is detected for each log-unit increase in male BPA. This inverse association between the paternal BPA concentrations and the effects on the embryo quality may be due according to the authors to a sperm integrity disruption, or to an unexplained biologic phenomenon. Nevertheless since the sample size is rather small, these results need to be confirmed.

Another recent retrospective study was performed by Miao *et al*. (2011). The participants for this sudy were recruited among the population of a previous study (Li *et al*., 2010b). A total of 153 boys were included in the final analysis. A total of 153 boys were included in the final analysis, among them 56 with parental occupational exposure during pregnancy and 97 without. Among the 56 boys who had parents who were exposed to BPA during pregnancy, 15 had a mother who was exposed to BPA in the workplace during the index pregnancy, 38 had a father who was exposed to BPA in the workplace while his wife was pregnant with the index child, and 3 had a mother and father who were both exposed to BPA in the workplace. The participation rate was 78.86% since first 194 boys were eligible. The BPA exposure of the parents was estimated using a personal air sample monitoring and also measured in urine among participants who provided an urine sample. Concerning the measure of the anogenital distance (AGD), it was measured from the center of the anus to the anterior base of the penis by the same physician who was blinded to the exposure status of the participants. And because AGD is a continuous variable, AGD for boys was compared between exposed and unexposed groups using multiple linear regression models after controlling for age and weight.

The age of the participants was ranging from 0 to 17 years old and 81% were younger than 10 years. After controlling for the boys' ages and weights using linear regression, parental occupational exposure to BPA during pregnancy was associated with shortened AGD in male offspring. Compared with boys coming from unexposed families, the AGD was 8.11 mm shorter on average for boys with maternal exposure (p = 0.003) and 2.87 mm shorter for those with paternal exposure (p = 0.15), then the association was stronger for maternal exposure. When the analysis was restricted to boys younger than 8 years in order to reduce the effects of the pubertal development, the results remained the same. There was also a dose-response relationship with increased BPA exposure levels in pregnancy associated with greater magnitude of shortened AGD in male offspring, with a statistically significant trend for the association (p = 0.008).

Fénichel *et al.* (2012) studied the relationship between fetal exposure to BPA and cryptorchidism in boys. Using a radioimmunoassay performed after extraction, validated by high-performance liquid chromatography and mass spectrometry, active levels of unconjugated BPA (uBPA) in cord blood (CB) were measured in 152 boys born after 34 weeks gestation, with cryptorchid or descended testes. Active uBPA was detectable in all CB samples, with values in the control group (n ¼ 106) of

0.14–4.76 ng/ml, median: 0.9 ng/ml; mean+SD: 1.12 ng/ml+0.86 ng/ml, which did not differ from cryptorchid boys (n ¼ 46, 1.26+1.13 ng/ml, P ¼ 0.38). uBPA in controls correlated with CB inhibin B (P , 0.01) and total testosterone (P , 0.05), and with maternal milk polychlorinated bisphenyl 138 (P , 0.03). uBPA did not correlate with clinical maternal or fetal parameters or with other steroid or polypeptide CB hormones assessed. The presence of uBPA in all CB samples suggests placental transfer and fetal exposure. Similar uBPA levels in the control and cryptorchid groups make the participation of fetal exposure to uBPA in the physiopathology of undescended testes unlikely. However, the observed nanomolar uBPA concentrations support assessment of epidemiological relationships between CB uBPA and other human diseases.

Conclusion in humans (men):

Although most of the studies available present limitations (sample size, very specific population, bias…) they all point out a correlation between higher BPA levels and different sexual parameters (quality of sperm, sex hormones, and sexual function and quality) and then strenghten the plausibility of causality.

Few epidemiological studies have been performed related to an exposure to BPA. Three studies by Meeker *et al.* (2010a), Mendiola *et al.* (2010) and Galloway *et al*. (2010) addressed the issue of the sex male hormones. According to Meeker *et al.*, when measured in the urine of men who belong to an infertile couple, the FSH levels was increased and the ratio estradiol/testosterone was decreased with increasing levels of BPA. In the study by Mendiola *et al*. (2010) a significant inverse association was found between the urinary BPA concentration of fertile men and the Free Androgen Index (FAI) or the FAI/LH ratio. A significant positive correlation between BPA concentration and SHBG was also observed. Finally in the study by Galloway *et al.* perfomed in the general Italian population, higher daily BPA excretion was associated with higher total testosterone concentrations in men, in models adjusted for age and study site, and in models additionally adjusted for smoking, measures of obesity, and urinary creatinine concentrations. Then, despite the fact that number of studies is limited and that they are sometimes performed in a very specific population, exposure to BPA has been demonstrated to induce effects on the male sex hormone levels.

In other epidemiological studies workers exposed occupationally to BPA showed a greater risk of sexual dysfunction, a declining sexual function or a decrease in sperm concentration, motility and vitality compared to unexposed workers (Li *et al*., 2010a, 2010b and 2011). It has also been shown that little boys (n=56) among whom one parent or both had an occupational exposure, exhibit a shorter AGD when compared to control boys (Miao *et al*., 2011). These results were almost irrevocable since when analysis was restricted to boys younger than 8 years (in order to reduce the effects of the pubertal development) the results remained the same. Moreover, a dose-response

relationship exists with increased BPA exposure levels in pregnancy associated with greater magnitude of shortened AGD in male offspring.

Unlike the workers, in a population of fertile men (they are partners of pregnant women) no effects were observed on semen volume and sperm concentration, on the percent of motile sperm, morphologically normal sperm, total motile count and total sperm count (Mendiola *et al.*, 2010). But of course there is a limitation since the population of these 375 men were recruited as fertile men. When a study was performed in 190 men consulting in an infertility clinic a link between urinary concentration of BPA and impaired sperm quality was established: a rise in urinary BPA concentration was associated with a decline of 23% in sperm concentration, of 7.5% in motility, and 13% in normal morphology.

Effects have also been seen on the issue of IVF since men with a greater serum concentration in BPA lead to higher embryo fragmentation score (EFS) and a reduced embryo cell number (ECN) (Bloom *et al*., 2011a).

Table 17: Summary of the studies performed on BPA effects on the human male reproductive system

Population and N	Results	Urinary BPA levels	Reference
General 715	men : ↗ total testosterone (n=316) subjects mostly aged 65-74 Y	1.3 – 11.5 ng/mL (24h)	Galloway *et al*., 2010
Infertile couples 167	↗ FSH, FSH/inhibin B and ↘ oestradiol/testosterone and inhibin B with ↗ of BPA levels	< 0.4– 36.4 ng/mL	Meeker *et al.*, 2010a
Fertile men 375	No relationship between the BPA exposure and the gametes quality ↘ Free T index (total testosterone/SHBG) ; ↗ SHBG	< 0.4– 6.5 ng/mL	Mendiola *et al.*, 2010
Workers 123 exposed to BPA 254 unexposed	Higher risk of male sexual dysfunction	Median TWA_8 in exposed workers = 4.57 µg/m^3	Li et al., 2010a
Workers 427	correlation between urine BPA level and declining male sexual function	Median = 53.7 µg BPA/g Cr	Li et al., 2010b
Workers 218	↘ concentration, motility, vitality of sperm	Median = 39 (6-354) µg/g creatinine	Li *et al.*, 2011
Men consulting in an infertility clinic Infertile men 190	↘ sperm concentration and percentage of typical shapes; ↗ DNA fragmentation in sperm nucleus	ND – 36.4 ng/mL	Meeker *et al.*, 2010b

Men implicated in an IVF 27	↘ of the embryo number and quality with ↗ conc. of BPA	Not provided	Bloom *et al*., 2011a
Boys with occupational exposure of the parents 153	↘ AGD distance in boys with parents exposed to BPA at work	3.7-72.3 µg/g creatinine	Miao *et al*., 2011
152 boys with cryptorchid or descended testes (born after 34 weeks of gestation)	Similar uBPA levels in the control and cryptorchid groups make the participation of fetal exposure to uBPA in the physiopathology of undescended testes unlikely.	Not provided	Fénichel *et al.*, 2012

4.11.3 Developmental toxicity

Difference between fertility effects and developmental effects are sometime subtle. Indeed, in utero effects of BPA on sexual organ development, sexual beahaviour or sexual efficiency might be considered as developmental toxicity. Similarly, the details given in the multigeneration study of BPA influence on pups BW could also be considered as developmental effect. However, as BPA has been tremendously studied and for seek of efficiency; fertility only is covered in this report. Therefore, the data presented here on development are not exhaustive and are presented because it was judge that they proved BPA toxicity of fertility.

4.11.4 Other relevant information

A number of effects of BPA in animals have been extensively investigated and target organs identified in repeat-dose animal studies include liver, kidney and sexual organs. However, the effects of most concern have been those related to the hormonal activity of BPA and potentially related effects on physical, neurological, behavioural and reproductive development. BPA acts as a weak oestrogen. It has a much lower affinity for the estrogen receptors (ERα and ERβ) than endogenous oestrogen and it is rapidly metabolized to BPA-glucuronide which is not hormonally active. More recently, BPA has been shown to bind with high affinity to estrogen-related receptor (ERR-γ), which may be related to its ability to act as an endocrine disruptor on fertility. According to *in vitro* and *in vivo* studies currently available, BPA did not demonstrate either androgenic or anti-androgenic activity. The consequences of these endocrine disrupting properties on fertility have been extensively studies and have been presented above.

4.11.5 Summary and discussion of reproductive toxicity

Regarding male fertility, despite the fact that some studies observe limited or no effects, there is a convergence in the effects observed independently from the period of exposure.

First, an exposure to low doses of BPA in males induces changes in the levels of the sexual hormones like the testosterone. It has been observed in animals exposed *in utero* or neonatally (Akingbemi *et al*., 2004), during puberty (Della Seta *et al*., 2006; Nakamura *et al*., 2010; Takahaski and Oshi, 2003; Akingbemi *et al*., 2004) and in adult animals (Herath *et al*., 2004). One study rapported no effect on the serum testosterone levels (LaRocca *et al*., 2011), but first the measure was done in a reduced number of animals (4-5) and moreover even the positive control group (treated with DES) remained unaffected. In human, effects of BPA on sexual hormones have been described in three studies (Meeker *et al.* (2010a), Mendiola *et al.* (2010) and Galloway *et al*. (2010) which were all consistent although in study with a smaller sample size (meeker et al.) the significance of the positive trend between BPA and testosterone was not reached.

This effect on testosterone levels could partly explain effects observed on the sperm production since this parameter has been affected following exposure to BPA. Sperm production has been decreased following a 5 weeks exposure in adult rats following two different routes of exposure. If in Chitra *et al.* (2003) rats have been exposed orally and in Herath *et al.* study (2004) they were exposed using sub-cutaneous injections. The reduction in sperm count was accompanied by a decrease of the testis weight and an increase of the ventral prostate weight.

