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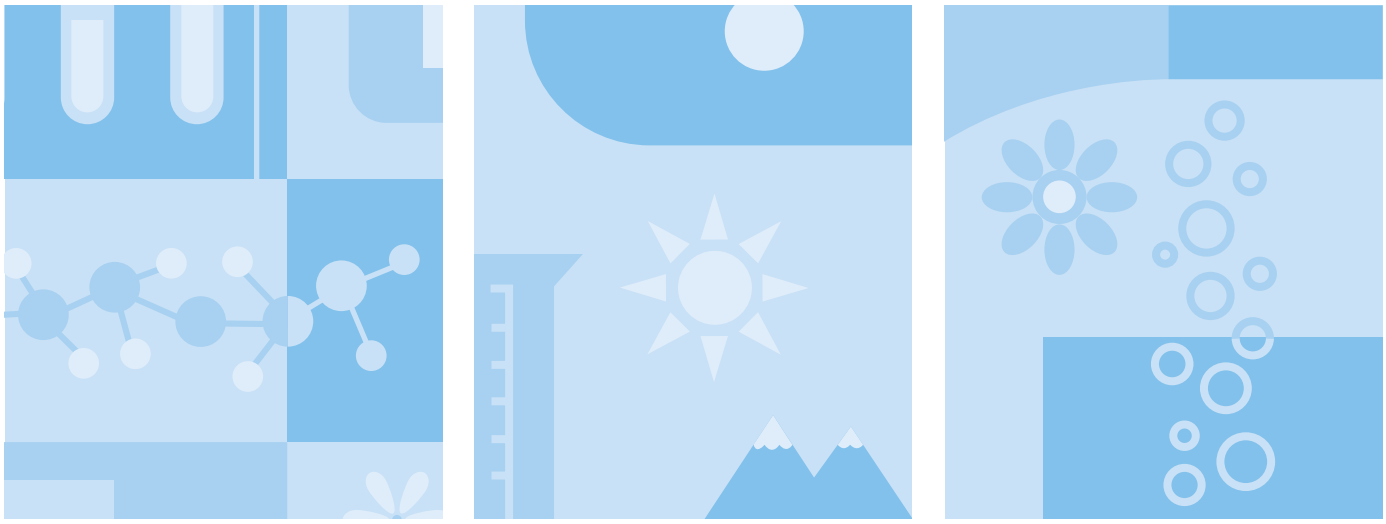
– identification of points of departure for the derivation of an alternative reference dose



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Preface

In April 2012 the Swedish government commissioned the Swedish Chemicals Agency (KemI) to investigate human exposure to bisphenol A (BPA) from thermal receipt papers, toys, children's products and drinking water from re-lined pipes as well as potential risks to human health. Consequently, in May 2012, KemI asked the Institute of Environmental Medicine (IMM) at Karolinska Institutet to review and evaluate scientific literature investigating the effects of BPA-exposure during early development on developmental neurotoxicity (DNT), as well as developmental effects on the mammary gland and female reproductive system and on lipogenesis. These effects have been reported in recent expert reports and risk assessments as being of potential concern for human health but have not been considered reliable enough to affect the current tolerable daily intake (TDI) for BPA established by the European Food Safety Authority (EFSA). The final report was to be provided to KemI by the end of June, 2012.

The purpose of this report is to review published studies investigating the effects of BPA in regard to the effects listed above, and to identify possible points of departure for the derivation of a reference dose to be considered in comparison with the current TDI for BPA. Several no observed (NOAEL) and lowest observed (LOAEL) adverse effect levels are identified. In addition, assessment factors are proposed and alternative reference doses are calculated to illustrate to what extent such values would deviate from the current TDI.

The uncertainties in the data material and its limitations for the derivation of a reference dose are discussed. Data gaps and future research needs are presented.

The views and recommendations expressed in this report are the authors' own and do not necessarily reflect the view of KemI.

Contents

Summary

This report presents a review and summary of studies investigating the effects of developmental exposure to bisphenol A (BPA) in regard to developmental neurotoxicity, as well as effects on the development of the mammary gland, the female reproductive system and lipogenesis. The purpose was to identify a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) for these different effects as well as to discuss how these NOAEL/LOAEL could be used as points of departure for the derivation of an alternative reference dose for BPA and to what extent this reference dose would deviate from the current tolerable daily intake (TDI) for BPA. The current TDI is based on the results of rodent studies that were conducted according to standardized toxicity test guidelines. In these studies effects on offspring body and organ weights were observed at doses above a NOAEL of 5 mg/kg bw/day. However, a large number of research studies conducted during the last decade have reported effects of BPA in animals at doses well below 5 mg/kg bw/day. But these data have so far not been considered relevant or reliable enough by regulatory bodies to affect the TDI or other health-based guidance values for BPA.

In this literature review studies using BPA-administration via the oral route to pregnant and/or lactating females or directly to neonatal offspring were primarily considered since these scenarios reflect relevant exposure scenarios in the general population. Dermal exposure may also be relevant but no animal studies where BPA was administered via the skin and investigating the chosen effects were available.

Many of the studies reviewed have methodological limitations or are poorly reported, which negatively impact their reliability and limit their utility for identifying a point of departure for the purpose of deriving a reference dose. No single study was considered reliable enough to serve as a key study for the identification of a NOAEL or LOAEL. The approach was instead to consider the data as a whole and to identify several NOAELs or LOAELs for each type of effect from different studies that were considered to be most reliable and relevant. In general, the selected NOAELs range from 2 to 50 µg/kg bw/day, which is 2 to 3 orders of magnitude lower than the NOAEL on which the current TDI is based. LOAELs range from 40 to 500 µg/kg bw/day.

Alternative reference doses calculated from these NOAELs/LOAELs range between 0.01 and 0.8 µg/kg bw/day and are considerably lower than the current TDI of 50 µg/kg bw/day. The lowest reference doses were calculated for developmental neurotoxicity.

Overall, it can be concluded that although no single study reviewed here was considered reliable enough to serve as a key study for the derivation of an alternative reference dose, if the data is considered as a whole, effects are consistently observed at doses well below those which serve as the basis for the current TDI for BPA. Even if confidence in a specific alternative reference dose based on this data material is low the results from this review indicate that considering a lower reference dose than the current TDI when conducting risk assessment of BPA may be prudent.

Sammanfattning

I denna rapport granskas och sammanfattas studier som undersökt effekterna av exponering för bisfenol A (BPA) under tidig utveckling med fokus på utvecklingsneurotoxicitet samt effekter på utvecklingen av bröstkörteln, det honliga reproduktionssystemet och fettomsättning. Syftet var att identifiera en no observed adverse effect level (NOAEL) eller lowest observed adverse effect level (LOAEL) för dessa olika effekter, samt att diskutera hur dessa NOAEL/LOAEL kan användas som utgångspunkt vid beräkning av en alternativ referensdos för BPA och i vilken utsträckning denna referensdos skulle avvika från det nuvarande tolerabla dagliga intaget (TDI) för BPA. Det nuvarande TDI-värdet har baserats på resultaten från studier i råttor och möss som utförts enligt standardiserade riktlinjer för toxicitetstestning. I dessa studier observerades effekter på kroppsvikt och organvikter hos avkomman vid doser över ett NOAEL på 5 mg/kg kroppsvikt/dag. Under det senaste decenniet har dock ett stort antal forskningsstudier publicerats där man observerat effekter av BPA i djur vid betydligt lägre doser än 5 mg/kg kroppsvikt/day. Dessa data har dock inte ansetts tillförlitliga nog av regulatoriska myndigheter för att påverka beräkningarna av TDI eller andra hälsobaserade riktvärden för BPA.

I den här litteraturgenomgången beaktades i första hand djurstudier där BPA administrerats oralt till dräktiga och/eller diande honor eller direkt till nyfödda ungar eftersom detta speglar relevanta exponeringsscenarion hos den allmänna befolkningen. Exponering via huden är också relevant men inga djurstudier där BPA administrerats via huden och som undersökt de utvalda effekterna kunde identifieras.

Många av studierna som granskats har metodologiska svagheter eller är bristfälligt rapporterade, vilket negativt påverkar tillförlitligheten och begränsar användbarheten för beräkningen av en referensdos. Ingen enskild studie ansågs tillförlitlig nog att på egen hand fungera som kritisk studie för att identifiera ett NOAEL eller LOAEL. Istället beaktades datamaterialet i sin helhet och flera NOAEL/LOAEL identifierades för varje typ av effekt baserat på de studier som ansetts vara mest tillförlitliga och relevanta. De NOAEL-värden som identifierades för de olika effekterna var generellt i samma storleksordning och varierade, med något undantag, mellan 2 och 50 µg/kg kroppsvikt/dag, vilket är 100 – 1000 gånger lägre än det NOAEL som ligger till grund för det nuvarande TDI. De LOAEL-värden som identifierades varierade generellt mellan 40 till 500 µg/kg kroppsvikt dag.

Alternativa referensdoser som beräknas från dessa NOAEL/LOAEL varierar mellan 0,01 och 0,8 µg/kg kroppsvikt/dag, vilket är betydligt lägre än nuvarande TDI som är 50 µg/kg kroppsvikt/dag. De lägsta referensdoserna beräknades för utvecklingsneurotoxicitet.

Slutsatsen från denna litteraturgenomgång är att effekter av BPA-exponering under tidig utveckling genomgående har rapporteras vid doser långt under det NOAEL på vilket TDI för närvarande baseras. Även om tillförlitligheten hos de enskilda studier som granskats här i många fall är låg visar ändå denna genomgång att det kan anses rimligt att beakta en lägre referens dos än det nuvarande TDI i riskbedömningen av BPA.

1. Introduction

1.1 Background

Bisphenol A (BPA) is one of the most highly produced industrial chemicals globally. It is used primarily for the manufacture of polycarbonate plastic and epoxy resins and as such is used in a wide variety of consumer products (Beronius and Hanberg, 2011). Measured concentrations of BPA in human blood, urine and other tissues confirm that exposure is widespread in the general human population (Vandenberg *et al.*, 2007; Calafat *et al.*, 2008). It is generally believed that consumer exposure to BPA occurs primarily via food in contact with BPA-containing materials, such as polycarbonate baby bottles, tableware and food containers as well as food and beverage cans lined with epoxy resins. Recently, it has also been shown that BPA can be transferred to the skin from certain types of thermal printing paper, such as some types of cashier's receipts, in significant amounts (Biedermann *et al.*, 2010).

