Annex XV report

PROPOSAL FOR IDENTIFICATION OF A SUBSTANCE OF VERY HIGH CONCERN ON THE BASIS OF THE CRITERIA SET OUT IN REACH ARTICLE 57

Substance Name(s): Benzyl butyl phthalate (BBP)

EC Number(s): 201-622-7

CAS Number(s): 85-68-7

Submitted by: Danish Environmental Protection Agency, Denmark

Date: 26 August 2014

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- It is proposed to identify the substance as a substance of equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of Regulation (EC) No 1907/2006 (REACH) according to Article 57(f) of REACH Regulation.

Summary of how the substance meets the criteria set out in Article 57 of the REACH Regulation

Benzyl butyl phthalate (BBP) is proposed to be identified as a substance of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) because it is an endocrine disruptor, i.e. it has endocrine disrupting properties for which there is scientific evidence of probable serious effects to human health and to the environment, and this gives rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 REACH.

BBP has been shown to adversely affect the endocrine system of mammals primarily through *in vivo* findings on reduced fetal testosterone. These findings are further substantiated by mechanistic findings, also *in vivo*, of down-regulation of genes in the steroidogenic biosynthesis pathway. The spectrum of adverse effects observed in rats include increased nipple retention, decreased anogenital distance, genital malformations, reduced number of spermatocytes and testicular changes including multinucleated gonocytes, tubular atrophy and Leydig cell hyperplasia.

In relation to the environment, adverse effects concerning development and reproduction are generally regarded as endpoints of particular relevance because such effects are likely to manifest themselves at the population level. The effects observed in rats are of particular concern for wildlife species with a natural low reproductive output, including top predators, primates and other larger mammals (including endangered species) as negative effects on reproduction has an even higher potential for causing long term negative effect at the population level for such taxa.

Adverse effects caused by exposure to BBP has not been observed in non-mammalian wildlife as no fish, amphibian or invertebrate studies including endocrine relevant endpoints has been found for BBP. However, cross-species extrapolation for hazard identification of endocrine disruptive properties seems relevant, e.g. between rodents and fish, since the key molecular initiating events (in this case inhibition of enzymes involved in steroid synthesis) are similar between species (even though apical responses vary across phyla and some differences in sensitivity to adverse effects have been observed). In addition, read across between structural analogues for hazard identification of the endocrine disruptive properties of BBP from other phthalates, such as DEHP and DBP, with sufficient experimental data in fish and rodents, supports that BBP has endocrine properties for which there is evidence of probable serious effects to wildlife species.

In conclusion, when available information from toxicological and ecotoxicological studies are combined, BBP can be considered an endocrine disruptor for both the environment and for human health as it fulfils the WHO/IPCS definition of an endocrine disruptor and the

recommendations from the European Commission's Endocrine Disrupters Expert Advisory Group for a substance to be identified as an endocrine disruptor.

BBP is considered as a substance giving rise to an equivalent level of concern because scientific evidence shows that exposure during sensitive time windows of development may cause irreversible developmental programming effects leading to severe effects on development and reproduction, regarded as particularly serious in relation to human health and wild life species, also because these adverse effects may first manifest themselves in later life stages as a consequence of exposure during early life stages.

Registration dossiers submitted for the substance: Yes

PART I

Justification

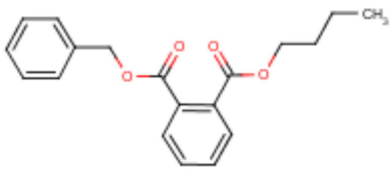
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	201-622-7
EC name:	Benzyl butyl phthalate
CAS number (in the EC inventory):	85-68-7
CAS number:	85-68-7
CAS name:	1,2-Benzenedicarboxylic acid, butyl phenylmethyl Ester
IUPAC name:	Benzyl butyl phthalate
Index number in Annex VI of the CLP Regulation	607-430-00-3
Molecular formula:	C19H20O4
Molecular weight range:	312.35 g/mol
Synonyms:	*BBP*

Structural formula:



1.2 Composition of the substance

Name: BBP

Description: The substance is a mono constituent substance (typical concentration of benzyl butyl phthalate 80-100%(w/w)).