Other studies reported effects on one or several male reproductive organs such as effects on the weight of the testes and the seminal vesicles, the ventral prostate or the epididymides following an *in utero* exposure (Kabuto *et al.*, 2004; Akingbemi *et al*., 2004; Timms *et al*., 2005), a pubertal exposure (Nakamura et al., 2010; Takahashi and Oishi, 2003) and multigeneration study (Tyl *et al.*, 2008). In its 3-generation study performed in Sprague-dawley rats (Tyl *et al.*, 2002), adverse effects on fertility were described by Tyl *et al*. at the highest dose only (7500 ppm). Like in other studies, effects were seen on the weight of the reproductive organs since there was a reduction in testes, epididymis, prostate and seminal vesicles weight in most of the generations of the 7500 ppm group (the only exception was F0). Nevertheless when the relative weight of these organs (% of the sacrifice weight) is analysed, a significant increase of the paired testis and the paired epididymides is observed for all 3 generations.

Effects on the sperm production or parameters were observed when animals were exposed prenatally, neonatally or post-natally (during lactation) (Tinwell *et al*., 2002; Salian *et al*., 2009c) and in the multigeneraion studies by Tyl *et al*. described a decrease in epididymal sperm concentration in F1 males and in the daily sperm production in F3 males of the 7500 ppm dose group (Tyl et al.; 2002) and in F0 males treated with 3500ppm of BPA (Tyl *et al*.; 2008). Abnormalities in the seminiferous tubules (Iida *et al*., 2002; Okada and Kai, 2008) were also reported. Effects on sperm morphology (Aikawa *et al*., 2004; Toyama and Yuasa, 2004b; Okada and kai, 2008) or motility (Chitra *et al*., 2003; Salian *et al*., 2009c) were not reproduced in the multigeneration studies. It should be noted that no positive control was used in this study, and then it is not possible in this study to know if effects would have been observed in the same rats treated

with ethynilestradiol or diethylstilbestrol (DES) and to have an explanation on the fact that effects were observed at the highest dose only.

When animals were exposed to BPA neonatally effects on fertility were generally observed (Salian *et al*., 2009b) with effect on the sperm parameters (Salian *et al*., 2009c; Aikawa *et al*., 2004), on the reproductive organ weight, the histology of the seminiferous tubules or on the level of testosterone (Nakamura *et al*., 2010; Toyama and Yuasa, 2004b) in rats or mice, except in Kato study (2006) where Sprague Dawley rats exposed to BPA through sub-cutaneous injections do not exhibit any effect on reproductive parameters. But it has been shown that apparently Charles-River Sprague-Dawley rats have a very low sensitivity to estrogenic compounds (Yamasaki *et al*., 2002; NTP, 2001). These rats need very high doses of ethinylestradiol to induce a response but ethinylestradiolis a very potent.

In human, cccupational exposure to BPA was associated with a greater risk of sexual dysfunction, a declining sexual function or a decrease in sperm concentration, motility and vitality compared to unexposed workers (Li *et al*., 2010a, 2010b and 2011) and dose-related shorter AGD in boys whom one parent or both had an occupational exposure (Miao *et al*., 2011). However, in a population of fertile men (375 partners of pregnant women) no effects were observed on semen volume and sperm concentration, on the percent of motile sperm, morphologically normal sperm, total motile count and total sperm count (Mendiola *et al.*, 2010) but a link between urinary concentration of BPA and impaired sperm quality was established in 190 men consulting in an infertility clinic (Meeker et al.2010a). Finally, effects have also been seen on the issue of IVF since men with a greater serum concentration in BPA lead to higher embryo fragmentation score (EFS) and a reduce embryo cell number (ECN) (Bloom *et al*., 2011a).

Despite all the limitations of the epidemiological studies, the dose-effect relations identified in these various studies should not be questioned and the effects seen in men are consistent with the one observed in animals like effects on the sexual hormones and on male sexual function including sperm parameters.

Concerning the effects of BPA in females, a large number of recent studies is available and several kinds of effects which may impair the fertility were observed leading to consider the weight of evidence instead of basing the conclusion on few key studies limited to very specific models. Most of the studies were performed in rodents, and sometimes in sheeps, during a prenatal, neonatal, or postnatal period. A very few were realized in order to assess the impact of a BPA exposure during adulthood.

The most consistent effect is the increased occurrence of ovarian cysts following a gestational or postnatal exposure. Indeed, this effect was found in all studies investigating the influence of BPA on ovarian morphology and can thus be considered as a proven effect (Fernandez *et al*., 2010;

Adewale *et al.*, 2009; Newbold *et al*., 2009; Newbold *et al*., 2007; Signorile *et al*., 2010). More severe lesions were also found in ovaries including progressive proliferative lesions of the oviduct but this effect was seen in only 2 studies (Newbold *et al*., 2007; Newbold *et al*., 2009). Concerning the oocytes development, meiotic abnormalities leading to aneuploidy were demonstrated in three studies with different exposure period (prenatal, postnatal or prepubertal exposure) (Hunt *et al*., 2003; Susiarjo *et al*., 2007; Rodriguez *et al*., 2010). However, too few studies on this subject are available and it is thus difficult to draw conclusions. Although rodent ovarian cysts are not the exact model for PCOS, it is interesting to note that in human, the two epidemiological studies available shown higher incidence of polycystic ovaries positively associated with higher serum BPA concentrations (Kandaraki *et al*., 2011 and Takeuchi *et al*., 2004).

Consistent results were reported in seven different studies for an adverse effect of BPA on the estrous cycle, including irregular and prolonged cycles, following either prenatal (Nikaido *et al*., 2004; Honma *et al*., 2002), perinatal (Mendoza-Rodriguez *et al*., 2011; Rubin *et al*., 2001; Tyl *et al*., 2008) or postnatal exposure (Adewale *et al*., 2009; Fernandez *et al*., 2009). The 2 studies failing to reveal an effect of BPA on the estrous cycle (Kwon *et al*., 2000 and Nikaido *et al*., 2005) were performed in to strains/species insensitive to estrogen. This demonstrate that the effect of BPA on estrous after prenatal, perinatal or postnatal exposure is probably due to its estrogenic properties.

BPA also induces changes in the uterus morphology in several studies. Benign lesion like endometrial hyperplasia, or atypical hyperplasia, which is a precursor lesion to adenocarcinoma, were revealed in three studies following *in utero* exposure (Newbold *et al*., 2009), perinatal exposure (Signorile *et al*., 2010) or postnatal exposure (Newbold *et al*., 2007). Malignant invasions (squamous metaplasia or polyps) were also described in the two studies performed by Newbold (Newbold *et al*., 2007; Newbold *et al*., 2009). Although these effects were significant, it is again difficult to conclude due to small number of animal studies. Epidemiological studies reported contradictory effects of BPA on the human endometrium. BPA was not detected in control women, whereas serum levels of BPA were above the detection limit in women with endometriosis (Cobellis *et al*. 2009) in a population of women from a gynecological-obstetric clinic, but no significant association between urinary BPA levels and the stage of the endometriosis was described by Itoh et al. (2007). A lower exposure to BPA was also associated with endometrium hyperplasia (Hiroi *et al*., 2004). In a limited sample size (16 cases versus 21 controls) circulating levels of BPA appeared to be lesser in women with more severe endometrium hyperplasia.

Concerning the age at puberty, a large discrepancy exists between the studies. A significant advanced age at puberty was reported for a broad range of doses (2µg/kg to 100mg/kg) following an *in utero* (Howdeshell *et al*., 1999; Honma *et al*., 2002; Nikaido *et al*., 2004) or an early postanal exposure period (Adewale *et al*., 2009; Fernandez *et al*., 2009; Nah *et al*., 2011). This effect is therefore considered as a proven effect for these two windows of exposition. In contrast, and without being able to explain, oral treatment during exposure period encompassing the second half of gestation and the lactation period does not reveal any effect of BPA on the puberty timing

(Yoshida *et al*., 2004; Rubin *et al*., 2001; Ryan *et al*., 2010; Kwon *et al*., 2000). Two recent studies performed in little girls (Wolff *et al.*, 2008 and 2010) demonstrated in an important number of little girls that there was no relationship between the urinary BPA concentration and the onset of their puberty.

The whole of studies investigating effect of BPA exposure on the hypothalamic-hypophyseal axis in rodents and in sheeps show that BPA can influence the pattern of GnRH or LH secretion but a subtle effect of BPA is difficult to demonstrate as it is interspersed in the feedback loop and it depends on both the exposure and observation periods. However, a decrease in LH concentration and in LH pulse amplitude, suggesting a negative feedback exerts by BPA, was found in several studies in sheeps and in rats after a gestational, perinatal postnatal and prepubertal exposure (Savabieasfahani *et al*., 2006; Rubin *et al*., 2001; Evans *et al*., 2004; Colet *et al*., 2010) and can thus be considered as a proven effect.

A decline in reproductive capacity, i.e. a decrease in the number of pregnancies and a decrease in the number of pups born, was observed when exposure occurs *in utero* and during the lactation period (Cabaton *et al*., 2010) or during the first days of life only (Fernandez *et al*., 2010). By contrast, in the study performed by Honma *et al*., *in utero* exposure to BPA did not affect the female reproductive ability assessed by the total number of pups born (Honma *et al*., 2002). However, the observations in this study were limited to the first pregnancy of the offspring exposed during gestation and both Honma *et al.* and Cabaton *et al.* highlighted the importance to include repetitive breeding protocol and to not limit the observations to the first pregnancy in order to observe an effect on fertility and/or fecundity. Another study by Ryan *et al*. (2010), failed to demonstrate an effect of BPA on the fertility. However, the authors emphasized that the sample sizes were very small leading to not very conclusive results. Similarly, the multigeneration studies performed by Tyl et al. were contradictory with ovarian weights and total pups and live pups/litter decreased on PND0 at 7500 ppm (Tyl et al., 2002) but effects not reproduced at 3500ppm during the next 3-gen study (Tyl et al., 2008). In human, implantation failures were reported in women undergoing a medical-assisted procreation with higher urinary levels of BPA than control women (Ehrlich *et al*., 2012), correlation between 3 or more consecutive spontaneous miscarriages and higher serum level of BPA compared to the control women was described (Sugiura-Ogasawara *et al.*, 2005), decreased ovarian stimulation as part of an in vitro fertilization (IVF) by the BPA exposure was reported by Mok-Lin *et al.* (2010). This was confirmed in another study (Fujimoto *et al*., 2011) in which a significant association between the serum BPA concentrations of the women and decreased oocyte fertilization was reported.