It is well known that BPA is hormonally active and can interact with nuclear and membrane-bound estrogen receptors. The toxicity of BPA is very well studied compared to other chemicals. Still, there is disagreement among scientists as well as regulators as to the nature and size of the health risks posed by this compound. The current tolerable daily intake (TDI) for BPA established by the European Food Safety Authority (EFSA, 2006) is 50 μg/kg bodyweight (bw). The TDI is based on the results from two multi-generation reproduction studies in rats and mice (Tyl *et al.*, 2002 and 2008) in which adverse effects were only observed above a no observed adverse effect level (NOAEL) of 5 mg/kg bw/day. These studies were carried out according to standardized toxicity test guidelines, such as the OECD test guidelines, and are generally considered to be of very good quality and reliable. However, there are a large number of non-guideline studies available, i.e. studies that were not conducted according to standardized test guidelines, reporting effects of BPA exposure at doses well below 5 mg/kg bw/day, sometimes around only a few μg/kg bw/day (reviewed in Richter *et al.*, 2007). This so called "low dose controversy" has made the risk assessment of BPA particularly difficult (Beronius *et al.*, 2010) and has led scientists and others to question the sufficiency of the current TDI for BPA. In this report "low-dose effects" refer to effects observed at oral doses below 50 mg/kg bw/day, which is the lowest observed adverse effect level (LOAEL) established in the critical studies by Tyl *et al.*

However, many of the studies reporting low-dose effects of BPA have so far not been considered adequate to serve as the basis for the derivation of health-based guidance values, such as TDI, in regulatory risk assessments of BPA. The reasons given are often that they suffer from methodological flaws, such as only using one or two dose groups and inappropriate statistical methods, and/or are poorly reported, which limit their reliability for risk assessment purposes.

1.2 Purpose of the report

The purpose of this report is to review and summarize the literature describing the effects of developmental exposure to BPA in regard to developmental neurotoxicity (DNT), effects on the development of the mammary gland and female reproductive system, as well as lipogenesis. The aim was to identify NOAELs or LOAELs for these different effects, to discuss how these could be used as points of departure for the derivation of an alternative reference dose of BPA and to compare how this value would deviate from the current TDI for BPA. The term "reference dose"

is used in this context in order to keep the discussion open and general and not limit the conclusions drawn to the derivation of a more specific "TDI" or "Derived No Effect Level (DNEL)".

1.3 Scope and limitations of the report

The toxicity of BPA has been extensively investigated and low-dose effects have been observed for a wide range of endpoints. Given the short time-frame allowed for this report it was necessary to limit the literature review to a few selected types of effects. It was decided, together with KemI, to focus on DNT as well as developmental effects on the mammary gland, the female reproductive system and on lipogenesis. These effects were chosen because they have recently been highlighted as being of potential concern for human health by several authorities and expert groups (e.g. ANSES, 2011; NTP, 2008; WHO, 2011).

For the purpose of the assignment commissioned to KemI by the Swedish government, four human exposure scenarios were considered relevant:

1. Indirect exposure of the developing embryo/fetus/breast-fed infant via oral exposure of the mother.
2. Indirect exposure of the developing embryo/fetus/breast-fed infant via dermal exposure of the mother.
3. Direct exposure of the infant/child via the oral route.
4. Direct exposure of the infant/child via the dermal route.

The focus of this literature review was therefore on studies investigating developmental exposure to BPA, i.e. via administration of BPA to pregnant and/or lactating females or direct exposure of pre-pubertal offspring.

For identification of possible points of departure studies using BPA-administration via the oral route were primarily considered since this directly reflects a relevant exposure scenario in humans. Studies using subcutaneous administration were considered as supportive in regard to the nature of effects observed. However, subcutaneous administration of BPA is expected to result in higher levels of free circulating BPA than the same dose given orally due to the extensive first pass metabolism via the oral route.

2. Methods

Toxicity studies were identified from the open literature via searches in PubMed. Recent literature reviews conducted for the purpose for health risk assessment by different expert groups and authorities were also consulted. The aim was to include all literature investigating developmental exposure in regard to the selected effects.

All identified studies were summarized in tables, describing the animal model, exposure duration, dose levels, NOAEL (if any), LOAEL (if any) and effects observed or not observed of each study. No study was excluded based on evaluation of robustness or quality at this step. The tables are presented at the end of each section for the different types of effects.

Several different NOAELs and/or LOAELs for each type of effect were identified based on what was considered to be the most reliable studies, i.e. studies without major methodological flaws or deficiencies in reporting, and excluding studies reporting extremely high or extremely low NOAELs. These values were carried forward to a discussion regarding derivation of an alternative reference dose.

3. Developmental Neurotoxicity of BPA

3.1 Associations between BPA exposure and developmental effects on behavior observed in children

Four epidemiological studies investigating the association between prenatal BPA exposure and behavior in children in prospective birth cohorts are available (Braun *et al.*, 2009 and 2011; Perera *et al.*, 2012; Yolton *et al.*, 2011). Braun *et al.* observed associations between mothers' urinary BPA concentrations during pregnancy and anxious, depressive and hyperactive behaviors at 2 (2009) and 3 (2011) years of age, which were more pronounced for girls than for boys. In contrast, Perera *et al.* reported decreased anxious/depressed and aggressive behaviors in 3-5 year old girls but increased aggression and emotionally reactive behaviors in 3-5 year old boys with higher prenatal exposure to BPA (measured in mothers' urine during pregnancy). Yolton *et al.*, found no association between mothers' urinary BPA concentrations and infant neurobehavior evaluated at 5 weeks of age. These human data, although inconsistent, indicate that prenatal BPA-exposure may affect child behaviors in a sex-dependent manner. However, they are not considered to be adequate for derivation of a reference dose.

3.2 Animal data

Fifty-three *in vivo* studies investigating effects on behavior and/or cerebral development after exposure to BPA were identified. These have been summarized in tables 1 – 5. The majority of these studies were conducted in rats and mice. One study was conducted in monkeys. For the purposes of identifying points of departure for the derivation of a reference dose for DNT the studies investigating effects on behavior were considered the most relevant. However, the French Agency for Food, Environmental and Occupational Health and Safety (ANSES, 2011) recently concluded that

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

Studies investigating functional and behavioral effects were separated into the following categories depending on dosing regime: oral exposure of pregnant and/or lactating females (Table

1), direct oral exposure to offspring (Table 2), subcutaneous exposure of pregnant and/or lactating females (Table 3) and direct exposure of the offspring via injection subcutanesously or intra-cisternally (Table 4). Studies investigating cerebral development after pre- and/or postnatal exposure to BPA have been summarized in Table 5. No animal studies investigating DNT effects after dermal BPA exposure to pregnant/lactating females or directly to offspring are available.

In the very comprehensive literature review conducted by the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) in conjunction with their report on potential human reproductive and developmental effects of BPA (NTP-CERHR, 2008) the studies by Kwon *et al.* (2000), Negishi *et al.* (2004), Palanza *et al.* (2002) and Ryan and Vandenbergh (2006) (all investigating effects in the offspring after oral exposure to the mother during gestation/lactation, Table 1) were considered to be adequate and of high utility for the health risk assessment of BPA.

3.3 Identification of NOAELs/LOAELs

3.3.1. Indirect exposure of offspring via oral administration to pregnant and/or lactating females

Thirty-three animal studies were identified that have investigated behavioral effects in offspring after oral exposure to pregnant and/or lactating females (Table 1). Only a few studies were able to identify a NOAEL, i.e. many studies observed effects at the lowest doses tested. It should also be noted that the majority of these studies only included one or two different dose groups, which limits the conclusions that can be made about a dose-response relationship and increases the uncertainties regarding the identification of a NOAEL or LOAEL for DNT effects. The studies that are considered contribute most to the derivation of a possible alternative reference dose for BPA, i.e. studies without major methodological flaws, including more than one dose of BPA and from which a NOAEL/LOAEL can be identified, are briefly summarized below.

The lowest NOAEL of 2 µg/kg bw/day was identified in the well-made study by Ryan and Vandenberg (2006) with a LOAEL of 200 µg/kg bw/day (only those two doses tested). At 200 µg/kg bw/day anxiety-related behaviors in the Elevated Plus Maze (EPM) and light-dark preference chamber were affected in female offspring, however effects were only statistically significant in the light-dark preference test. Spatial memory was also tested using the radial-arm maze and Barnes maze but were unaffected by BPA exposure in this study.

Jones *et al.* (2011) studied effects on several different parameters of male sexual behavior after administration of four different doses ranging from 5 to 5000 µg/kg bw/day. No effects were observed at the lowest dose of 5 µg/kg bw/day. However, the dose-response for all effects observed was reported to be non-monotonic, i.e. U-shaped or inverted U-shaped. In a later study by Jones *et al.* (2012) the same doses were tested and anxiety-related and depressive behaviors were evaluated. In this study diminished sex-differences in behaviors were reported at the lowest dose of 5 µg/kg bw /day. However, the actual changes in behaviors in males and females do not seem to have been statistically significant, which makes these results difficult to interpret for the purposes of setting a LOAEL. Nevertheless, this study can be considered to support the results in the study by Ryan and Vandenberg that effects on anxiety-related behaviors are sensitive to the exposure of BPA and can be observed in rodents at doses around a few µg/kg bw/day.

The study by Xu *et al.*, (2010b) investigated effects on spatial as well as passive avoidance learning and memory after gestational and lactational exposure to 50, 500, 5000, and 50 000 µg BPA/kg bw/day. Significant but marginal effects on spatial memory were observed at 500 µg/kg bw/day and above. Effects on passive avoidance memory were observed at 5000 µg/kg bw/day and above.

A NOAEL of 2 µg/kg bw/day for effects on anxiety-like behaviors in offspring after oral exposure to pregnant and/or lactating females, can be identified based on the mouse study by Ryan and Vandenbergh (2006) and supported by the study by Jones *et al.* (2011). However, it should also be noted that there are studies that did not observe any effects on anxiety in the same dose range.