1.3 Physico-chemical properties

Table 2: Overview of physicochemical properties

Property	Value	IUCLID section	REACH ref Annex, §
Physical state at 20°C and 101.3 kPa	Liquid	3.1	VII, 7.1
Melting/freezing point	< -35C	3.2	VII, 7.2
Boiling point	370C at 10.10 hPa	3.3	VII, 7.3
Vapour pressure	0.00112 Pa at 20C	3.6	VII, 7.5
Water solubility	2.8 mg/l	3.8	VII, 7.7
Partition coefficient n-octanol/water (log value)	Log Kow 4.84	3.7 partition coefficient	VII, 7.8
Dissociation constant	-	3.21	XI, 7.16

2 HARMONISED CLASSIFICATION AND LABELLING

BBP is listed in Regulation (EC) No 1272/2008 as follows:

Classification and labelling of BBP according to Annex VI, Table 3.1 of Regulation (EC) No 1272/2008

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling		Specific Conc. Limits, M-factors
				Hazard Class and Category Code(s)	Hazard Statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	
607-430-00-3	benzyl butyl phthalate; BBP	201-622-7	85-68-7	Repr. 1B Aquatic Acute 1 Aquatic Chronic 1	H360Df H400 H410	GHS08 GHS09 Dgr	H360Df H410	

Classification and labelling of BBP according to Annex VI, Table 3.2 of Regulation (EC) No 1272/2008 (The list of harmonized classification and labelling of hazardous substances from Annex I to Directive 67/548/EEC)

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration limits
607-430-00-3	BBP; benzyl butyl phthalate;	201-622-7	85-68-7	Repr. Cat. 2; R61 Repr. Cat. 3; R62 N; R50-53	T; N R: 61-62-50/53 S: 53-45-60-61	

3 ENVIRONMENTAL FATE PROPERTIES

3.1 Environmental fate

The environmental fate of BBP as concluded in the EU RAR for degradation, distribution and bioaccumulation is cited in the sections below (EU RAR 2007).

BBP may be released into the environment during its production and subsequent life cycle stages, including disposal. Emissions to water and air are expected to be the most important entry routes of BBP (EU RAR 2007). General characteristics of BBP which are relevant for the exposure assessment are given below.

3.2 Degradation

"The contribution of hydrolysis and photolysis in water to the overall environmental degradation of phthalate esters, including BBP, is expected to be low. Photo-oxidation by OH radicals contributes to the elimination of BBP from the atmosphere. An atmospheric half-life of about 1.5 days has been estimated for the photo-oxidation reaction. The metabolic pathway of aerobic and anaerobic biodegradation of phthalates can be summarised as follows. First the di-ester is hydrolysed into the mono-esters (monobutyl phthalate and monobenzyl phthalate) by esterases with low substrate specificity. Subsequently the mono-esters are converted into phthalic acid. There is ample evidence that BBP is ready biodegradable under aerobic conditions fulfilling the 10-day window criterion. Anaerobic test indicate that biodegradation of BBP is slower in the anaerobic environment e.g. sediments or deeper soil or groundwater layers". Citation from EU RAR 2007

3.3 Distribution

"The Henry's law constant of 0.176 Pa m3*mol-1 indicates that BBP is not likely to volatilize from surface waters. BBP is emitted to air during production, formulation and processing due to elevated processing temperatures. In the air BBP is removed by both wet and dry deposition, but long distance transport is unlikely due to low volatility and short half life in the atmosphere.