When exposure occurs during adulthood, BPA consistently induces a decrease of the number of pregnancies and implantations following either intragastrically administration (Al Hiyasat *et al*., 2004) or subcutenaous injections (Berger *et al*., 2007, 2008 and 2010). This effect was also found after a postnatal exposure (Bosquiazzo *et al*., 2010 and Varayoud *et al*., 2011) and can thus be considered as proven for these two windows of exposure. However, in the 3-generations study

performed by Tyl *et al*., although the percent of post-implantation loss was unaffected, the number of implants, total pups, and live pups per litter were significantly reduced at 7500 ppm in all generations. The authors do not explain this difference. The pre-implantation loss could not be determined in this study, by the time the parental females were scarified. However, as demonstrated in the studies performed by Berger *et al*., decrase in litter size seems to be mediated by a disruption of intrauterine blastocyst implantation rather than by a post-implantation effect. Therefore it can not be excluded that the decrease in implants and the reduced live litter size at birth observed in this study may result from a pre-implantation disruption.

Several of the epidemiological studies performed in women have methodological limitations, this could be because of the small sample sizes, limited details on subject selection criteria, and they generally are cross-sectional designs that include limited control for potential confounders. These limitations in design limit our ability to make strong conclusions on how to weight the epidemiology of potential health risks of BPA in women in our conclusion. Nevertheless they confirm the effects of BPA observed on female animals and manage to show that the hazard demonstrated in animals can be observed as a risk in humans.

Fertility is gouverned by many parmeters and a lot of cofounding factors intervene in its modulation rendering difficult to reproduce the effects observed from one study to another: strain, route of exposure, alimentation, and windows of exposure are as many parameters that impact this endpoint. Rodents, in particular certain strains are not the most sensitive specie for fertility endpoints (Yamasaki *et al*., 2002; NTP, 2001). Historically, the last time the classification of BPA for human health was discussed and decided was in 2002. At the timebeing, the three guideline studies presented led UK to propose a Repr. Cat. 2; R60 (67/548) classification. In the mean-time a new key study was produced that showed male reprotoxicity (Tyl;, 2008) and a bunch of supportive publication were produced, showing sometimes diverging results difficult to explain but also confirming findings. Indeed, the results presented are therefore strong weight of evidence in favor of BPA effects on various endpoints of fertility both in male and female.

Independantly of the context (environmental or occupational) of exposure, most of the epidemiological studies manage to raise a risk between BPA and fertility.

4.11.6 Comparison with criteria

Annex I part 3 of CLP states for the hazard class reproductive toxicity that “Substances are classified in **Category 2** for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects".

Annex VI of CLP states for the hazard class reproductive toxicity that
"Substances are classified in **Category 1B** for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans… The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide **clear evidence** of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered **not to be a secondary non-specific consequence of other toxic effects**. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate".

Based on the weight of evidence of numerous animal studies, it apparears that BPA impacts the male reproductive system with effects on the seminiferous tubules, the reproductive hormones levels and the quantity and quality of sperm following an *in utero* exposure at doses that do not lead to major toxicity and in specific models that can predict human toxicity. When the exposure occurs neonatally, effects on fertility and on the organs of the reproductive tract are observed. An exposure during the puberty leads to effects on the levels of the reproductive hormones, on the seminal vesicles, prostate, testis and epididymis weights, and on sperm quality. Finally, when exposed during adulthood BPA induces effects on the plasma testosterone levels, on the organs of the reproductive tract and on the sperm production and quality.
Based on human studies, it appeared that exposure to BPA affects men fertility and reproductive hormone levels in specific population.

In female animals, following a pre- and post-natal exposure only, an increased occurrence of ovarian cysts or disturbance of estrous cycles are observed in all the animal studies presented in the report (exhaustive literature search from 2002 to 2011). These observations corroborate risks identified in human through epidemiological studies.
When the exposure occurs at the adult or postnatal age decrease in the number of pregnancies and implantations was systematically reported. This seemed to be contradicted by multigeneration studies, although pre-implementation loss was not assessed. In fact, pre-implementation loss seems to be responsible of the effect of BPA on fecundity in rodents. Implantation failures were reported in a study conducted in women undergoing a medical-assisted procreation, and same kind of women seemed to have a worse ovarian response (number of ovocytes collected and amplitude of the preovulatory oestradiol peak) when the urinary levels of BPA were higher. And the pregnancy

outcome seems to be also affected by an exposure to BPA because miscarriages and premature birth were observed in different studies.
In the few animal studies describing this endpoint, endometrial hyperplasia was observed. Concerning the epidemiological studies in women, endometriosis and hyperplasia were reported.
Finally, advencement of the age at puberty, or changes in the sex hormones levels were observed in animals but contradicted or not corroborated by epidemiological studies.

Depending on the effect defined as the leading effect for classification, human data corroborate or validate the finding on animal, when others are more debatable. We therefore propose that a classification **Repr. 1B–H360F** is warranted (Repr. Cat. 2; R60 according to Directive 67/548/EEC) but welcome a discussion regarding a classification in Repr. 1A; H360F. Depending on how the uncertainties on human data are considered, we suggest that a classification of BPA in Repr. 1A; H360F is discussed.

Classification in Repr. 2 is not appropriate since there is more than some evidence of effects in animals or humans, the effects observed are sufficiently convincing to propose at least a classification in category 1B.

4.11.7 Conclusions on classification and labelling

In the light of all these new data, a classification **Repr. 1B–H360F** is proposed (Repr. Cat. 2; R60 according to Directive 67/548/EEC) with no specific route of exposure added.

4.12 Other effects

Not evaluated in this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

6 OTHER INFORMATION

The information included in this report is based on a bibliographic search perfomed from January 2002 up to December 2011.

7 REFERENCES

Adewale HB, Jefferson WN, Newbold RR, Patisaul HB (2009) Neonatal Bisphenol-A Exposure Alters Rat Reproductive Development and Ovarian Morphology Without Impairing Activation of Gonadotropin-Releasing Hormone Neurons. Biology of Reproduction 81, 690-699.

Adewale HB, Todd KL, Mickens JA, Patisaul HB (2011) The impact of neonatal bisphenol-A exposure on sexually dimorphic hypothalamic nuclei in the female rat. Neurotoxicology (Amsterdam) 32, 38-49.

Agence nationale de sécurité sanitaire des aliments (AFSSA) (2010) Saisine n°2010-SA-0041:, (AFSSA, Maisons-Alfort) Exposition de la population française au Bisphénol A et aux teneurs maximales en bisphénol A dans les aliments *in french*.

Agence nationale de sécurité sanitaire (ANSES) (2011) Rapport d'étude : Effets sanitaires du Bisphénol A. Rapport d'expertise collective. ANSES, (Anses, Maisons-Alfort) *in french*.

Aikawa H, Koyama S, Matsuda M, Nakahashi K, Akazome Y, Mori T (2004) Relief effect of vitamin A on the decreased motility of sperm and the increased incidence of malformed sperm in mice exposed neonatally to bisphenol A. Cell and Tissue research 315, 119-124.

Akingbemi B, Sottas C, Koulova A, Klinefelter G, Hardy M (2004) Inhibition of testicular steroidogenesis by the xenoestrogen Bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. Endocrinology 145, 592-603.

Aldad TS, Rahmani N, Leranth C, Taylor HS, 2011. (2011) Bisphenol-A exposure alters endometrial progesterone receptor expression in the nonhuman primate. Fertility and Sterility, 96, 175-179.

Al-Hiyasat AS, Darmani H, Elbetieha AM (2004). Leached components from dental composites and their effects on fertility of female mice. Eur J Oral Sci. 112(3), 267-72.

Alonso-Magdalena P, Vieira E, Soriano S, Menes L, Burks D, Quesada I, Nadal A (2010) Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environmental Health Perspectives 118, 1243-1250.

Aschberger K, Castello P, Hoeskstra E, Karakitsios S, Munn S, Pakalin S, Sarigiannis D (2010) Bisphenol A and baby bottles: challenges & perspectives. JRC scientific and technical reports. (Publications Office of the European Union, Luxembourg)

Ashby J, Tinwell H, Haseman J (1999) Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed in utero. Regulatory Toxicology and Pharmacology 30, 156-166.

Aubele MM, Cummings MC, Mattis AE, Zitzelsberger HF, Walch AK, Kremer M, Hofler H, Werner M (2000) Accumulation of chromosomal imbalances from intraductal proliferative lesions to adjacent in situ and invasive ductal breast cancer. Diagnostic Molecular Pathology 9, 14-19.

Balakrishnan B., Henare K., Thorstensen E.B., Ponnampalam A.P., Mitchell M.D. (2010) Transfer of bisphenol A across the human placenta. Am J Obstet Gynecol 202:393.e1-7.

Berger RG, Hancock T, deCatanzaro D (2007) Influence of oral and subcutaneous bisphenol-A on intrauterine implantation of fertilized ova in inseminated female mice. Reproductive Toxicology 23, 138-144.

Berger RG, Shaw J, Catanzaro D (2008). Impact of acute bisphenol-A exposure upon intrauterine implantation of fertilized ova and urinary levels of progesterone and 17beta-estradiol. Reprod Toxicol. 26(2), 94-9.

Berger RG, Foster WG, deCatanzaro D (2010) Bisphenol-A exposure during the period of blastocyst implantation alters uterine morphology and perturbs measures of estrogen and progesterone receptor expression in mice. Reproductive Toxicology 30, 393-400.

Berkowitz G (2006) Limitations of a case-control study on bisphenol A (BPA) serum levels and recurrent miscarriage. Human Reproduction 21, 565-566.

Bloom MS, vom Saal FS, Kim D, Taylor JA, Lamb JD, Fujimoto VY (2011a) Serum unconjugated bisphenol A concentrations in men may influence embryo quality indicators during in vitro fertilization. Environmental Toxicology and Pharmacology 32, 319–323.

Bloom MS, Kim D, vom Saal FS, Taylor JA, Cheng G, Lamb JD, Fujimoto VY (2011b) Bisphenol A exposure reduces the estradiol response to gonadotropin stimulation during *in vitro* fertilization. Fertility and Sterility 96 (3), 672-677.

Bosquiazzo V.L., Varayoud J., Munoz-de-Toro M., Luque E.H., and Ramos J.G. (2010) Effects of Neonatal Exposure to Bisphenol A on Steroid Regulation of Vascular Endothelial Growth Factor Expression and Endothelial Cell Proliferation in the Adult Rat Uterus. Biology of reproduction 82, 86–95.