A NOAEL of 50 µg/kg bw/day for effects on learning and memory can be identified from the study by Xu *et al.* (2010b). Considering that effects observed at 500 µg/kg bw/day in this study were marginal and limited to a few parameters of spatial memory and no effects on learning and memory were observed at 200 µg/kg bw/day in the study by Ryan and Vandenberg (2006), this NOAEL can be considered to be conservative.

3.3.2. Direct developmental exposure of the offspring via the oral route

Four studies investigating behavioral effects after postnatal developmental exposure to BPA via direct oral administration to the pups were identified (Table 2). One study (Ema *et al.*, 2001) did not observe any effects.

In the study by Viberg *et al.* (2011) relatively high doses were used. However, the NOAEL from this study is 320 µg/kg bw/day, i.e. one order of magnitude below the NOAEL on which the current TDI is based. This study used a single postnatal exposure, which may well represent a relevant exposure scenario for humans, i.e. an infant being exposed to a relatively high dose of BPA at an isolated time-point during critical windows of development.

In the study by Carr *et al.* (2003) newborn rats were exposed to BPA orally for 14 days. Diminished sex differences in spatial learning were observed at the lowest dose tested (100 µg/kg bw/day). However, no statistically significant changes in either sex were observed at this dose. At the higher dose of 250 µg/kg bw/day impaired spatial learning was observed in females. It was concluded that 100 µg/kg bw/day is the most appropriate NOAEL from this study.

No NOAEL was established in the study in mice by Xu *et al.* (2011). Males in this study showed significantly reduced passive avoidance memory, an effect that was more pronounced at 40 µg/kg bw/day than at 400 µg/kg bw/day. Further, elimination of sex-differences in anxiety-related behaviors and exploration as well as in spatial learning and memory was observed. A LOAEL of 40 µg/kg bw/day can be derived from this study. It should be noted that in this study young mice were exposed to BPA during puberty/adolescence rather than shortly after birth

3.3.3. Studies using subcutaneous administration of BPA

3.3.3.1. Indirect exposure of offspring via subcutaneous administration to pregnant or lactating females

Five studies investigating DNT effects after subcutaneous administration to the mother were identified (Table 3). The study by Nakamura *et al.* (2012) present very inconsistent results concerning decreased motor activity and is not considered reliable for a derivation of a point of departure. One study investigated only a very high dose (Sato *et al.*, 2001). The study by Rubin *et al.* (2006) investigated effects of BPA in mice at very low doses, 0.025 and 0.25 µg/kg bw/day. Diminished sex-differences in exploratory behaviors measured in the open field were observed at the higher dose. However, the main focus of this study was to investigate effects on neuronal structure in certain parts of the brain and behavioral tests were secondary. There are some weaknesses in the behavioral testing, e.g. methodological difficulties preventing the investigators to evaluate effects on motor activity, which introduces uncertainties regarding the reliability of the observed effects on exploration. Further, the actual changes in exploration in males and females do not seem to have been statistically significant. In all, these results are difficult to interpret for the purposes of setting a NOAEL and the study is of questionable reliability. However, it should be noted that behavioral effects were observed in this study at doses that are two orders of magnitude lower than in other DNT studies of BPA.

The study by Nakagami *et al.* (2009) reported feminization of behavior in male infant cynomolgus monkeys after a dose of 10 µg/kg bw/day (the only dose tested) administered to pregnant mothers from gestational day (GD) 20 to term via subcutaneous pumps. Infant and maternal behaviors were evaluated 2 and 3 months after birth. In this study the behavior both of male infants as well as their mothers were affected to make them more alike female infant – mother pairs. It can therefore be discussed whether effects on maternal behavior influenced changes in infant behavior, or vice versa. However, the authors argue that had the main effect been on maternal behavior this should also have been seen in mothers of female infants. They therefore conclude that the effects observed are due to disturbed behavioral sexual differentiation in male fetuses. In BPA-exposed male infants "clinging" at 2 months after birth and "outward looking" both at 2 and 3 months after birth were significantly different from unexposed male infants and similar to female infants. Exposed female infants showed no differences from unexposed female infants. "Outward looking" was also the main affected behavioral parameter in BPA-exposed mothers of male infants, making them significantly different from unexposed mothers of male infants and similar to mothers of female infants. There are a few uncertainties in the evaluation of the results from this study. The meaning and potential adversity of the observed changes in behavior is not very well described and many behavioral parameters were not affected. In addition, only one low dose was investigated. It should also be noted that this study does not provide information on effects after lactational exposure in neonates since exposure was stopped at parturition.

3.3.3.2 Direct developmental exposure of the offspring via subcutaneous administration

Two studies (Monje *et al.*, 2009; Patisaul and Bateman, 2008) were identified that investigated behavioral effects after direct neonatal subcutaneous injection to offspring (Table 4). Monje *et al.* reported altered sexual behavior in females at the lowest dose tested (50 µg/kg bw/day). Patisaul

and Bateman reported effects on anxiety but not on aggression in males (only males tested) at 50 µg/kg bw/day, the only dose tested. Effects were observed at doses in the same range as in studies investigating oral and subcutaneous exposure to mothers and oral exposure to offspring. However, both these studies are of questionable quality due to study design, statistical approach and poor reporting.

Six studies administered BPA directly to neonatal rats via intra-cisternal injection (Table 4) but were not considered for the identification of a point of departure. This route of exposure was considered to be of questionable relevance for health risk assessment.

3.4 Tables summarizing literature on developmental neurotoxicity of BPA

Table 1. Studies investigating behavioral effects in offspring after oral BPA exposure to pregnant and/or lactating females.

Study	**Animal model**	**Exposure duration**	**Dose** (μg/kg bw/d)	**NOAEL** (μg/kg bw/d)	**LOAEL** (μg/kg bw/d)	**DNT effects observed**
Adriani *et al.*, 2003	Rat (Sprague-Dawley)	Mating – PND 25	40	--	40	Increased novelty-induced hyperactivity, neophobia in females, decreased impulsivity in both sexes, impairment of the classical response to AMPH in male subjects
Cox *et al.*, 2010	Mouse (C57BL/6J)	Mating – PND 0	8000	--	8000	Diminished sex-differences in social behavior, increased anxiety
Dessí-Fulgheri *et al.*, 2002	Rat (Sprague-Dawley)	2 groups 1) Mating – PND 21 2) GD 14 – PND 6	Group 1) 40 Group 2) 400	--	40	Several factors of male and female play behavior affected in both groups
Ema *et al.*, 2001	Rat (Sprague-Dawley)	Mating – PND 21 (Oral to offspring from PND 23)	0.2, 2, 20, 200	200	--	*No effects observed*
Farabollini *et al.*, 1999	Rat (Sprague-Dawley)	2 groups 1) Mating – PND 21 2) GD 14 – PND 6	Group 1) 40 Group 2) 400	--	40	Decreased motor activity and motivation to explore in females, decreased motivation to explore and anxiety in males
Farabollini *et al.*, 2002	Rat (Sprague-Dawley)	2 groups 1) Mating – PND 0 2) PND 0 – 21	40	--	40	Marginally affected sociosexual behavior in males and females
Fujimoto *et al.*, 2006	Rat (Wistar)	GD 14 – PND 0	15	--	15	Diminished sex-differences in exploratory behavior and stress-response, enhanced depression-like behavior. Unaffected: motor activity, passive avoidance learning/memory, anxiety in EPM
Gioiosa *et al.*, 2007	Mouse (CD-1)	GD 11 – PND 8	10	--	10	Diminished sex-differences in exploratory behavior in novelty-seeking test, open-field and elevated plus maze
Goncalves et al,. 2010	Rat (Wistar)	3 groups 1) Mating – PND 0 2) PND 0 – PND 21 3) Mating – PND 21	40	--	40	Impaired memory and reduced locomotion and exploratory behavior. Unaffected: “emotional state”

Jasarevic *et al.*, 2011	Wild deer mouse (Peromyscus maniculatus)	Mating – PND 21	20000[a]	--	20000	Impaired learning and memory, increased anxiety-like behavior/ decreased exploration, decreased "attractiveness" to females
Jones *et al.*, 2011	Rat (Long-Evans)	GD 7 – PND 14	5, 50, 500, 5000	5	50	Deficits in several parameters of male sexual behavior (non-monotonic dose-response)
Jones *et al.*, 2012	Rat (Long-Evans)	GD 7 – PND 14	5, 50, 500, 5000	--	5	Diminished sex-differences in anxiety-related behaviors (fecal boli and activity/immobility) and forced swim test (non-monotonic dose-response)
Kawai *et al.*, 2003	Mouse (CD-1) Male offspring investigated only	GD 11 – PND 17	2, 20	--	2	Increased aggression at 8 weeks (but not at 12 or 16 weeks)
Kubo *et al.*, 2001	Rat (Wistar)	Mating – PND 21	1500	--	1500	Diminished sex differences of sexually dimorphic behaviors in the open field and passive avoidance test
Kubo *et al.*, 2003	Rat (Wistar)	Mating – PND 21	30, 300	--	30	Diminished sex-differences in locomotor activity and exploratory behavior Unaffected: male and female sexual behavior
Kwon *et al.*, 2000	Rat (Sprague-Dawley)	GD 11 – PND 20	3200, 32000, 320000	320 000	--	*No effects observed*
Laviola *et al.*, 2005	Mouse (CD-1)	GD 11 – PND 18	10	--	10	Disruption of normal drug-conditioned place preference response in females
Miyagawa *et al.*, 2007	Mouse (C57/Bl-6) Male offspring investigated only	Mating – PND 21	12, 800000[a]	--	12	Impaired memory Unaffected: anxiety-related behaviors
Mizuo *et al.*, 2004	Mouse (ddY) Male offspring investigated only	Mating – PND 21	800, 200000, 800000[a]	800	200000	Enhanced morphine-induced place preference compared to controls
Narita *et al.*, 2006	Mouse (ddY) Male offspring investigated only	Mating – PND 21	12, 120, 1200, 200000, 800000[a]	--	12	Supersensitivity of morphine-induced pharmacological actions on locomotor activity and place preference test. Unaffected: motor activity without pharmacological challenge