The octanol/water partition coefficient (Kow) of BBP is high and consequently the equilibrium between water and organic carbon in soil or sediment will be in favour of the soil or sediment. A Koc of 10,500 l/kg can be calculated using the log Kow of 4.84." Citations from EU RAR 2007

3.4 Bioaccumulation

"The measured bioconcentration factors (BCF) based on total radioactivity are in the range 135-663 l/kg. The BCF-value of 12 l/kg, taking only into account the accumulation of the parent compound, would mean that BBP is not considered to biomagnify. Based on the data from the Human Health Risk Assessment, it cannot be excluded that the metabolites can give endocrine/reproductive toxicity effects to other species like birds, fish etc, as they do to mammals. Therefore the BCF-value used should cover the BCF of the parent compound and the accumulation of the two monoester metabolites (MBuP and MBeP). The experimental BCF of 449 l/kg using 14C-labelled BBP is therefore used for estimating secondary poisoning in EUSES." Citation from EU RAR 2007

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The toxicokinetics as described in the EU RAR for human health is cited below (EU RAR 2007):

"In rats, the kinetics of BBP after oral administration was dose-dependent. Excretion of radiolabelled BBP in the urine was between 70% and 80% when BBP was given at doses from 2 mg/kg p.o. to 200 mg/kg p.o., whereas 22.4% were excreted in the urine after administration of 2,000 mg/kg p.o. The excretion of radioactivity in the feces was 20% after intravenous administration which indicates that the absorption in the dose range between 2 mg/kg p.o. and 200 mg/kg p.o. is nearly complete. After dermal application approximately 5% of the applied dose was absorbed each day. After 7 days approximately 30-40% of the applied amount seemed to be absorbed and reached the systemic circulation. BBP is rapidly metabolised and after 7 days 30% of the applied dose was excreted in the urine or faeces. 45% of the applied dose was found at the skin area of application. For the risk characterisation 5% dermal absorption is used. The extent of systemic availability of the substance administered by inhalation is not known as specific data are lacking. BBP is metabolized to monobutyl phthalate (MBuP) and monobenzyl phthalate (MBeP). This metabolism may take place in the gut wall and/or liver. In adult and immature rats, the ratio of monobutyl phthalate to monobenzyl phthalate found in the urine was 3:1. Both metabolites were found in the bile. Reabsorption from gut lumen may take place. There is no evidence of tissue accumulation. The percentage of excreted metabolites (MBuP and MBeP) in the urine in adult rats was shown to be higher compared to immature rats. The excretion of BBP metabolites in urine has also been studied in humans. Contrary to the metabolism of BBP in rats, BBP is mainly metabolised to MBeP in humans. However, limited data on the metabolism of BBP in humans are available. No half-life of BBP in the body has been calculated. However, the available data indicate a half-life of less than 24 hours. In the risk characterisation, 100% absorption is assumed for both inhalation and oral exposure, whereas the absorption for dermal exposure is set at 5%."

4.2 Other effects: Endocrine disruption

4.2.1 General approach –Human Health

Criteria on how to assess whether or not a substance has endocrine disrupting properties and/or is an endocrine disruptor are currently being developed in the European Union. The timeline for the finalization of the process is not currently known.

The basis for the criteria is envisaged to be the widely accepted definition of an endocrine disruptor by the WHO/IPCS:

An endocrine disruptor is an exogenous substance or mixture that

1) alters function(s) of the endocrine system and

2) consequently causes

3) adverse health effects in an intact organism, or its progeny, or (sub)populations.

The European Commission's Endocrine Disrupters Expert Advisory group agreed in 2013 "that the elements for identification of an endocrine disrupter were demonstration of an adverse effect for which there was convincing evidence of a biologically plausible causal link to an endocrine disrupting mode of action and for which disruption of the endocrine system was not a secondary consequence of other non-endocrine-mediated systemic toxicity. Relevance of the

data to humans should be assumed in the absence of appropriate data demonstrating non-relevance." (JRC 2013)

As it is assumed in this report that a substance should fulfil the recommendations from the European Commission's Endocrine Disrupters Expert Advisory group outlined above in order to be identified as an endocrine disruptor, available information is assessed based on the following topics:

1) Adverse health effects
2) Endocrine mode of action
3) Plausible link between adverse effects and endocrine mode of action
4) Human relevance

The most marked adverse effects of BBP have been described for the male reproductive system and most work performed to elucidate the mode of action of BBP has been carried out in experimental tests studying developing male rats. The following discussion therefore focuses on endocrine disrupting effects on male reproduction. BBP may also have other endocrine disrupting modes of action, but these will only be discussed briefly here.