Braun JM, Yolton K, Dietrich KN, Hornung R, Ye X, Calafat AM, Lanphear BP (2009) Prenatal bisphenol A exposure and early childhood behavior. Environmental Health Perspectives 117, 1945-1952.

Bredhult C, Sahlin L, Olovsson MDO (2009) Gene expression analysis of human endometrial endothelial cells exposed to Bisphenol A. Reproductive Toxicology 28, 18-25.

Burstein HJ, Polyak K, Wong JS, Lester SC, Kaelin CM (2004) Ductal carcinoma in situ of the breast. New England Journal of Medicine 350, 1430-1441.

Cabaton NJ, Wadia PR, Rubin BS, Zalko D, Schaeberle CM, Askenase MH, Gadbois JL, Tharp AP, Whitt GS, Sonnenschein C, Soto AM (2010) Perinatal Exposure to Environmentally Relevant Levels of Bisphenol-A Decreases Fertility and Fecundity in CD-1 Mice. Environmental Health Perspectives 119, 547-552.

Cagen SZ, Waechter JM, Jr., Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, Harris LR (1999a) Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A. Toxicological Sciences 50, 36-44.

Cagen SZ, Waechter JM, Jr., Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, Harris LR (1999b) Normal reproductive organ development in Wistar rats exposed to bisphenol A in the drinking water. Regulatory Toxicology and Pharmacology 30, 130-139.

Cantonwine D, Meeker JD, Hu H, Sanchez BN, Lamadrid-Figueroa H, Mercado-Garcia A, Fortenberry GZ, Calafat AM, Tellez-Rojo MM (2010) Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study. Environmental Health 9, 62.

Cao J, Guo LH, Wan B, Wei Y (2011) In vitro fluorescence displacement investigation of thyroxine transport disruption by bisphenol A. Journal of Environmental Sciences (China) 23, 315-321.

Cardiff RD, Anver MR, Gusterson BA, Hennighausen L, Jensen RA, Merino MJ, Rehm S, Russo J, Tavassoli FA, Wakefield LM, Ward JM, Green JE (2000) The mammary pathology of genetically engineered mice: the consensus report and recommendations from the Annapolis meeting. Oncogene 19, 968-988.

Cha BS, Koh SB, Park JH, Eom A, Lee KM, Choi HS (2008) Influence of occupational exposure to bisphenol A on the sex hormones of male epoxy resin painters. Molecular and Cellular Toxicology 4, 230-234.

Chitra KC, Latchoumycandane C, Mathur PP (2003) Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. Toxicology 185, 119-127.

Clayton EM, Todd M, Dowd JB, Aiello AE (2011) The impact of bisphenol A and triclosan on immune parameters in the U.S. population, NHANES 2003-2006. Environmental Health Perspectives 119, 390-396.

Cobellis L, Colacurci N, Trabucco E, Carpentiero C, Grumetto LD (2009) Measurement of bisphenol A and bisphenol B levels in human blood sera from endometriotic women. Biomedical Chromatography 23, 1186-1190.

Colborn T, vom Saal FS, Soto AM (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environmental Health Perspectives 101, 378-384.

Colerangle JB, Roy D (1997) Profound effects of the weak environmental estrogen-like chemical bisphenol A on the growth of the mammary gland of Noble rats. Journal of Steroid Biochemistry and Molecular Biology 60, 153-160.

Collet SH, Picard-Hagen N, Viguie C, Lacroix MZ, Toutain PL, Gayrard V (2010) Estrogenicity of Bisphenol A: A Concentration-Effect Relationship on Luteinizing Hormone Secretion in a Sensitive Model of Prepubertal Lamb. Toxicological Sciences 117, 54-62.

Commission européenne (CE) DG Environnement (2002) Endocrine Disrupters: study on gathering information on 435 Substances with insufficient data. Final report. RPS BKH Consulting Engineers, No. B4-3040/2001/325850/MAR/C2 (RPS BKH Consulting Engineers, Delft)

Commission européenne (CE) Joint Research Centre (2003) European Union Risk Assessment Report. 4,4'-Isopropylidenediphenol (Bisphenol-A). Office for Official Publications of the European Communities, No. EUR 20843 EN (Office for Official Publications of the European Communities, Luxembourg)

Commission européenne (CE) Joint Research Centre (2010a) European Union Risk Assessment Report - Environment Addendum of April 2008 - 4,4'-ISOPROPYLIDENEDIPHENOL (Bisphenol-A) - Part 1 Environment. Publications Office of the European Union, JRC Scientific and Technical Reports (EUR collection) No. EUR 24588 EN (Publications Office of the European Union, Luxembourg)

Commission européenne (CE) Joint Research Centre (2010b) European Union Risk Assessment Report - Human Health of April 2008 - 4,4'-ISOPROPYLIDENEDIPHENOL (Bisphenol-A) - Part 2 Human Health. Publications Office of the European Union, JRC Scientific and Technical Reports (EUR collection) No. EUR 24589 EN (Publications Office of the European Union, Luxembourg)

Cox KH, Gatewood JD, Howeth C, Rissman EF (2010) Gestational exposure to bisphenol A and cross-fostering affect behaviors in juvenile mice. Hormones and Behavior 58, 754-761.

Decatanzaro D, Berger RG, Guzzo AC, Thorpe JB, Khan A.(2012) Perturbation of male sexual behavior in mice (Mus musculus) within a discrete range of perinatal bisphenol-A doses in the context of a high- or low-phytoestrogen diet. Food Chem Toxicol. 2013 Jan 8. doi:pii: S0278-6915(13)00004-5. 10.1016/j.fct.2012.12.046.

De Mascarel I, MacGrogan G, Mathoulin-Pelissier S, Vincent-Salomon A, Soubeyran I, Picot V, Coindre JM, Mauriac L (2007) Epithelial atypia in biopsies performed for microcalcifications. Practical considerations about 2,833 serially sectioned surgical biopsies with a long follow-up. Virchows Archiv 451, 1-10.

Della Seta D, Minder I, Belloni V, Aloisi AM, Dessi-Fulgheri F, Farabollini F (2006) Pubertal exposure to estrogenic chemicals affects behavior in juvenile and adult male rats. Hormones and Behavior 50, 301-307.

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

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

Doerge DR, Vanlandingham M, Twaddle NC, Delclos KB (2010b) Lactational transfer of bisphenol A in Sprague-Dawley rats. Toxicology Letters 199, 372-376.

Doerge DR, Twaddle NC, Vanlandingham M, Fisher JW. Pharmacokinetics of bisphenol A in serum and adipose tissue following intravenous administration to adult female CD-1 mice. Toxicol Lett. 2012 Mar 20. [Epub ahead of print] Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR 72079, United States.

Duft M, Schulte-Oehlmann U, Weltje L, Tillmann M, Oehlmann J (2003) Stimulated embryo production as a parameter of estrogenic exposure via sediments in the freshwater mudsnail Potamopyrgus antipodarum. Aquatic Toxicology 64, 437-449.

Edlow AG, Chen M, Smith NA, Lu C, McElrath TF. Fetal bisphenol A exposure: concentration of conjugated and unconjugated bisphenol A in amniotic fluid in the second and third trimesters. Reprod Toxicol. 2012 Aug. Epub 2012 Apr 10. *Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA.* http://www.ncbi.nlm.nih.gov/pubmed/22516041

Ehrlich S, Williams PL, Missmer SA, Flaws JA, Berry KF, Calafat AM, Ye X, Petrozza JC, Wright D, Hauser R (2012) Urinary Bisphenol A Concentrations and Implantation Failure among Women Undergoing in Vitro Fertilization. Environmental Health Perspectives 120 (7), 978-983.

Ema M, Fujii S, Furukawa M, Kiguchi M, Ikka T, Harazono A. Rat Two-Generation Reproductive Toxicity Study Of Bisphenol A. Reprod Toxicol. 2001 Sep-Oct;15(5):505-23

European Food Safety Authority (EFSA, 2006). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-BIS(4-HYDROXYPHENYL)PROPANE (Bisphenol A), question number EFSA-Q-2005-100, adopted on 29 November 2006. EFSA Journal.

European Food Safety Authority (EFSA, 2008). Toxicokinetics of Bisphenol A - Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food, question number EFSA-Q-2008-382, adopted on 9 July 2008. EFSA Journal.

European Food Safety Authority (EFSA, 2010). Scientific Opinion on Bisphenol A: evaluation of a study investigating its neurodevelopmental toxicity, review of recent scientific literature on its toxicity and advice on the Danish risk assessment of Bisphenol A, question number EFSA-Q-2009-00864, EFSA-Q-2010-01023, EFSA-Q-2010-00709, adopted on 23 September 2010. EFSA Journal.

Evans NP, North T, Dye S, Sweeney T (2004) Differential effects of the endocrine-disrupting compounds bisphenol-A and octylphenol on gonadotropin secretion, in prepubertal ewe lambs. Domestic Animal Endocrinology 26, 61-73.

Farbos M., (2012) Interspecies comparison of Bisphenol A toxicokinetic in five species and extrapolation from animal to human. THESIS: 2012 – TOU 3 – 4025

Fénichel P, Déchaux H, Harthe C, Gal J, Ferrari P, Pacini P, Wagner-Mahler K, Pugeat M, Brucker-Davis F, 2012. Unconjugated bisphenol A cord blood levels in boys with descended or undescended testes. Human Reproduction, 27, 983-990.

Fernandez M, Bourguignon N, Lux-Lantos V, Libertun C (2010) Neonatal Exposure to Bisphenol A and Reproductive and Endocrine Alterations Resembling the Polycystic Ovarian Syndrome in Adult Rats. Environmental Health Perspectives 118, 1217-1222.

Fiorini C., Tilloy-Ellul A., Chevalier S., Charuel C., PointisG., (2004) Sertoli cell junctional proteins as early targets for different classes of reproductive toxicants. Reproductive Toxicology 18, 413–421.

Food and Agriculture Organization of the United Nations (FAO), Organisation mondiale de la santé (OMS) (2010) Joint FAO/WHO Expert Meeting to Review Toxicological and Health Aspects of Bisphenol A: Summary Report. OMS, (OMS, Genève)

Fujimoto VY, Kim D, vom Saal FS, Lamb JD, Taylor JA, Bloom MS (2011) Serum unconjugated bisphenol A concentrations in women may adversely influence oocyte quality during in vitro fertilization. Fertility and Sterility 95, 1816-1819.

Galloway T, Cipelli R, Guralnik J, Ferrucci L, Bandinelli S, Corsi AM, Money C, McCormack P, Melzer D (2010) Daily Bisphenol A Excretion and Associations with Sex Hormone Concentrations: Results from the InCHIANTI Adult Population Study. Environmental Health Perspectives 118, 1603-1608.