Negishi *et al.*, 2003	Rat (Fischer 344)	GD 10 – PND 20	4000, 40000, 400000	--	4000	Altered spontaneous activity in females, lower response in active avoidance test and increased grooming at 8 weeks in males
Negishi *et al.*, 2004	Rat (Fischer 344) Male offspring investigated only	GD 3 – PND 20	100	--	100	Impaired learning, altered response to disruption of the monoaminergic system. Unaffected: motor activity without pharmacological challenge
Palanza *et al.*, 2002	Mouse (CD-1) Female offspring investigated only	GD 14 – 18	10	--	10	Disrupted maternal behavior Unaffected: anxiety pre-weaning (ontogeny)
Poimenova., 2010	Rat (Wistar)	GD 0 – PND 22	40	--	40	Impaired spatial recognition memory, decreased exploration, increased anxiety in females
Porrini *et al.*, 2005	Rat (Sprague-Dawley) Female offspring investigated only	Mating – PND 21	40	--	40	Changes in several factors identified for social behavior
Ryan and Vandenbergh, 2006	Mouse (C57/Bl-6) Female offspring investigated only	GD 3 – PND 21	2, 200	2	200	Increased anxiety Unaffected: spatial learning/memory
Ryan *et al.*, 2010	Rat (Long-Evans) Female offspring investigated only	GD 7 – PND 18	2, 20, 200	200	--	*No effects observed*
Stump *et al.*, 2010	Rat (Sprague-Dawley)	GD 0 – PND 21	20, 185, 9475, 92700, 287000	287000	--	*No effects observed*
Suzuki *et al.*, 2003	Mouse (ddY) Male offspring investigated only	Mating – PND 21	800, 200000, 800000[a]	--	800	Enhancement of the rewarding effect by methamphetamine Unaffected: motor activity without pharmacological challenge
Tian *et al.*, 2010	Mouse (ICR)	GD 7 – PND 21 (Oral to offspring PND 22 – 36)	100, 500	--	100	Reduced anxiety, decreased novel object recognition, reduced working memory (analyzed males and females together)
Wolstenholme *et al.*, 2011	Mouse (C57BL/6J)	Not clearly stated, implied: "last 10 days of gestation"	~220[b]	--	220	Social interactions in females affected Unaffected: social preference, anxiety

Xu *et al.*, 2007	Rat (Sprague-Dawley)	GD 11 – PND 21	15, 7600[c]	--	15	Increased locomotion and exploration in males, impaired learning and memory in males (effects only at the low dose)
Xu *et al.*, 2010b	Mouse (ICR) Male offspring investigated only	GD 7 – PND 21	50, 500, 5000, 50000	50	500	Impaired spatial memory. Impaired passive avoidance memory observed at higher doses (at 5000 µg/kg bw/d and above).

-- not established

[a] Dose reported as mg/g food and converted here assuming an average food intake during gestation and lactation of 9 g/day (Hau & Shapiro, 2011) and a body weight of 0.0225 kg (US EPA, 1988)

[b] Dose reported as 5 µg/d and converted here assuming a body weight of 0.0225 kg (US EPA, 1988). Reported to produce an internal exposure within the range detected in human maternal blood

[c] Dose reported as mg/L drinking water and converted here assuming a water intake for Sprague-Dawley rats of 0.152 L/kg bw/day (US EPA, 1988) (NOTE: not adjusted for gestation/lactation)

Table 2. Studies investigating behavioral effects in offspring after direct oral BPA exposure.

Study	**Animal model**	**Exposure duration**	**Dose** (µg/kg bw/d)	**NOAEL** (µg/kg bw/d)	**LOAEL** (µg/kg bw/d)	**DNT effects observed**
Carr *et al.*, 2003	Rat (Fischer 344)	PND 1 - 14	100, 250	100	250	Impaired spatial learning in females at the high dose. NOTE: Diminished sex-differences in spatial learning without statistically significant changes in either sex were observed at the low dose,
Ema *et al.*, 2001	Rat (Sprague-Dawley)	From PND 23 to termination (to dam mating – PND 21)	0.2, 2, 20, 200	200	--	*No effects observed*
Viberg *et al.*, 2011	Mouse (NMRI) Male offspring investigated only	PND 10	320, 3200, 4800	320	3200	Altered spontaneous behavior in novel home environment and reduced habituation. Unaffected: anxiety, spatial learning, nicotine-induced behavior
Xu *et al.*, 2011	Mouse (ICR)	PND 32 – 87 (pubertal exposure)	40, 400	--	40	Elimination or reversal of sex-differences in anxiety-like behaviors and exploration as well as spatial learning and memory, reduced passive avoidance memory in males (larger effects in lowest dose)

-- not established

Table 3. Studies investigating behavioral effects in offspring after subcutaneous BPA exposure to pregnant and/or lactating females.

Study	**Animal model**	**Exposure duration**	**Dose** (µg/kg bw/d)	**NOAEL** (µg/kg bw/d)	**LOAEL** (µg/kg bw/d)	**DNT effects observed**
Nakagami *et al.*, 2009	Monkey (*Macaca fascicularis*)	GD 20 – 160 (pump)	10	--	10	Feminization of behavior in male infants
Nakamura *et al.*, 2012	Mouse (ICR/Jcl)	GD 0 – PND 21 (injection)	20	--	20	Inconsistent results: decreased motor activity at some time points Unaffected: exploration, anxiety, spatial learning and memory
Rubin *et al.*, 2006	Mouse (CD-1)	GD 8 – PND 22 (pump)	0.025, 0.25	0.025	0.25	Diminished sex-differences in exploration
Sato *et al.*, 2001	Mouse (Jcl-ICR)	GD 11 – 19 (injection)	100000	--	100 000	Increased grooming as well as decreased defecation in open-field test (males and females analyzed together). Unaffected: motor activity

-- not established

Table 4. Studies investigating behavioral effects in offspring after direct BPA exposure via injection subcutaneously or intra-cisternally.

Study	Animal model	Exposure duration	Dose (µg/kg bw/d)	NOAEL (µg/kg bw/d)	LOAEL (µg/kg bw/d)	DNT effects observed
Intra-cisternal injection (NOTE: not considered relevant for health risk assessment)						
Ishido *et al.*, 2004	Rat (Wistar) Male offspring investigated only	PND 5	2, 20, 200, 2000[a]	2	20	Motor hyperactivity
Ishido *et al.*, 2011	Rat (Wistar) Male offspring investigated only	PND 5	20	--	20	Motor hyperactivity
Kiguchi *et al.*, 2007	Rat (Wistar)	PND 5	2000, 4000	4000	--	*No effects observed*
Kiguchi *et al.*, 2008	Rat (Wistar) Male offspring investigated only	PND 5	2000, 4000[a]	--	2000	Motor hyperactivity, reduced habituation Unaffected: response to pharmacological challenge
Masuo *et al.*, 2004a	Rat (Wistar) Male offspring investigated only	PND 5	2, 20, 200, 2000[a]	2	20	Motor hyperactivity
Masuo *et al.*, 2004b	Rat (Wistar) Male offspring investigated only	PND 5	2000[a]	--	2000	Motor hyperactivity
Subcutaneous injection						
Monje *et al.*, 2009	Rat (inbred Wistar-derived strain) Female offspring investigated only	PND 1 - 7	50, 20000	--	50	Altered sexual behavior in females.
Patisaul and Bateman, 2008	Rat (Long-Evans) Male offspring investigated only	PND 0 - 3	50	--	50	Increased anxiety Unaffected: aggression

-- not established

[a]Dose reported as µg/rat and converted here assuming a body weight of 10g for a 5d-old rat (Falkmer & Waller, 1994)

Table 5. Studies investigating effects on cerebral development after perinatal exposure to BPA.

Study	Animal model	Exposure	Dose (µg/kg bw/d)	NOAEL (µg/kg bw/d)	LOAEL (µg/kg bw/d)	DNT effects observed
Adewale *et al.*, 2011	Rat (Long Evans)	Subcutaneous to offspring PND 0 – 3 (injection)	50, 50000	--	50	Increased number of oxytocin neurons in the paraventricular nucleus. No changes in ER-alpha receptor density. No effects on serotonin fibre density in the ventrolateral subdivision of the ventromedial nucleus.
Bai *et al.*, 2011	Rat (Sprague-Dawley)	Subcutaneous to dams GD 10 – PND 7 (injection)	2	--	2	Increased kisspeptin neurons and reduced GnRH neurons in the AVPV (hypothalamus), feminization of the nervous component of the gonadotropic axis.
Kim *et al.*, 2009	Mouse (ICR)	Subcutaneous to dams GD 14.5 – 18.5 (injection)	5000, 10000, 20000	10000	20000	Acceleration of the formation of the dentate gyrus (only on observed on PND 1 and not at later time points).
Kubo *et al.*, 2001	Rat (Wistar)	Oral to dams Mating – PND 21	1500	--	1500	Reversal of gender differences in LC volume and number of neurons.
Kubo *et al.*, 2003	Rat (Wistar)	Oral to dams Mating – PND 21	30, 300	--	30	LC volume/number of neurons
Monje *et al.*, 2009	Rat (inbred Wistar-derived strain) Female offspring investigated only	Subcutaneous to offspring PND 1 – 7 (injection)	50, 20000	--	50	ER-alpha expression down-regulated, repressor of estrogen receptor activity (REA) expression up-regulated in the hypothalamus
Nakamura *et al.*, 2010	Mouse (Jcl-ICR)	Subcutaneous to dams GD 0 – PND 21 (injection)	20	--	20	Increase of dopamine and serotonin in different areas of the brain
Rubin *et al.*, 2006	Mouse (CD-1)	Subcutaneous to dams GD 8 – PND 22 (pump)	0.025, 0.25	0.025	0.25	Diminished or eliminated sex-differences in the number of tyrosine hydroxylase (TH) neurons in the rostral periventricular preoptic area of the brain.