Gayrard V., Lacroix M. Z., Collet S. H., Virguié C., Bousquet-Melou A., Toutain P-L., Picard-Hagen N. ; High bioavailability of Bisphenol A from sublingual exposure. ENVIRONMENTAL HEALTH PERSPECTIVES; http://dx.doi.org/10.1289/ehp.1206339; Online 12 June 2013

Ghibellini G, Leslie EM, and Brouwer KL (2006). Methods to evaluate biliary excretion of drugs in humans: an updated review. Mol.Pharm. 3, 198-211.

Ginsberg G, Rice DC (2009) Does rapid metabolism ensure negligible risk from bisphenol A? *Environmental Health Perspectives* **117,** 1639-1643.

Glavinas H., Krajcsi P., Cserepes J., and Sarkadi B. (2004). The role of ABC transporters in drug resistance, metabolism and toxicity. *Curr. Drug Deliv.* **1**, 27-42.

Gupta C (2000) Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. Proceedings of the Society for Experimental Biology and Medicine 224, 61-68.

Hanaoka T, Kawamura N, Hara K, Tsugane S (2002) Urinary bisphenol A and plasma hormone concentrations in male workers exposed to bisphenol A diglycidyl ether and mixed organic solvents. Occupational and Environmental Medicine 59, 625-628.

Hanioka N, Naito T, Narimatsu S (2008) Human UDP-glucuronosyltransferase isoforms involved in bisphenol A glucuronidation. Chemosphere 74, 33-36.

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

Hiroi H, Tsutsumi O, Takeuchi T, Momoeda M, Ikezuki Y, Okamura A, Yokota H, Taketani Y (2004) Differences in serum bisphenol a concentrations in premenopausal normal women and women with endometrial hyperplasia. Endocrine Journal 51, 595-600.

Hirom,PC, Millburn,P., and Smith,RL (1976). Bile and urine as complementary pathways for the excretion of foreign organic compounds. Xenobiotica 6, 55-64.

Hiyama M, Choi EK, Wakitani S, Tachibana T, Khan H, Kusakabe KT, Kiso Y (2011) Bisphenol-A (BPA) affects reproductive formation across generations in mice. J Vet Med Sci. 73(9), 1211-5.

Honma S, Suzuki A, Buchanan DL, Katsu Y, Watanabe H, Iguchi T (2002) Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. Reproductive Toxicology 16, 117-122.

Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, vom Saal FS (1999) Environmental toxins: Exposure to bisphenol A advances puberty. Nature 401, 763-764.

Howdeshell KL, Furr J, Lambright CR, Wilson VS, Ryan BC, Gray LE, Jr. (2008) Gestational and lactational exposure to ethinyl estradiol, but not bisphenol A, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male long evans hooded rat. Toxicological Sciences 102, 371-382.

Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Ilagan A, Voigt RC, Thomas S, Thomas BF and Hassold TJ. (2003). Bisphenol A exposure causes meiotic aneuploidy in the female mouse. Current Biology 13: 546-553.

Hunt PA, Lawson C, Gieske M, Murdoch B, Smith H, Marre A, Hassold T, VandeVoort CA, 2012. Bisphenol A alters early oogenesis and follicle formation in the fetal ovary of the rhesus monkey. Proceedings of the National Academy of Sciences of USA, In Press.
Iida H, Mori T, Kaneko T, Urasoko A, Yamada F, Shibata Y (2002) Disturbed spermatogenesis in mice prenatally exposed to an endocrine disruptor, Bisphenol A. Mammal Study 27(1), 73-82.

Ikezuki Y., Tsutsumi O., Takai Y., Kamei Y., Taketani Y. Determination of Bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. Hum Reprod. 2002; 17:2839-41

Institut National de Recherche et de Sécurité (INRS) (2010) Fiche toxicologique FT 279 - Bisphénol A. INRS, (INRS, Paris)

Institut national de la santé et de la recherche médicale (Inserm) (2011) Reproduction et environnement. Expertise collective. Inserm, (Inserm, Paris)

International Programme on Chemicals Safety (IPCS) (1999) Principles for the assessment of risks to human health from exposure to chemicals. Environmental health criteria 210. OMS, (OMS, Genève).

Itoh H, Iwasaki M, Hanaoka T, Sasaki H, Tanaka T, Tsugane S (2007) Urinary bisphenol-A concentration in infertile Japanese women and its association with endometriosis: A cross-sectional study. Environmental Health and Preventive Medicine 12, 258-264.

Kabuto H, Amakawa M, Shishibori T (2004) Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. Life Sciences 74, 2931-2940.

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

Kandaraki E, Chatzigeorgiou A, Livadas S, Palioura E, Economou F, Koutsilieris M, Palimeri S, Panidis D, amanti-Kandarakis E (2011) Endocrine disruptors and polycystic ovary syndrome (PCOS): elevated serum levels of bisphenol A in women with PCOS. Journal of Clinical Endocrinology & Metabolism 96, E480-E484.

Karavan JR, Pepling ME, 2012. Effects of estrogenic compounds on neonatal oocyte development. Reproductive Toxicology, 34, 51-56.

Kato H, Furuhashi T, Tanaka M, Katsu Y, Watanabe H, Ohta Y, Iguchi T (2006) Effects of bisphenol A given neonatally on reproductive functions of male rats. Reproductive Toxicology 22, 20-29.

Kim YH, Kim CS, Park S, Han SY, Pyo MY, Yang M (2003) Gender differences in the levels of bisphenol A metabolites in urine. *Biochemical and Biophysical Research Communications* **312,** 441-448.

Kobayashi K, Miyagawa M, Wang RS, Sekiguchi S, Suda M, Honma T (2002) Effects of in utero and lactational exposure to bisphenol A on somatic growth and anogenital distance in F1 rat offspring. Industrial Health 40, 375-381.

Kobayashi K, Ohtani K, Kubota H, Miyagawa M (2010) Dietary exposure to low doses of bisphenol A: effects on reproduction and development in two generations of C57BL/6J mice. Congenital Anomalies 50, 159-170.

Kobayashi K, Kubota H, Ohtani K, Hojo R, Miyagawa M (2012) Lack of effects for dietary exposure of bisphenol A during in utero and lactational periods on reproductive development in rat offspring.

Kwon S, Stedman DB, Elswick BA, Cattley RC, Welsch F (2000) Pubertal development and reproductive functions of Crl:CD BR Sprague-Dawley rats exposed to bisphenol A during prenatal and postnatal development. Toxicological Sciences 55, 399-406.

LaRocca J, Boyajian A, Brown C, Smith SD, Hixon M (2011) Effects of in utero exposure to bisphenol A or diethylstilbestrol on the adult male reproductive system. Birth Defects Research (part B) 92, 526-533.

Li DK, Zhou Z, Qing D, He Y, Wu T, Miao M, Wang J, Weng X, Ferber JR, Herrinton LJ, Zhu Q, Gao E, Checkoway H, Yuan W (2010a) Occupational exposure to bisphenol-A (BPA) and the risk of self-reported male sexual dysfunction. Human Reproduction 25, 519-527.

Li DK, Zhou Z, Miao M, He Y, Qing D, Wu T, Wang J, Weng X, Ferber J, Herrinton LJ, Zhu Q, Gao E, Yuan W (2010b) Relationship between urine bisphenol-A level and declining male sexual function. Journal of Andrology 31, 500-506.

Li DK, Zhou Z, Miao M, He Y, Wang J, Ferber J, Herrinton LJ, Gao E, Yuan W (2011) Urine bisphenol-A (BPA) level in relation to semen quality. Fertility and Sterility 95 (2), 625-630.

Li MW, Mruk DD, Lee WM, Cheng CY (2009) Disruption of the blood-testis barrier integrity by bisphenol A in vitro: is this a suitable model for studying blood-testis barrier dynamics? International Journal of Biochemistry and Cell Biology 41, 2302-2314.

Maffini MV, Rubin BS, Sonnenschein C, Soto AM (2006) Endocrine disruptors and reproductive health: the case of bisphenol-A. Molecular and Cellular Endocrinology 254-255, 179-186.

Mahoney MM, Padmanabhan V (2010) Developmental programming: impact of fetal exposure to endocrine-disrupting chemicals on gonadotropin-releasing hormone and estrogen receptor mRNA in sheep hypothalamus. Toxicology and Applied Pharmacology 247, 98-104.

Markey CM, Wadia PR, Rubin BS, Sonnenschein C, Soto AM (2005) Long-term effects of fetal exposure to low doses of the xenoestrogen bisphenol-A in the female mouse genital tract. Biology of Reproduction 72, 1344-1351.

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

Mazur CS, Marchitti SA, Dimova M, Kenneke JF, Lumen A, Fisher J. Human and rat ABC transporter efflux of bisphenol a and bisphenol a glucuronide: interspecies comparison and implications for pharmacokinetic assessment. Toxicol Sci. 2012 Aug;128(2):317-25. doi: 10.1093/toxsci/kfs167. Epub 2012 May 2.

Meeker JD, Calafat AM, Hauser R (2010a) Urinary Bisphenol A Concentrations in Relation to Serum Thyroid and Reproductive Hormone Levels in Men from an Infertility Clinic. Environmental Science & Technology 44, 1458-1463.

Meeker JD, Ehrlich S, Toth TL, Wright DL, Calafat AM, Trisini AT, Ye X, Hauser R (2010b) Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. Reproductive Toxicology 30, 532-539.

Mendiola J, Jorgensen N, Andersson AM, Calafat AM, Ye X, Redmon JB, Drobnis EZ, Wang C, Sparks A, Thurston SW, Liu F, Swan SH (2010) Are environmental levels of bisphenol a associated with reproductive function in fertile men? Environmental Health Perspectives 118, 1286-1291.

Mendoza-Rodríguez CA, García-Guzmán M, Baranda-Avila N, Morimoto S, Perrot-Applanat M, Cerbón M (2011) Administration of bisphenol A to dams during perinatal period modifies molecular and morphological reproductive parameters of the offspring. Reproductive Toxicology 31, 177-183.

Miao M, Yuan W, He Y, Zhou Z, Wang J, Gao E, Li G, Li D-K (2011) In Utero Exposure to Bisphenol-A and Anogenital Distance of Male Offspring. Birth Defects Research (Part A) 91, 867-872.

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

Mita L, Baldi A, Diano N, Viggiano E, Portaccio M, Nicolucci C, Grumiro L, Menale C, Mita Dg, Spugnini Ep, Viceconte R, Citro G, Pierantoni R, Sica V, Marino M, Signorile Pg, Bianco M. (2010) Differential accumulation of BPA in some tissues of offspring of Balb-c mice exposed to different BPA doses. Environ Toxicol Pharmacol 33(1): 9-15Mok-Lin E, Ehrlich S, Williams PL, Petrozza J, Wright DL, Calafat AM, Ye X, Hauser R (2010) Urinary bisphenol A concentrations and ovarian response among women undergoing IVF. International Journal of Andrology 33, 385-393.

Mose T., Mathiesen L., Karttunen V., Nielsen J.K.S., Sieppi E., Kummu M., Morck T.A., Myohanen K., Partanen H., Vahakangas K., Knudsen L.E., Myllynen P. (2012) Meta-analysis of data from human ex vivo placental perfusion studies on genotoxic and immunotoxic agents within the integrated European project NewGeneris; *Placenta* **33(5),** 433-439

Munoz del Toro M, Markey CM, Wadia PR, Luque EH, Rubin BS, Sonnenschein C, Soto AM (2005) Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. Endocrinology 146, 4138-4147.

Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM (2007) Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. Reproductive Toxicology 23, 383-390.

Myers JP, vom Saal FS, Akingbemi BT, Arizono K, Belcher S, Colborn T, Chahoud I, Crain DA, Farabollini F, Guillette Jr. LJ, Hassold T, Ho S, Hunt P, Iguchi T, Jobling S, Kanno J, Laufer H, Marcus M, McLachlan JA, Nadal A, Oehlmann J, Olea N, Palanza P, Parmigiani S, Rubin BS, Schoenfelder G, Sonnenschein C, Soto AM, Talsness CE, Taylor JA, Vandenberg LN, Vandenbergh JG, Vogel S, Watson CS, Welshons WV, Zoeller RT (2009) Why Public Health Agencies Cannot Depend on Good Laboratory Practices as a Criterion for Selecting Data: The Case of Bisphenol A. Environmental Health Perpectives 117(3), 309-315.

Nagao T, Saito Y, Usumi K, Yoshimura S, Ono H (2002) Low-dose bisphenol A does not affect reproductive organs in estrogensensitive C57BL/6N mice exposed at the sexually mature, juvenile, or embryonic stage. Reproductive Toxicology 16, 123-130.

Nah WH, Park MJ, Gye MC (2011). Effects of early prepubertal exposure to bisphenol A on the onset of puberty, ovarian weights, and estrous cycle in female mice. Clin Exp Reprod Med. 38(2), 75-81.

Nakamura D, Yanagiba Y, Duan Z, Ito Y, Okamura A, Asaeda N, Tagawa Y, Li CM, Taya K, Zhang S-Y, Naito H, Ramdhan DH, Kamijima M, Nakajima T (2010) Bisphenol A may cause testosterone reduction by adversely affecting both testis and pituitary systems similar to estradiol. Toxicology Letters 194, 16–25.

National Toxicology Program (NTP) (2001) National Toxicology Program's Report of the Endocrine Disruptors Low-Dose Peer Review. NIEHS, (NIEHS, Research Triangle Park, NC)

National Toxicology Program (NTP) (2008) NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A. NIH, No. 08-5994 (NIH, Research Triangle Park, NC)

National Toxicology Program (NTP). NTP Historical Controls for NTP-2000 Diet [base de données en ligne]. En ligne : http://ntp. niehs. nih.gov/?objectid=92E705C7-F1F6-975E-72D23026B1645EB9 [Date de consultation : mars 2011] . 2010.

Navarro V. M., Sanchez-Garrido M. A, Castellano J. M., Roa J., García-Galiano D., Pineda R., Aguilar E., Pinilla L., and Tena-Sempere M. (2009) Persistent Impairment of Hypothalamic KiSS-1 System after Exposures to Estrogenic Compounds at Critical Periods of Brain Sex Differentiation. Endocrinology 150: 2359–2367.

Newbold RR, Jefferson WN, Padilla-Banks E (2007) Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. Reproductive Toxicology 24, 253-258.

Newbold RR, Jefferson WN, Padilla-Banks E (2009) Prenatal Exposure to Bisphenol A at Environmentally Relevant Doses Adversely Affects the Murine Female Reproductive Tract Later in Life. Environmental Health Perspectives 117, 879-885.

Nikaido Y, Yoshizawa K, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, Tsubura A (2004) Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. Reproductive Toxicology 18, 803-811.

Nikaido Y, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, Tsubura A (2005) Effects of prepubertal exposure to xenoestrogen on development of estrogen target organs in female CD-1 mice. In Vivo 19, 487-494.

Norazit A, Mohamad J, Razak SA, Abdulla MA, Azmil A, Mohd MA, 2012. Effects of Soya Bean Extract, Bisphenol A and 17β-Estradiol on the Testis and Circulating Levels of Testosterone and Estradiol Among Peripubertal Juvenile Male Sprague-Dawley Rats. Sains Malaysiana, 41, 63-69.

Office of Environmental Health Hazard Assessment California Environmental Agency (OEHHA) Reproductive and Cancer Hazard Assessment Branch (2009) Evidence on the developmental and reproductive toxicity of Bisphenol A. OEHHA, (OEHHA, Sacramento)

Okada A, Kai O (2008) Effects of estradiol-17beta and bisphenol A administered chronically to mice throughout pregnancy and lactation on the male pups reproductive system. Asian Journal of Andrology 10, 271-276.

Patisaul HB, Todd KL, Mickens JA, Adewale HB (2009). Impact of neonatal exposure to the ERalpha agonist PPT, bisphenol-A or phytoestrogens on hypothalamic kisspeptin fiber density in male and female rats. Neurotoxicology. 30(3), 350-7.

Patterson TA, Twaddle NC, Roegge CS, Callicott RJ, Fisher JW, Doerge DR.Concurrent determination of bisphenol A pharmacokinetics in maternal and fetal rhesus monkeys. Toxicol Appl Pharmacol. 2012 Dec 19. doi:pii: S0041-008X(12)00533-9. 10.1016/j.taap.2012.12.006.

Pottenger LH, Domoradzki JY, Markham DA, Hansen SC, Cagen SZ, Waechter JM, Jr. (2000) The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. Toxicological Sciences 54, 3-18.

Ramakrishnan S, Wayne NL (2008) Impact of bisphenol-A on early embryonic development and reproductive maturation. Reproductive Toxicology 25, 177-183.

Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenbergh JG, Walser-Kuntz DR, vom Saal FS (2007) In vivo effects of bisphenol A in laboratory rodent studies. Reproductive Toxicology 24, 199-224.

Rodríguez H.A., Santambrosio N., Santamaría C.G., Munoz-de-Toro M., Luque E.H. (2010) Neonatal exposure to bisphenol A reduces the pool of primordial follicles in the rat ovary. Reproductive Toxicology 30, 550–557.

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

Ryan BC, Hotchkiss AK, Crofton KM, Gray LE (2010) In Utero and Lactational Exposure to Bisphenol A, In Contrast to Ethinyl Estradiol, Does Not Alter Sexually Dimorphic Behavior, Puberty, Fertility, and Anatomy of Female LE Rats. Toxicological Sciences 114, 133-148.

Sakaue M, Ohsako S, Ishimura R, Kurosawa S, Kurohmaru M, Hayashi Y, Aoki Y, Yonemoto J, Tohyama C (2001) Bisphenol-A affects spermatogenesis in the adult rat even at low dose. Journal of Occupational Health 43, 185-190.

Salian S, Doshi T, Vanage G (2009a) Impairment in protein expression profile of testicular steroid receptor coregulators in male rat offspring perinatally exposed to Bisphenol A. Life Sciences 85, 11-18.

Salian S, Doshi T, Vanage G (2009b) Neonatal exposure of male rats to Bisphenol A impairs fertility and expression of sertoli cell junctional proteins in the testis. Toxicology 265, 56-67.

Salian S, Doshi T, Vanage G (2009c) Perinatal exposure of rats to Bisphenol A affects the fertility of male offspring. Life Sciences 85, 742-752.

Savabieasfahani M, Kannan K, Astapova O, Evans NP, Padmanabhan V (2006) Developmental programming: Differential effects of prenatal exposure to bisphenol-A or methoxychlor on reproductive function. Endocrinology 147, 5956-5966.

Schaefer WR, Fischer L, Deppert WR, Hanjalic-Beck A, Seebacher L, Weimer M, Zahradnik HP (2010) In vitro-Ishikawa cell test for assessing tissue-specific chemical effects on human endometrium. Reproductive Toxicology 30, 89-93.

Schonfelder G., Friedrich K., Paul M. and Chahoud I.(2004) Developmental Effects of Prenatal Exposure to Bisphenol A on the Uterus of Rat Offspring. Neoplasia. Vol. 6, No. 5, pp. 584 – 594.

Shimizu M, Ohta K, Matsumoto Y, Fukuoka M, Ohno Y and Ozawa S (2002) Sulphation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF-7 cells. Toxicol In Vitro 16, 549-556.

Signorile PG, Spugnini EP, Mita L, Mellone P, D'Avino A, Bianco M, Diano N, Caputo L, Rea F, Viceconte R, Portaccio M, Viggiano E, Citro G, Pierantoni R, Sica V, Vincenzi B, Mita DG, Baldi F, Baldi A (2010) Pre-natal exposure of mice to bisphenol A elicits an endometriosis-like phenotype in female offspring. General and Comparative Endocrinology 168, 318-325.

Smith C. C. and Taylor H; S. (2007) Xenoestrogen exposure imprints expression of genes (Hoxa10) required for normal uterine development. The FASEB Journal Vol. 21.

Snyder RW, Maness SC, Gaido KW, Welsch F, Sumner SC and Fennell TR (2000) Metabolism and disposition of bisphenol A in female rats. Toxicol Appl Pharmacol 168, 225-234.

Study report (1988). REACH join submission registration dossier: http://apps.echa.europa.eu/registered/data/dossiers/DISS-9dbe071c-c12d-0fe1-e044-00144f67d249/AGGR-035f3645-9f43-458b-9587-d7cdceabd46c_DISS-9dbe071c-c12d-0fe1-e044-00144f67d249.html#AGGR-035f3645-9f43-458b-9587-d7cdceabd46c

Sugiura-Ogasawara M, Ozaki Y, Sonta S, Makino T, Suzumori K (2005) Exposure to bisphenol A is associated with recurrent miscarriage. Human Reproduction 20, 2325-2329.

Susiarjo M, Hassold TJ, Freeman E and Hunt PA. (2007). Bisphenol A Exposure In Utero Disrupts Early Oogenesis in the Mouse. PLoS Genetics, 3(1): e5 doi:10.1371/journal.pgen.0030005.