Sato *et al.*, 2001	Mouse (Jcl-ICR)	Subcutaneous to dams GD 11 – 19 (injection)	100000	--	100 000	Increased grooming as well as decreased defecation in open-field test (males and females analyzed together). Unaffected: motor activity
Tian *et al.*, 2010	Mouse (ICR)	Oral to dams GD 7 – PND 21 Oral to offspring PND 22 – 36	100, 500	--	100	Decrease in dopamine D2 receptors and dopamine transporters in the putamen. Decreased NMDA receptor in the frontal cortex, dentate gyrus and hippocampus
Xu *et al.*, 2010a and b	Mouse (ICR)	Oral to dams GD 7 – PND 21	50, 500, 5000, 50000, 200000	--	50	Decreased NMDA receptor expression in the hippocampus, less marked effect at the high doses. Decreased ER-beta expression and increased aromatase in the hippocampus.

-- not established

4. Effects of BPA on the developing mammary gland

4.1 Associations between BPA and risk for breast cancer in humans

The associations between BPA exposure and breast cancer have been investigated in one case-control study in Korean women (Yang *et al.*, 2009). BPA levels did not differ between cases and controls in this study. However, major methodological limitations, such as low statistical power, undetectable BPA levels in many of the subjects and cross-sectional design prevents any conclusions to be drawn from this study. No human studies investigating the association between developmental exposure to BPA and effects on the mammary gland were identified.

4.2 Animal data

Seven in vivo studies investigating the effects on the mammary gland in female offspring after oral exposure to pregnant and/or lactating mothers were identified and have been summarized in Table 6. These studies have primarily been considered for the identification of a point of departure for the derivation of a reference dose. In addition, 6 studies investigating effects on the mammary gland in offspring after subcutaneous exposure to pregnant and lactating females are available and have been summarized in Table 7. Although not directly considered in the identification of a NOAEL or LOAEL these studies serve as additional information regarding the type of effects in the mammary gland that may result from developmental BPA-exposure.

Acceleration of the mammary gland's structural maturation and development of intraductal hyperplastic lesions, which may result in an increased susceptibility to mammary carcinogenesis, after perinatal exposure to BPA are effects that have been reported in several of the studies.

All 6 studies investigating developmental effects on the mammary gland after subcutaneous exposure to the pregnant and lactating females were conducted by many of the same investigators. There is a general agreement between most of these studies that subcutaneous exposure to BPA during gestation and lactation lead to increased proliferation and the formation of hyperplastic ducts. Effects were observed in all doses investigated and no NOAEL was identified in any of these studies. It can be noted that effects were observed at lower doses than after oral exposure to pregnant or lactating females. This is reasonable since subcutaneous injection would result in higher circulating concentrations of free BPA than the same oral dose.

4.3 Identification of NOAELs/LOAELs

Four rat studies, 2 mouse studies and one study in monkeys have investigated effects on the mammary gland in female offspring after oral BPA exposure to pregnant and/or lactating females (Table 6). These studies can be considered to be in general agreement regarding the effects of BPA on the mammary gland as well as, to some extent, the doses at which such effects may occur. All 4 rat studies were conducted by investigators mainly from the same laboratory.

Changes in gene expression and protein levels relevant for signaling pathways involved in mammary carcinogenesis seem to be more sensitive effects than structural changes, after oral as well as subcutaneous administration of BPA. In two of the oral studies these "early effects" were reported at 25 µg/kg bw, the lowest dose investigated (Betancourt *et al.*, 2010b; Moral *et al.*,

2008). However, whether such effects can be considered adverse and relevant for a derivation of a reference dose is uncertain.

Structural changes, e.g. changes in the number of terminal end buds, the total area covered by terminal end buds and their density, were observed after oral administration of BPA in 2 rodent studies. Moral *et al.* (2008) observed such structural changes in immature and adult rats at doses of 250 µg/kg bw/day. In the mouse study by Ayyan *et al.* (2011), structural effects were observed at 3 µg/kg bw/day. However, the reliability of this study is challenged by the fact that the exposure duration, dose levels, number of dose groups as well as number of animals per dose group were poorly reported. It also seems that different endpoints were investigated at different doses in the study by Ayyan *et al*.

Susceptibility to the development of tumors after co-exposure to the known carcinogenic agent 7, 12-dimethylbenz(a)anthracene (DMBA), after oral administration of BPA to pregnant and lactating dams were observed in 3 rodent studies. Two studies investigated tumor susceptibility in rats after pre- or postnatal exposure, respectively (Betancourt *et al.*, 2010a; Jenkins *et al.*, 2009). Effects were observed at 250 but not 25 µg/kg bw/day in both studies. One study using the FVB/N mouse strain, which has intrinsic propensity to develop mammary tumors, observed effects also at 25 µg/kg bw/day (Lozada and Keri, 2011). Because of the unusual and sensitive rodent model this study is considered to provide information on the potential of BPA to sensitize the mammary gland to tumorigenesis but is less reliable in terms of determining a point of departure for health risk assessment. Collectively, these findings suggest that the structural changes of the rodent mammary gland observed after exposure to BPA alone may make the gland sensitive to the development of tumors in response to future exposure to carcinogens.

The study in monkeys by Tharp *et al.* (2012) can be considered particularly relevant for health risk assessment considering the similarities in BPA metabolism as well as mammary gland morphology between monkeys and humans as compared to rodents and humans. In this study pregnant rhesus monkeys were fed a dose of 400 µg BPA/kg bw/day. Exposure resulted in serum levels of 0.68±0.312 ng free BPA/ml and are comparable to those measured in the general population. It is interesting to note that these levels resulted from an exposure that is 8 times higher than the current TDI of 50 µg/kg bw/day. The mammary glands of female offspring (n=4, controls = 5) were collected 1 – 3 days after birth. Effects similar to those seen in rodents were observed, e.g. increased number of terminal end buds, terminal ends, branching points, as well as total mammary gland area, ductal area and number of ductal units.

No individual study is considered robust enough to serve as critical study for the identification of a point of departure in regard to effects on the developing mammary gland. Further, the studies that are in agreement were generally conducted in the same laboratories (Betancourt *et al.*, 2010a and b; Jenkins *et al.*, 2009; Moral *et al.*, 2008). In conclusion, based collectively on the rodent studies available an overall NOAEL of 25 µg/kg bw/day can be identified for effects on the development of the mammary gland after perinatal BPA exposure via oral exposure to the mother.

As an alternative, considering that the study in monkeys has high relevance for humans, e.g. more similarity in BPA metabolism and mammary gland morphology compared to rodents, a LOAEL of 400 µg/kg bw/day can be identified from this study.

4.4 Tables summarizing literature on the effect of BPA on the developing mammary gland

Table 6. Studies investigating effects on mammary gland development after pre- and/or postnatal exposure to BPA administered orally to pregnant or lactating females.

Study	**Animal model**	**Exposure duration**	**Dose** (µg/kg bw/d)	**Additional chemical challenge**	**NOAEL** (µg/kg bw/d)	**LOAEL** (µg/kg bw/d)	**Effects on mammary gland observed**
Ayyanan *et al.*, 2011	Mouse (C57Bl6)	Pre- and postnatally until weaning. NOTE: Exposure duration not clearly described	0.6 – 1200 NOTE: number of dose groups not clearly described.	--	Cannot be determined due to lack of information regarding dose levels.	3	Changes in transcription of ERα-regulated genes at 1200 µg/kg bw/day, increased number of terminal end buds at 3 µg/kg bw/day. Increased cell proliferation at 6 and 12 µg/kg bw/day (dose groups pooled) and above. Enhanced transcriptional response to progesterone at 6 µg/kg bw/day.
Betancourt *et al.*, 2010a	Rat (Sprague Dawley)	GD 10 - 21	25, 250	Single dose of DMBA[a] on PND 50 or 100	25	250	*With DMBA at PND 100 (but not 50):* increase in incidence in mammary tumors and shorter tumor latency. *Without DMBA:* Increase in cell proliferation and overexpression of some proteins at both time-points.
Betancourt *et al.*, 2010b	Rat (Sprague Dawley)	GD 10 - 21	25, 250	--	--	25	Changes in the expression of some proteins that are important for signaling pathways involved in mammary carcinogenesis, such as cell proliferation.
Jenkins *et al.*, 2009	Rat (Sprague Dawley)	PND 2 – 202	25, 250	Single dose of DMBA on PND 50	25	250	*With DMBA:* dose dependent increase in incidence in mammary tumors and shorter tumor latency. *Without DMBA:* Increase in proliferation and decreased apoptosis and overexpression of some proteins.

Lozada and Keri, 2011	Mouse (FVB/N)[b]	GD 8 – PND 0	25, 250	DMBA once at 5 and at 6 weeks of age	*Without DMBA:* 25 With DMBA: --	*Without DMBA:* -- With DMBA: 25	*Without DMBA (NOTE: only the low dose investigated!): no overt morphological effect on the development of the mammary gland with 25 µg/kg bw/day at any age (pre-puberty to adulthodd anf pregnancy/lactation).* *With DMBA:* increase of susceptibility to tumor formation and decreased tumor latency in both dose groups. Also observed in separate study that BPA (dose not clearly reported) can promote tumor growth in a xenograft model using MCF-7 human breast cancer cells and an immunocompromized mouse model.
Moral *et al.*, 2008	Rat (Sprague Dawley)	GD 10 - 21	25, 250	--	Structural changes: 25 Gene expression: --	Structural changes 250 Gene expression: 25	Changes in number of undifferentiated epithelial structures at the high dose, no effect on proliferation/apoptosis. Changes in gene expression signature also at the low dose. Different patterns in gene expression at high and low doses.
Tharp *et al.*, 2012	Rhesus monkey (*M. mulatta*)	GD 100 to term	400	--	--	400	Increased density of mammary buds, overall accelerated development of mammary gland

-- not established

[a]DMBA = dimethylbenz[a]anthracene

[b]Specific mouse strain shown to have an intrinisc property to develop mammary tumors.

Table 7. Studies investigating effects on mammary gland development after pre- and postnatal exposure to BPA administered subcutaneously to pregnant or lactating females.

Study	**Animal model**	**Exposure duration**	**Dose** (µg/kg bw/d)	**Additional chemical challenge**	**NOAEL** (µg/kg bw/d)	**LOAEL** (µg/kg bw/d)	**Effects on mammary gland observed**
Durando *et al.*, 2007	Rat (Wistar)	Subcutaneously to mother (pump) GD 8 – 23	25	Single subcarcinogenic dose of NMU[a] on PND 50	--	25	*With NMU:* increased hyperplastic ducts, development of neoplastic lesions. *Without NMU:* increased proliferation/apoptosis ratio, ductal hyperplasia, signs of desmoplasia
Muñoz-de-Toro *et al.*, 2005	Mouse (CD1)	Subcutaneously to mother (pump) GD 8 – 18	0.025, 0.25	--	--	0.025	At the low dose: decreased apoptosis, increased PR expression. At the high dose also increased number of terminal end buds.
Murray *et al.*, 2007	Rat (Wistar-Furth)	Subcutaneously to mother (pump) GD 9 – PND 1	2.5, 25, 250, 1000	--	--	2.5	Hyperplastic ducts at PND 50 and 95. At the two highest doses carcinoma *in situ* was also identified at both time points.
Vandenberg *et al.*, 2008	Mouse (CD1)	Subcutaneously to mother (pump) GD 8 – PND 16	0.25, 2.5, 25	--	--	0.25	Altered mammary phenotype, intraductal hyperplasias ("beaded ducts") with increased proliferation indexes.
Vandenberg *et al.*, 2007	Mouse (CD1)	Subcutaneously to mother (pump) GD 8 – 18	0.25	--	--	0.25	Altered mammary phenotype, delay in lumen formation, increased ductal area, decreased cell size.
Wadia *et al.*, 2007	Mouse (CD1 and C57Bl6)	Subcutaneously to mother (pump) GD 8 – PND 2	0.25	17 β-estradiol for 10 days after ovariectomy at PND 25	--	0.25	Increased response to estradiol treatment observed as increased number, total area covered and density of terminal end buds.

-- not established

[a]NMU = *N*-nitroso-*N*-methylurea

5. Effects of BPA on the developing female reproductive system

5.1 Associations between BPA and reproductive health in women

Several studies in humans report a link between BPA exposure and effects on the reproductive system in adult women, e.g. endometrial hyperplasia (Hiroi *et al.*, 2004), recurrent miscarriages (Sugiura-Ogasawara *et al.*, 2005) and polycystic ovary syndrome (Takeuchi *et al.*, 2004; Kandaraki *et al.*, 2011). Although these studies provide important information about the potential health effects of BPA they have major weaknesses that limit their usability for risk assessment, including questionable methodology and statistics, small sample numbers, lack of information on dose-response relationships, and cross-sectional design. There are no human studies investigating the association between developmental exposure to BPA and effects on the female reproductive system.

5.2 Animal data

Eleven rodent studies investigating effects on the female reproductive tract and onset of puberty in offspring of mothers exposed to BPA orally during gestation and lactation were identified (Table 8).

In addition, 17 studies investigating effects in female offspring after subcutaneous administration of BPA to the mother (Table 9) or directly to offspring shortly after birth are available (Table 10). These studies have primarily been included to provide further information about the nature of effects and were not considered in the identification of a NOAEL/LOAEL.

There are two multi-generation reproductive studies investigating a wide range of BPA doses and carried out according to standardized test guidelines available (Tyl *et al.*, 2002; 2008). These two studies currently serve as the basis for regulatory risk assessment of BPA in the EU (ECB, 2008; EFSA, 2006) and the US (US FDA, 2008). No effects on the female reproductive system were observed in the study in mice (Tyl *et al.*, 2008). Delayed vaginal patency was observed only at very high doses in the study in rats but there were no other effects on female reproduction parameters in this study (Tyl *et al.*, 2002).

5.3 Identification of NOAELs/LOAELs

The 11 studies in rats and mice where BPA was administered orally to pregnant and lactating dams were considered as the main basis for the identification of a point of departure. However, the majority of studies only included one or two dose groups and do not provide reliable information about the shape of the dose-response curve. The 5 studies that have included 3 or more dose groups often observed no effects on the endpoints investigated (Kwon *et al.*, 2000; Ryan *et al.*, 2010 Takagi *et al.*, 2004; Tyl *et al.*, 2002; 2008). It should however be noted that no effects were observed in the positive control (DES) group in the study by Kwon *et al.*

Some contradictory results have been reported. For example, decreased thickness of the uterine epithelia and increased expression of ERα expression was reported at 50 000 (but not 100) µg/kg

bw/day by Schönfelder *et al.* in 2004 while increased thickness of the uterine epithelia and down-regulation of ERα expression in epithelial cells was reported at 1200 µg/kg bw/day (the only dose tested) by Mendoza-Rodriguez *et al.* in 2011. While the large differences in doses administered in these studies could possibly explain the opposite effects on the uterine epithelium, assuming a non-monotonic dose-response curve in this case, too few and widely spaced dose groups prevents drawing any such conclusions. The confidence in the results regarding effects on the uterine epithelia is thus very low.

More convergent results and clearer dose-response relationships have been reported in studies using subcutaneous administration of BPA (Tables 9 and 10) in terms of effects on the female reproductive tract. For example, changes in ovarian morphology at a very low dose of 0.025 µg/kg bw/day and an increase of ovarian cysts at higher doses were observed in different studies (Markey *et al.*, 2005; Newbold *et al.*, 2009; Signorile *et al.*, 2010). There is however little support for the effects observed in the oral studies on estrous cyclicity or morphological changes of the uterus in studies using subcutaneous administration. Considering the limitations of the studies using oral BPA-exposure, studies where BPA was administered subcutaneously may be particularly important in providing information regarding effects on the female reproductive system.

One explanation for the divergent results in regard to developmental effects of BPA on the female reproductive system may be that such effects are intrinsically difficult to study due to natural variability in these endpoints with cycle stage and "interference" from circulating endogenous hormones. It should however be noted that Mendoza-Rodriguez *et al*. (2011) and Schönfelder *et al.* (2004) both state that measurements of uterine epithelia and ERα-expression were conducted during estrus, so differences in cycle stage does not seem to explain the contradictory results in those studies.

Given the low reproducibility of studies investigating effects of BPA on the female reproductive system, it is difficult to establish a reliable NOAEL or LOAEL below those identified in the studies by Tyl *et al.* (2002 and 2008) for these effects.

5.4 Tables summarizing literature on the effect of BPA on the developing female reproductive system

Table 8. Studies investigating developmental effects on the reproductive tract and onset of puberty in female offspring after oral BPA exposure to pregnant and/or lactating females.

Study	**Animal model**	**Exposure duration**	**Dose** (µg/kg bw/d)	**NOAEL** (µg/kg bw/d)	**LOAEL** (µg/kg bw/d)	**Effects on female reproductive tract**
Howdeshell *et al.*, 1999	Mouse (CF-1)	GD 11 – 17	2.4	--	2.4	Decreased interval between vaginal opening and age at first estrus (also depending on intrauterine position in combination with BPA-exposure). *No effect on age at vaginal opening.*
Kwon *et al.*, 2000	Rat (Sprague-Dawley)	GD 11 – PND 20	3200, 32 000, 320 000	320 000	--	*No effect of BPA on vaginal opening or age at first estrus.* NOTE: no effect in positive control (DES).
Mendoza-Rodríguez *et al.*, 2011	Rat (Wistar)	GD 6 – PND 21	1200	--	1200	Irregular estrus cycle. Increased thickness of the uterine epithelia and stroma as well as decreased apoptosis in the uterine epithelia during estrus. Downregulation of ER-α receptor expression in the epithelial cells of the uterus during estrus.
Rubin *et al.*, 2001	Rat (Sprague-Dawley)	GD 6 – weaning	100, 1200	100	1200	Altered estrous cyclicity. Decreased LH levels at adulthood after castration. *No difference in age at puberty or anogenital distance at birth*
Ryan *et al.*, 2010	Rat (Long-Evans)	GD 7 – PND 18	2, 20, 200	200	--	*No effects on vaginal opening or fertility observed.*
Schönfelder *et al.*, 2002	Rat (Sprague-Dawley)	GD 6 – PND 21	100, 50 000	--	100	Morphological changes in the vagina at estrus but not diestrus. Effects more pronounced in the low dose compared to the high dose. ERα downregulation in the vagina (not seen in animals treated with estradiol as positive control).
Schönfelder *et al.*, 2004	Rat (Sprague-Dawley)	GD 6 – PND 21	100, 50 000	100 (-- for ERβ expression)	50 000 (100 for ERβ expression)	Reduced thickness of uterine epithelium during estrus at the high dose, increased ERα expression at the high dose (and positive control), decreased ERβ expression at both doses and in positive control (17α-ethinyl estradiol).

Takagi *et al.*, 2004	Rat (Sprague-Dawley)	GD 15 – PND 10	During gestation: ~5000, 49 000, 232 000 During lactation: ~9000, 80 000, 384 000	5000	--	*No effects on onset of vaginal opening, estrous cyclicity or weights of female reproductive organs.*
Tyl *et al.*, 2008	Mouse (CD-1)	Mating – PND 21 (then oral to offspring, for 2 generations)	3, 30, 300, 5000, 50 000, 600 000	600 000	--	*No effects on ovarian primordial follicle counts, estrous cyclicity, or reproductive function*.
Tyl *et al.*, 2002	Rat (Sprague-Dawley)	Mating – PND 21 (then oral to offspring, for 3 generations)	1, 20, 300, 5000, 50 000, 500 000	50 000	500 000	Delayed vaginal patency. *No effects on ovarian primordial follicle counts, estrous cyclicity, or reproductive function.*
Yoshida *et al.*, 2004	Rat (Donryu)	GD 2 – PND 20	6, 6000	6000	--	*No effects on estrus cyclicity, morphology of reproductive organs or uterine carcinogenesis.*

-- not established

Table 9. Studies investigating developmental effects on the reproductive tract in female offspring after subcutaneous BPA exposure to pregnant and/or lactating females.