Tachibana T, Wakimoto Y, Nakamuta N, Phichitraslip T, Wakitani S, Kusakabe K, Hondo E, Kiso Y (2007). Effects of bisphenol A (BPA) on placentation and survival of the neonates in mice. J Reprod Dev. 53(3), 509-14.

Takagi H, Shibutani M, Masutomi N, Uneyama C, Takahashi N, Mitsumori K, Hirose M (2004) Lack of maternal dietary exposure effects of bisphenol A and nonylphenol during the critical period for brain sexual differentiation on the reproductive/endocrine systems in later life. *Archives of Toxicology* **78**, 87-105.

Takahashi O, Oishi S (2001) Testicular toxicity of dietary 2,2-bis(4-hydroxyphenyl)propane (bisphenol A) in F344 rats. Archives of Toxicology 75, 42-51.

Takahashi O, Oishi S (2003) Testicular toxicity of dietarily or parenterally administered bisphenol A in rats and mice. Food and Chemical Toxicology 41 (7), 1035-44.

Takeuchi T, Tsutsumi O, Ikezuki Y, Takai Y, Taketani Y (2004) Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. Endocrine Journal 51, 165-169.

Tan BLL, Kassim NM, Mohd MA (2003) Assessment of pubertal development in juvenile male rats after sub-acute exposure to bisphenol A and nonylphenol. Toxicology Letters 143, 261-270.

Taylor JA, Welshons WV, vom Saal FS (2008) No effect of route of exposure (oral; subcutaneous injection) on plasma bisphenol A throughout 24h after administration in neonatal female mice. *Reproductive Toxicology* **25,** 169-176.

Taylor JA, vom Saal FS, Welshons WV, Drury B, Rottinghaus G, Hunt PA, Toutain PL, Laffont CM, Vandevoort CA (2011) Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. *Environmental Health Perspectives* **119,** 422-430.

Teeguarden J.G., Calafat A.M., Ye X., Doerge D.R., Churchwell M.I., Gunawan R., Graham M.K. (2011) Twenty-four hour human urine and serum profiles of bisphenol A during high-dietary exposure. Toxicological Sciences **123(1),** 48-57.

Thigpen JE, Haseman JK, Saunders HE, Setchell KD, Grant MG, Forsythe DB (2003) Dietary phytoestrogens accelerate the time of vaginal opening in immature CD-1 mice. Comparative Medicine 53, 607-615.

Thigpen JE, Setchell KD, Padilla-Banks E, Haseman JK, Saunders HE, Caviness GF, Kissling GE, Grant MG, Forsythe DB (2007) Variations in phytoestrogen content between different mill dates of the same diet produces significant differences in the time of vaginal opening in CD-1 mice and F344 rats but not in CD Sprague-Dawley rats. Environmental Health Perspectives 115, 1717-1726.

Timms BG, Howdeshell KL, Barton L, Bradley S, Richter CA, vom Saal FS (2005) Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. Proceedings of the National Academy of Sciences of the United States of America 102, 7014-7019.

Tinwell H, Haseman J, Lefevre PA, Wallis N, Ashby J (2002) Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. Toxicological Sciences 68, 339-348.

Toutain PL, Ferran A, Bousquet-Melou A (2010). Species differences in pharmacokinetics ans pharmacodynamics. *Handbook of Experimental Pharmacology* **199**, 19-48.

Toyama Y, Suzuki-Toyota F, Maekawa M, ito C, Toshimori K (2004) Adverse effects of bisphenol A to spermiogenesis in mice and rats. Archivesin Histology and Cytology 64(4), 373-381.
Toyama Y, Yuasa S (2004b) Effects of neonatal administration of 17β-estradiol, β-estradiol 3-benzoate, or bisphenol A on mouse and rat spermatogenesis. Reproductive Toxicology 19, 181-188.

Tsukioka T, Terasawa J, Sato S, Hatayama Y, Makino T, Nakazawa H (2004) Development of analytical method for determining trace amounts of BPA in urine samples and estimation of exposure to BPA. Journal of Environmental Chemistry 17, 57-63.

Tsutsumi O.; Assessment of human contamination of estrogenic endocrine-disrupting chemicals and their risk for human reproduction. J Steroid Biochem Mol Biol. 2005; 93:325-30.

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 (2002) Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicological Sciences 68, 121-146.

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 (2008) Two-generation reproductive toxicity study of dietary bisphenol a in CD-1 (Swiss) mice. Toxicological Sciences 104, 362-384.

Vandenberg LN, Maffini MV, Wadia PR, Sonnenschein C, Rubin BS, Soto AM (2007a) Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters development of the fetal mouse mammary gland. Endocrinology 148, 116-127.

Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV (2007b) Human exposure to bisphenol A (BPA). Reproductive Toxicology 24, 139-177.

Vandenberg LN, Maffini MV, Schaeberle CM, Ucci AA, Sonnenschein C, Rubin BS, Soto AM (2008) Perinatal exposure to the xenoestrogen bisphenol-A induces mammary intraductal hyperplasias in adult CD-1 mice. Reproductive Toxicology 26, 210-219.

Varayoud J, Ramos JG, Bosquiazzo VL, Lower M, Muñoz-de-Toro M, Luque EH (2011). Neonatal exposure to bisphenol A alters rat uterine implantation-associated gene expression and reduces the number of implantation sites. Endocrinology. 152(3), 1101-11.

Volkel W, Colnot T, Csanady GA, Filser JG, Dekant W (2002) Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. Chemical Research in Toxicology 15, 1281-1287.

Volkel W, Bittner N, Dekant W (2005) Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by high performance liquid chromatography-tandem mass spectrometry. Drug Metabolism and Disposition: The Biological Fate of Chemicals 33, 1748-1757.

Vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, Dhar MD, Ganjam VK, Parmigiani S, Welshons WV (1997) Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proceedings of the National Academy of Sciences of the United States of America 94, 2056-2061.

Vom Saal FS, Cooke PS, Buchanan DL, Palanza P, Thayer KA, Nagel SC, Parmigiani S, Welshons WV (1998) A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. Toxicology and Industrial Health 14, 239-260.

Vom Saal FS, Akingbemi BT, Belcher SM, Birnbaum LS, Crain DA, Eriksen M, Farabollini F, Guillette LJ, Jr., Hauser R, Heindel JJ, Ho SM, Hunt PA, Iguchi T, Jobling S, Kanno J, Keri RA, Knudsen KE, Laufer H, LeBlanc GA, Marcus M, McLachlan JA, Myers JP, Nadal A, Newbold RR, Olea N, Prins GS, Richter CA, Rubin BS, Sonnenschein C, Soto AM, Talsness CE, Vandenbergh JG, Vandenberg LN, Walser-Kuntz DR, Watson CS, Welshons WV, Wetherill Y, Zoeller RT (2007) Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reproductive Toxicology 24, 131-138.

Walton,K, Dorne,JL, and Renwick,AG.(2001). Uncertainty factors for chemical risk assessment: interspecies differences in glucuronidation. Food Chem.Toxicol. 39, 1175-1190.

Watanabe S, Wang RS, Miyagawa M, Kobayashi K, Suda M, Sekiguchi S, Honma T (2003) Imbalance of testosterone level in male offspring of rats perinatally exposed to bisphenol A. Industrial Health 41, 338-341.

Welsh M, Saunders PT, Fisken M, Scott HM, Hutchison GR, Smith LB, Sharpe RM (2008) Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. Journal of Clinical Investigation 118, 1479-1490.

Willhite,CC, Ball,GL, and McLellan,CJ (2008). Derivation of a bisphenol A oral reference dose (RfD) and drinking-water equivalent concentration. J.Toxicol.Environ.Health B Crit Rev. 11, 69-146.

World Health Organization (WHO) (1999) WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge University Press, (Cambridge University Press,New York)

Wolff MS, Britton JA, Boguski L, Hochman S, Maloney N, Serra N, Liu Z, Berkowitz G, Larson S, Forman J (2008a) Environmental exposures and puberty in inner-city girls. Environmental Research 107, 393-400.

Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, Wetmur J, Calafat AM (2008b) Prenatal phenol and phthalate exposures and birth outcomes. Environmental Health Perspectives 116, 1092-1097.

Wolff MS, Teitelbaum SL, Pinney SM, Windham G, Liao L, Biro F, Kushi LH, Erdmann C, Hiatt RA, Rybak ME, Calafat AM (2010) Investigation of Relationships between Urinary Biomarkers of Phytoestrogens, Phthalates, and Phenols and Pubertal Stages in Girls. Environmental Health Perspectives 118.

Yamasaki K, Sawaki M, Noda S, Inmatanaka N, Takatsuki M (2002). Subacute oral toxicity study of ethinylestradiol and bisphenol A, based on the draft protocol for the "Enhanced OECD Test Guideline No. 407." Archives of Toxicology 76, 65-74.

Ye X, Kuklenyik Z, Needham LL, Calafat AM (2005) Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. Analytical and Bioanalytical Chemistry 383, 638-644.

Ye X, Wong LY, Bishop AM, Calafat AM (2011) Variability of Urinary Concentrations of Bisphenol A in Spot Samples, First-morning Voids, and 24-Hour Collections. Environmental Health Perspectives 119 (7), 983-988.

Yoshida M, Shimomoto T, Katashima S, Watanabe G, Taya K, Maekawa A (2004) Maternal exposure to low doses of bisphenol a has no effects on development of female reproductive tract and uterine carcinogenesis in Donryu rats. Journal of Reproduction and Development 50, 349-360.

Zalko D, Soto AM, Dolo L, Dorio C, Rathahao E, Debrauwer L, Faure R, Cravedi JP (2003) Biotransformations of bisphenol A in a mammalian model: answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice. Environmental Health Perspectives 111, 309-319.

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

8 ANNEX 1:

Summary of the reproductive toxicity assessment in the EU RAR from 2003 provided by UK

No human data are available. Bisphenol-A has been shown to have endocrine modulating activity in a number of *in vitro* and *in vivo* screening assays. The potency of this activity in these assays generally ranged from 3 to 5 orders of magnitude less than that of oestradiol. No significant oestrogenic activity has been observed with bisphenol-A glucuronide *in vitro.* The available data also indicate that there is a marked strain difference in the response to bisphenol-A in rats. However, there are no data to indicate the underlying reasons for such differences.

It should be noted that these studies investigating endocrine modulating activity are essentially screening tests and many of them employ experimental protocols, which have not undergone any international validation. Whilst these assays can be used to indicate potential oestrogenic activity, they do not measure functional changes in reproductive parameters and therefore cannot in themselves be used for risk characterisation purposes. In addition, many of the available *in vivo* studies have used parenteral routes of exposure, the relevance of which are uncertain with respect to relevant routes of human exposure.