Study	**Animal model**	**Exposure duration**	**Dose** (µg/kg bw/d)	**NOAEL** (µg/kg bw/d)	**LOAEL** (µg/kg bw/d)	**Effects on female reproductive tract**
Cabaton *et al.*, 2011	Mouse (CD-1)	GD 8 – PND 16 (pump)	0.025, 0.25, 25	--	0.025	Reduced fertility and fecundity apparent after 5-6 pregnancies at the low and high but not middle dose.
Ema *et al.*, 2001	Rat (Sprague Dawley)	Mating – PND 21 (Oral to offspring from PND 23)	0.2, 2, 20, 200	200	--	*No effects on vaginal opening, estrous cyclicity or reproductive function observed*
Honma *et al.*, 2002	Mouse (ICR)	GD 11 – 17	2, 20	2	20	Advanced vaginal opening and age at first estrus.
Markey *et al.*, 2005	Mouse (CD-1)	GD 9 – PND 4 (pump)	0.025, 0.25	--	0.025	Changes in ovarian morphology at 3 and 6 months. Reduced dry weight of the vagina and endometrial volume. Increased expression of ERα and PR in uterine epithelium, impairment of DNA synthesis in the uterine epithelium.
Newbold *et al.*, 2009	Mouse (CD-1)	GD 9 – 16	0.1, 1, 10, 100, 1000	0.1	1	Ovarian cysts in the 1 µg/kg group. Increased endometrial hyperplasias in all groups except the lowest but did not reach significance.
Nikaido *et al.*, 2004	Mouse (CD-1)	GD 15 – 18	500, 10 000	--	500	Advanced puberty at the high dose, irregular estrus cyclicity and lack of corpora lutea and vaginal cornification at both doses.
Signorile *et al.*, 2010	Mouse (Balb-C)	GD 1 – PND 7	100, 1000	--	100	Endometriosis-like structures (with both glands and stroma expressing both estrogen receptor and HOXA-10) in adipose tissue surrounding genital tracts. Increased incidence of endometrial hyperplasia and cystic ovaries
Suzuki *et al.*, 2002	Mouse (ICR)	GD 10 – 18	10 000, 100 000	--	10 000	Reduced number of mice with corpora lutea but *no effect on fertility or morphology of vagina or uterus*
Tachibana *et al.*, 2007	Mouse (ICR)	GD 0 – 7	10 000	--	10 000	Reduced number of embryos, reduced uterine weight and marked modifications of placental structure.

-- not established

Table 10. Studies investigating developmental effects on the reproductive tract in females after neonatal BPA exposure via subcutaneous injection.

Study	**Animal model**	**Exposure duration**	**Dose** (µg/kg bw/d)	**NOAEL** (µg/kg bw/d)	**LOAEL** (µg/kg bw/d)	**Effects on female reproductive tract**
Adewale *et al.*, 2009	Rat (Long-Evans)	PND 0 - 3	50, 50 000	--	50	Advanced puberty at the low dose, irregular estrus cycle at both doses, effects on ovaries, e.g. large antral-like follicles and lower numbers of corpora lutea, which are more pronounced at the high dose.
Fernandez *et al.*, 2009	Rat (Sprague-Dawley)	PND 1 - 10	2500 – 6200, 25 000 – 62 500*	--	2500 – 6200	Accelerated puberty onset, more pronounced at the high dose. Altered estrous cyclicity, with the high dose causing permanent estrus.
Fernandez *et al.*, 2010	Rat (Sprague-Dawley)	PND 1 - 10	250-620, 2500 – 6200, 25 000 – 62 500*	250-620	2500 – 6200	Reduced fertility at the middle dose, abnormal morphology of the ovaries with many cysts (morphology similar to that observed in the case of polycystic ovaries in women) and all females sterile at the high dose.
Newbold *et al.*, 2007	Mouse (CD-1)	PND 1 - 5	10, 100, 1000	10	100	Increase in cystic ovaries and cystic endometrial hyperplasia in animals at 18 months.
Nikaido *et al.*, 2005	Mouse (CD-1)	PND 15 – 19	10 000	10 000	--	Annovulary state of 80 % of BPA-treated females. No effects on age at puberty, morphology of uterus or vagina, or estrus cyclicity.
Rivera *et al.*, 2011	Lamb	PND 1 – 14	50	--	50	Decreased ovarian weight, increased primordial-to-primary follicle transition, decreased primordial follicle reserve, increased incidence of multiovular follicles.
Rodríguez *et al.*, 2010	Rat (Wistar)	PND 1 – 7	50, 20 000	50	20 000	Increased follicle recruitment and decreased primordial follicle reserve
Suzuki *et al.*, 2002	Mouse (ICR)	PND 1 – 5	10 000, 100 000	10 000	100 000	Ovary-independent vaginal epithelial stratification and polyovular follicles.

-- not established

*Three doses given as µg BPA/µL vehicle. Exposure calculated to these ranges by the study authors. Basis for calculations unclear.

6. Developmental effects of BPA on lipogenesis

6.1 Associations between BPA and obesity in humans

A positive relationship between BPA exposure and obesity has been reported in the US general population (Carwile *et al.*, 2011) and in a small Japanese study in women with and without ovarian dysfunction (Takeuchi *et al.*, 2004). However, associations with markers of changes in fat metabolism, such as circulating levels of lipids, cholesterol or leptin, were not investigated in these studies. No human studies investigating the association between developmental exposure to BPA and effects on body weight or fat metabolism were identified.

6.2 Animal data

Three in vivo studies have investigated the effects of exposure to BPA on lipogenesis (Miyawaki *et al.*, 2007; Somm *et al.*, 2009; Wei *et al.*, 2011). These studies were conducted in rat offspring exposed during gestation and lactation to BPA administered orally to the dam and have been summarized in Table 11. A large number of studies have investigated the effect of developmental exposure to BPA on body weight. These studies have not been reviewed and summarized here due to time constraints. However, both decreases and increases in body weight have been observed in animal studies after developmental exposure to BPA, making it difficult to draw clear conclusions regarding the effects of BPA on body weight.

The results from Miyawaki *et al.* and Somm *et al.* suggest that female rodents may be more sensitive than males to perturbations in lipogenesis.

6.3 Identification of NOAELs/LOAELs

Some weaknesses can be identified in these studies which limit their usability for identifying a point of departure. For example, Miyawaki *et al.* only included 3 dams per dose group and do not seem to have made considerations for the influence of litter effects in their analyses. The number of dams per dose group is not clearly reported in the study by Somm *et al.* It is only stated that offspring from "at least three litters" in each group were analyzed. However, litter effects were controlled for in this study. The study by Wei *et al.* included 3 dose groups and 8 dams per dose group. Litter effects seem to have been appropriately controlled for in this study. However, effects were only observed at the lowest dose of 50 µg/kg bw/day and not at the two higher doses, which, in lack of other reliable data complicates the interpretation of results.

No NOAELs were identified in any of the three rat studies by Miyawaki *et al.* (2007), Somm *et al.* (2009) and Wei *et al.* (2011). However, Somm *et al.* and Wei *et al.* report similar LOAELs of 70 and 50 µg/kg bw/day, respectively. Based on study design and level of reporting these studies are the most reliable of the three.

The fact that Wei *et al.* report effects only at the lowest dose tested and that no clear dose-response relationship can be established from these studies increases the uncertainty in the results from this study and in the LOAEL.

6.4 Tables summarizing literature on the developmental effects of BPA on body weight and lipogenesis

Table 11. Studies investigating effects on lipogenesis after pre- and postnatal exposure to BPA administered orally to pregnant and lactating females.

Study	**Animal model**	**Exposure duration**	**Dose** (µg/kg bw/d)	**NOAEL** (µg/kg bw/d)	**LOAEL** (µg/kg bw/d)	**Observed effects on lipogenesis or bw**
Miyawaki *et al.*, 2007	Mouse (ICR)	GD 10 – weaning	260, 2720	--	260	Increased mean bw in males at the high dose and in females at both doses. Increased mean adipose tissue weight in males at the high dose and in females at the low dose. Increased serum cholesterol in females at both doses and increased leptin levels at the low dose. Increased serum triacylglycerol in males at the low dose. No effects on serum glucose levels.
Somm *et al.*, 2009	Rat (Sprague-Dawley)	GD 6 – weaning	70	--	70	Increased bw at birth in both sexes and at PND 21 in females. Increased parametrial white adipose tissue weight in females on PND 21 associated with adipocyte hypertrophy and overexpression of several lipogenic genes. No change in circulating lipids or glucose. Between 4 – 14 weeks no change in bw in males on standard diet, increased bw in males on high-fat diet (weeks 9 – 14) and in females both on standard and high-fat diet.
Wei *et al.*, 2011	Rat (Wistar)	GD 0 – PND 21	50, 250, 1250	--	50	With both normal and high-fat diet: increased bw, elevated serum insulin, and impaired glucose tolerance in adult offspring. Also with high-fat diet: obesity, dyslipidemia, hyperleptindemia, hyperglycemia, hyperinsulinemia, and glucose intolerance. *NOTE: no effects observed at 250 or 1250 µg/kg bw/day*

-- not established

7. Overall summary and conclusions

The purpose of this report was to review and summarize the literature describing the effects of developmental exposure to BPA in regard to DNT, as well as effects on the development of the mammary gland, the female reproductive system and lipogenesis. These types of effects were chosen based on previous expert reports and risk assessments where they have been identified as being of potential concern for humans (e.g. ANSES, 2011; NTP, 2008; WHO, 2011). The aim was to identify NOAELs or LOAELs for these different effects, to discuss how these could be used as points of departure for the derivation of an alternative reference dose of BPA and to compare how this value would deviate from the current TDI for BPA, which is 50 μg/kg bw/day (EFSA, 2006). The current TDI is based on the results from two multi-generation reproduction studies in rats and mice (Tyl *et al.*, 2002 and 2008) conducted according to standardized toxicity test guidelines where effects on offspring body and organ weights were observed at doses above a NOAEL of 5 mg/kg bw/day. Effects on reproductive parameters were only observed above 50 mg/kg bw/day in these studies. However, a large number of research studies conducted during the last decade have reported effects of BPA in animals at very low doses well below 5 mg/kg bw/day.

For the identifications of points of departure for the derivation of a reference dose studies using BPA-administration via the oral route were primarily considered since this directly reflects a relevant exposure scenario in humans. Dermal exposure may also be relevant but no animal studies using dermal exposure were identified. BPA administered via the oral route undergoes extensive first-pass metabolism in the liver and gut and BPA-metabolites, primarily BPA-glucuronide, do not show any biological activity (WHO, 2011). Hence, an oral dose of BPA results in much lower circulating levels of free BPA that can interact with estrogen receptors and other molecular targets than the same dose given subcutaneously, which by-passes first-pass metabolism. Subcutaneous doses would have to be extrapolated into an oral dose in order to provide information for setting an oral reference dose. However, for the risk assessment of exposure to BPA via the dermal route studies using subcutaneous administration may be particularly useful since exposure via the skin also by-passes metabolism in the gut and liver. Reliable data regarding dermal uptake and metabolism of BPA is still scant, but BPA has been shown to be absorbed through the skin (Marquet *et al.*, 2011).

The data set for DNT is more extensive than for the other effects included and covers oral and subcutaneous exposure to pregnant and lactating females as well as directly to offspring. For the other effects direct early exposure of offspring was not as well studied. Developmental effects on the female reproductive system after direct exposure to offspring have been studied using subcutaneous but not oral administration. However, for developmental effects on the mammary gland and lipogenesis no studies were found which investigate direct exposure in offspring. This lack of data may have implications for risk assessment since direct exposure of infants and children at critical times of development as well as exposure of the developing fetus and newborn via the mother are relevant exposure scenarios in this case.

In regard to establishing a point of departure for the derivation of a reference dose, many of the studies reviewed here have methodological limitations or suffer from poor reporting, which negatively impact their reliability and limit their utility for this purpose. It is difficult to identify a single study that would be reliable and relevant enough to serve as a key study for the

identification of a NOAEL or LOAEL. The approach was therefore to consider the data as a whole for each type of effect and, excluding studies with major methodological flaws, as well as some studies that reported an extremely high or low NOAEL/LOAEL, identify several NOAELs and LOAELs from different studies that were considered to be most reliable and relevant. These values are considered to be representative of the data reviewed and have been summarized in table 12.

No NOAEL/LOAEL for developmental effects on the female reproductive system was carried forward to table 12. The reason is that uncertainty in the studies using oral administration of BPA was considered to be too large due to contradictory data and low reproducibility of results. For example, effects on estrous cyclicity were reported in a few studies (Mendoza-Rodriguez *et al.*, 2011; Rubin *et al.*, 2001) but these effects were not observed in other studies in the same dose range (Tyl *et al.*, 2002 and 2008; Yoshida *et al.*, 2004). Also, inconsistent results on morphological changes in the vagina were reported (Mendoza-Rodriguez *et al.*, 2011; Schönfelder *et al.*, 2004). Studies using subcutaneous administration of BPA provide more consistent data in terms of effects on the female reproductive tract. For example, changes in ovarian morphology at a very low dose of 0.025 µg/kg bw/day and an increase of ovarian cysts at higher doses were observed in different studies (Markey *et al.*, 2005; Newbold *et al.*, 2009; Signorile *et al.*, 2010). There is however little support for the effects observed in the oral studies on estrous cyclicity or morphological changes of the uterus in studies using subcutaneous administration.

In general (excluding the study by Viberg *et al.*, 2001) the selected NOAELs in table 12 range from 2 to 50 µg/kg bw/day and LOAELs from 40 to 500 µg/kg bw/day. These values are within the same range for DNT as well as developmental effects on the mammary gland and lipogenesis. NOAELs from this data set are, in general, 2 to 3 orders of magnitude lower than the NOAEL on which the current TDI is based.

Several studies reviewed here have reported effects at the lower dose levels tested which were less pronounced or not observed at higher dose levels (e.g. Jones *et al.*, 2011 and 2012; Wei *et al.*, 2011; Xu *et al.*, 2011). Non-monotonic dose-response curves is an issue that is currently under debate (Kortenkamp *et al.*, 2012; Vandenberg *et al.*, 2012), especially for endocrine active substances, and these types of observations do not seem entirely unlikely for BPA. However, for many effects of BPA there is a lack of data, i.e. for a wide range of appropriately spaced dose levels, preventing any conclusions about the shape of the dose-response curve.

Alternative reference doses were calculated from the selected NOAELs/LOAELs and are also presented in table 12. In the selection of assessment factors for the calculations of reference doses the following considerations were made:

- Humans cannot be assumed to be less sensitive than the rat, mouse or monkey
- For inter-species extrapolation allometric scaling, to correct for differences in metabolic rate, and an additional factor of 2.5 for other interspecies differences, i.e. toxicokinetic differences not related to metabolic rate (small part) and toxicodynamic differences (larger part) was applied according to recommendations by the European Chemicals Agency (ECHA, 2008).

- The default factor of 10 has been used for intra-species extrapolation due to lack of information regarding inter-individual variability
- When extrapolating from LOAEL to NOAEL an additional assessment factor of 10 has been chosen. There is a general lack of NOAELs among the available studies and in many studies only one dose or large dose-intervals have been studied. Thus, there is large uncertainty regarding the actual steepness of the dose-response curve. Non-monotonic dose-response relationships have been observed, but this has not been considered further in the dose-extrapolation.

The reference doses calculated for indirect exposure of offspring via the pregnant and/or lactating female range between 0.01 and 0.8 µg/kg bw/day and are considerably lower than the current TDI of 50 µg/kg bw/day. It can be noted that the lowest reference doses were calculated for DNT.

The Danish EPA recently calculated an alternative DNEL for BPA based on effects on the developing mammary gland (Danish EPA, 2011). From the studies by Durando *et al.* (2007) and Murray *et al.* (2007) (both using subcutaneous administration of BPA to pregnant Wistar rats) a LOAEL of 25 µg/kg bw/day was identified, resulting in an alternative DNEL of 0.083 µg/kg bw/day (assessment factor = 300). Because of the lower doses administered in studies using subcutaneous exposure, and because a LOAEL rather than a NOAEL was used, implying a higher assessment factor, the Danish EPA DNEL is one order of magnitude lower than the reference doses for developmental effects on the mammary gland calculated here.

In regard to DNT it was considered relevant to calculate reference doses for direct developmental exposure of offspring from three studies covering different time periods, i.e. repeated neonatal exposure (Carr *et al.*, 2003), a single neonatal exposure (Viberg *et al.*, 2011) and pre-pubertal exposure of older offspring (Xu *et al.*, 2011). Calculations based on these studies resulted in alternative reference doses of 1, 1.83 and 0.023 µg/kg bw/day, respectively. It is noteworthy that even calculations based on the "high" NOAEL in this context of 320 µg/kg bw/day identified from the Viberg study generates a reference dose much below the current TDI.

Overall, it can be concluded that although no single study reviewed here was considered reliable enough to alone serve as a key study for the derivation of an alternative reference dose, if the data is considered as a whole, effects are consistently observed at doses well below those which serve as the basis for the current TDI for BPA. Even if confidence in a specific alternative reference dose based on this data material is low the results from this review indicate that considering a lower reference dose than the current TDI when conducting risk assessment of BPA is prudent.

Table 12. Summary of identified points of departure for the derivation of an alternative reference dose for BPA.

Reference	**Exposure**	**Animal model**	**Endpoint**	**NOAEL (µg/kg bw/day)**	**LOAEL (µg/kg bw/day)**	**Assessment factor**	**Reference dose (µg/kg bw/day)**
Indirect exposure of offspring via oral administration to pregnant and lactating females							
Ryan and Vandenbergh, 2006	Oral to pregnant and lactating females	Mouse (C57/Bl-6) Female offspring only.	Increased anxiety	2	200	175 (7 x 2.5 x 10)	0.01
Xu *et al.*, 2010b	Oral to pregnant and lactating females	Mouse (ICR) Male offspring only.	Impaired spatial memory	50	500	175 (7 x 2.5 x 10)	0.29
Betancourt *et al.*, 2010a Moral *et al.*, 2008	Oral to pregnant females	Rat (Sprague-Dawley)	Morphological changes in the mammary gland, increased susceptibility to tumorigenesis	25	250	100 (4 x 2.5 x 10)	0.25
Jenkins *et al.*, 2009	Oral to lactating females	Rat (Sprague-Dawley)	Morphological changes in the mammary gland, increased susceptibility to tumorigenesis	25	250	100 (4 x 2.5 x 10)	0.25
Tharp *et al.*, 2012	Oral to pregnant females	Rhesus monkey	Morphological changes and accelerated development of the mammary gland	--	400	500 (10 x 2 x 2.5 x 10)	0.8

Somm *et al.*, 2009	Oral to pregnant and lactating females	Rat (Sprague-Dawley)	Increased bw and adipose tissue weight, adipocyte hypertrophy, overexpression of genes	--	70	1000 (10 x 4 x 2.5 x 10)	0.07
Wei *et al.*, 2011	Oral to pregnant and lactating females	Rat (Wistar)	obesity, dyslipidemia on a high fat diet	--	50	1000 (10 x 4 x 2.5 x 10)	0.05
Direct oral exposure in offspring							
Carr *et al.*, 2003	Oral to offspring on PND 1- 14	Rat (Fischer 344)	Impaired spatial learning in females	100	250	100 (4 x 2.5 x 10)	1
Viberg *et al.*, 2011	Oral to offspring on PND 10	Mouse (NMRI)	Altered spontaneous behavior and reduced habituation	320	3200	175 (7 x 2.5 x 10)	1.83
Xu *et al.*, 2011	Oral to pre-pubertal offspring	Mouse (ICR)	Impaired passive avoidance memory	--	40	1750 (10 x 7 x 2.5 x 10)	0.023

-- not established

8. Data gaps and research needs

From the literature review conducted here for the purposes of identifying possible points of departure for the derivation of an alternative reference dose for BPA some data gaps and research needs were identified.

- In regard to the developmental effects of BPA reviewed in this report there is a lack of studies including more than two dose groups. In order to reliably assess the effects of BPA, as well as the shape of the dose-response curves, at the low doses implicated more studies are needed that investigate several and adequately spaced dose levels.
- Given that direct exposure to BPA in infants and young children at critical periods of development is a relevant exposure scenario for human health risk assessment more studies are needed that investigate developmental effects after direct early exposure in offspring.
- Much of the limitations for risk assessment purposes identified in the studies reviewed here stems from inadequate reporting of study design, methodology and results. In order to improve the utility of research studies for risk assessment one important aspect is to improve the reporting of research studies published in scientific journals.
- Reports of low-dose effects are emerging for a wide range of chemicals. Risk assessment methodologies need to be revised in order to adequately assess health risks from chemical exposure to low doses.

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