The effects of bisphenol-A on fertility and reproductive performance have been investigated in three good quality studies: two generation and multigeneration studies in the rat, and a continuous breeding study in the mouse. Although no effect on fertility was seen in the rat two generation study, low dose levels were employed (0.2-200 μg/kg/day). In the multigeneration study, an effect on fertility (reduction in litter size) was seen in all three generations at the top dose of 500 mg/kg. Although this effect was seen only at a dose level causing parental toxicity (a reduction in body weight gain (>13%) in both sexes and renal tubule degeneration in females only), it is not clear whether or not the finding could be a secondary consequence of parental toxicity, or a direct effect of bisphenol-A. In the light of this uncertainty, and given that an adverse effect of fertility has been seen in the mouse, it is prudent to assume that bisphenol-A may be having a direct effect on fertililty in this study. No effects on fertility were seen at 50 mg/kg. The continuous breeding study in the mouse provides some evidence that bisphenol-A can cause adverse effects on fertility. In the F_0 generation, no effects on fertility were seen at 300 mg/kg/day, but at dose levels of approximately 600 mg/kg/day and above, reductions in the numbers of litters produced, litter size and numbers of live pups per litter were observed in each of the 4-5 litters produced. These effects were observed in the absence of significant parental toxicity. In contrast, no adverse effects on fertility were observed in the single litter tested at each dose level from the F_1 generation. A statistically significant and dose-related decrease in epididymal weight was seen at all doses in the F_1 generation. However, the significance of this finding is uncertain given that there was no effect on fertility in the F_1 generation, and where an adverse effect on fertility was seen (in the F_0 generation), there was no effect on epididymal weight.. In spite of the uncertainty, the epididymis is associated with sperm transport and storage, and any reduction in the weight of this organ would be of concern. […]

Some studies have investigated the potential of bisphenol-A to affect male reproductive tract development in rats and mice. Conflicting results have been reported in both species. In mice, adverse effects on male reproductive tract development (an increase in prostate weight in two studies and a reduction in epididymis weight in one study) have been reported at dose levels in the range 2 – 50 μg/kg. However, these results have not been reproducible in two other studies, one of which included additional dose levels, and using larger group sizes compared with those used in either of the two studies showing effects.

In rats, although one study showed effects on male reproductive tract development (reductions in testis size and in daily sperm production), the author later reported that these effects may have been influenced by uncontrolled biological factors. A more recent study, which also used additional dose levels, was unable to reproduce these results. Furthermore, no functional changes in reproductive parameters or reproductive organ development were observed in a recent rat two-generation study and multigeneration study at similar or higher dose levels.

Overall, there is no convincing evidence in postnatal rats and mice of effects on male reproductive tract development, although the available and apparently conflicting data do raise uncertainties. Recent evidence suggests that there are differences in the sensitivity of different mice strains to the effects of oestrodiol and that this sensitivity may be related to the selection of strains for large litter size. The relevance of these differences in relation to human health is unclear. Reassurance of an absence of effects is provided by the results of recent, good quality two-generation and multigeneration studies in the rat, covering a very wide range of dose levels and involving investigations in very large numbers of animals.

9 ANNEX 2: WORKING GROUP ON THE CLASSIFICATION AND LABELLING OF DANGEROUS SUBSTANCES: MAY 2001 MEETING

Agenda Item 3.2

K007 4,4'-Isopropylidenediphenol [Bisphenol-A] Index No. 604-030-00-0]

Current Annex I entry:	Xi; R36/37/38 : R43
UK preliminary position:	Repr. Cat. 3; R62 : Xi; R36/37 : R43
UK classification proposal:	Repr. Cat. 2; R60 : Xi; R37/41 : R43

Summary of previous discussions

HSE reviewed the hazardous properties of bisphenol A (BPA) as part of the UK's commitment towards the 3rd Priority List of the Existing Substances Regulation (ESR). The key endpoint of concern has been reproductive toxicity, and the possibility of effects occurring at relatively low exposure levels received much attention at the ESR Technical Meetings. Of more relevance to classification of bisphenol A, however, clear adverse effects on fertility have been seen in fertility studies in both rats and mice at relatively high dose levels (see below).

Summary of current health classification issues

A comprehensive appraisal of the available data for each endpoint is presented in the draft ESR risk assessment report (RAR) and therefore we provide here only a summary of the key issues for classification & labelling**.**

Fertility:

The effects of BPA on fertility and reproductive performance have been investigated in three good quality studies. There are both a 2- and a 3-generation study in rats (Chemical Compound Safety Research Institute, 2000 and Tyl et al., 2000[2], respectively) and a continuous breeding study in the mouse (NTP, 1985b).

In the rat 2-generation study, no adverse effects on fertility were observed up to and including the relatively low top dose level of 200 μg/kg.

In the rat 3-generation study, using doses of 0.001-500 mg/kg, the key finding was a statistically significant reduction in litter size observed in all three generations at the highest dose level (F_1: 11.5 live pups/litter compared to 14.3 for controls; F_2: 10.8 compared to 14.6; F_3: 10.9 compared to 14.8). No increases in numbers of dead pups/litter were seen. Alongside this, there was evidence of general toxicity in adults in all generations at 500 mg/kg (a reduction in body weight gain ≥13% in both sexes and renal tubule degeneration in females only).

In the mouse study, at the mid and high dose levels of approx. 600 and 1300 mg/kg, there were clear reductions in both the numbers of litters produced per Fo pair and in litter size (live pups/litter). The litter size reductions occurred across all matings and were dose-related (see ESR RAR for details). No effects were seen on the number of dead pups/litter. No effects were seen at the lowest dose level (approx 300 mg/kg). Decreases in litter size were also seen in additional "cross-over" experiments in which treatment of females clearly had the most significant effect. There were some signs of toxicity seen at the highest dose level only in the livers and kidneys of the

[2] Equivalent to Tyl et al. 2002

parental Fo animals, but the changes seen were not graded as either severe or high (see ESR RAR for details). No adverse effects on fertility were observed in the single litter tested at each dose level from the F_1 generation in this study.

Our assessment of the data suggests that clear evidence of impaired fertility has been seen in one species, the mouse. The adverse effects on numbers of litters and litter size seen in the continuous breeding study were seen at both the mid and high dose levels. There is no information about the mechanism of this action, but it is not possible to attribute these findings to general systemic toxicity. In this study, general toxicity was not evaluated comprehensively, but the findings reported do not suggest severe toxicity and were limited to the high dose groups. Classification is justified for adverse effects on fertility at least in category 3. The findings in rats are less straightforward to interpret, given that the deficits in fertility indices were only seen in dose groups that also exhibited signs of systemic toxicity. We view these findings as being supportive to those in the mouse and, therefore, suggest overall that the criteria for a category 2 classification are met. We would welcome further discussion in the Working Group of this interpretation of the criteria.

Developmental toxicity:
A relatively large number of developmental studies with BPA are available (see the ESR RAR for details) in addition to the detailed fertility studies conducted in rats.

No evidence of developmental toxicity has been observed in well-conducted, standard, oral studies in rats (NTP, 1985c) and mice (NTP, 1985a).

In addition to the standard studies, a number of other investigations in rats and mice have presented equivocal findings in the male reproductive tract at lower exposure levels.

A poorly reported study in rats, available only as an abstract (Sharpe et al, 1996), showed a 'highly significant' decrease in testes weight (5-15%) and daily sperm production (not quantified), in the absence of any morphological changes, in male pups from dams exposed to approximately 0.114 mg/kg BPA in drinking water during gestation. There were a number of design weaknesses in this study: the group sizes were not reported, the pups were apparently not weight-matched at the start of treatment, and the type of statistical analysis used is not known. The authors subsequently reported that a change to the laboratory water supply resulted in a reduction in the normal range of control testes weight and it is likely that this change preceded the commencement of this study. The authors maintained their confidence in this study but have acknowledged that factors in drinking water for which they had not controlled have the potential to cause reductions in testes weight in pups of comparable magnitude to that caused by exposure to potent estrogens during gestation and therefore may have confounded their findings (Sharpe *et al.*, 1998). In addition, these findings were not reproduced in a later, well reported, good quality study conducted using the same protocol and higher dose levels, up to 1.8 mg/kg (Cagan *et al.*, 1999a).

There have been similar conflicting findings in mice. In mice exposed to 2 or 20 μg/kg during days 11-17 gestation (Nagel *et al.*, 1997; Vom Saal *et al.*, 1998) or 50 μg/kg during days 16-18 gestation (Gupta, 2000) the most prominent finding was a statistically significantly increased prostate weight (30 and 35% at 2 and 20 μg/kg, respectively; 101% at 50 μg/kg), and decreased epididymis weight at 50 μg/kg (35%). Two other studies in mice, conducted with larger group sizes but otherwise comparable protocols to those of as Nagel *et al.* (1997) and Vom Saal *et al.* (1998), did not reproduce these findings and provided no evidence of developmental effects at the same or higher doses (up to 200 μg/kg) (Ashby *et al.*, 1999; Cagen *et al.*, 1999).

In the 2-generation and 3-generation studies conducted in rats (Chemical Compound Safety Research Institute, 2000; Tyl et al, 2000) there was no clear evidence of developmental toxicity. Observations in these studies included both increases and decreases in ano-genital distance, decreases in the weight of seminal vesicles, and delays in the time of preputial separation. As discussed in the ESR RAR in some detail, none of these observations were regarded by us as toxicologically significant.

Overall, our view of the classification criteria for this endpoint is that they emphasise the importance of the results of standard, well-conducted, validated studies in decision making. The criteria for category 2, which require clear evidence of an adverse effect, are not met. The changes seen at low doses only in male rodents in some of the non-standard studies have been offered by some as evidence of developmental toxicity in the various debates that have been taking place on this subject over recent years. It is not possible to be entirely dismissive of these findings but, especially because there appears to be a consensus that the standard tests for developmental toxicity have given clear, reproducible negative results, we also believe there is insufficient evidence to meet the criteria for classification in category 3.

UK proposal: Repr. Cat. 2; R60 : Xi; R37-41 R43

References cited

As listed in the ESR RAR, except for:

Haseman J.K, Hailey J.R and Morris R.W (1998). Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: a National Toxicology Program Update. Toxicologic Pathology, 26 (3), 428-441.

Takahashi O and Oishi S (2001). Testicular toxicity of dietary 2,2-bis(4-hydroxyphenyl)propane (bisphenol A) in F344 rats. Archives in Toxicology, 75, 42-51.

Takahashi S, Chi XJ, Yamaguchi Y, Suzuki H, Kita K et al (2001). Mutagenicity of bisphenol A and its suppresion by interferon-alpha in human RSa cells. Mutation Research, 490, 199-207.

HSE, Bootle

10th May, 2001