4.2.2 Adverse health effects – Analysis of available information from *in vivo* studies

a) Background

BBP is classified as a substance toxic to reproduction (Repr. 1B, H360Df) based on evidence of adverse effects on the reproductive organs in adult and developing rodents. The evidence of reproductive toxicity and a thorough discussion of endocrine activity were presented in the EU risk assessment report from 2007 (EU RAR, 2007):

"Reproductive effects of BBP and its major metabolites MBuP and MBeP in rats following oral administration both by gavage or in the diet have been investigated in studies of different duration (from 4 days to 26 weeks, and in 2-generation studies). The main effects reported include a decrease in the relative weight of testis, damage to the testis, epididymis, prostate, seminal vesicle and to reduced epididymal sperm concentrations, and at high BBP concentrations reduced fertility, in addition to increases in relative liver and kidney weights." (…) "Effects on male reproductive organs and/or fertility are reported after administration of BBP in doses equal to or higher than those which induce minimal systemic toxicity such as relative organ weight changes, and in some studies histopathological changes in the liver and pancreas. Furthermore, since signs of testicular toxicity, evident as a dose-dependent decrease in epididymal spermatozoa concentration and atrophy of the testis, and decreased testosterone and FSH levels, are reported in the absence of effects in other organs, BBP may affect fertility." (…) "BBP was shown in one *in vitro* study to be a potent anti-androgen in yeast cells expressing the androgen receptor. Nine *in vivo* studies are available which indicate an anti-androgen-like activity of BBP or its major metabolites in rats, MBuP and MBeP (Piersma et al., 2000; Gray et al., 2000; Parks et al., 2000; Imajima et al., 1997; Shono et al., 2000; Nagao et al., 2000; Tyl et al., 2004; Ema et al., 2002; Ema et al., 2003)" (EU RAR, BBP, 2007, pp. 165-210).

An overview of the key studies on effects of BBP on fertility and reproductive organs were given in tables in the EU risk assessment report for BBP (2007) and are presented in Annex 1 to this report. An overview of the studies on effects of BBP on development were also given in tables in the EU risk assessment report for BBP (2007) and the studies including endpoints of special relevance for endocrine disruption are presented in table 3. These studies are considered reliable (i.e. in most cases with a Klimisch score 1 or 2). Detailed study summaries can be found in the EU risk assessment report.

Several studies included in the EU risk assessment report (EU RAR, 2007) found reduced anogenital distance (AGD) (Ema et al. 2002 & 2003; Gray et al. 2000; Nagao et al. 2000; Parks et al. 2000; Tyl et al. 2004) and two studies found increased nipple retention in male

pups (Gray et al. 2000; Parks et al. 2000). AGD and nipple retention in male pups are generally known to be androgen dependant and associated with an anti-androgenic mode of action, respectively (Bowman et al. 2003; Imperato-McGinley et al. 1985; Imperato-McGinley et al. 1986), and the findings on AGD and nipple retention thus strengthens the hypothesis of BBP as an endocrine disruptor.

The reproductive toxicity of BBP was thus evaluated to be likely induced via an endocrine disrupting mode of action, i.e. interference with steroid hormone synthesis. This conclusion is further substantiated by studies published after data was collected for the EU risk assessment report for BBP (see next section and table 4).

Table 3. Summary of *in vivo* developmental studies included in the EU risk assessment report on BBP (EU RAR, 2007) showing adverse effects and/or showing an *in vivo* endocrine mode- and/or mechanism of action.

Study Design	**Effect Level**	**Critical Effect**	**Reference**
Cpb-WU pregnant rats; Administration of BBP by gavage gd 6-15 or 6-20; 0, 270, 350, 450, 580, 750, 970, 1,250, 1,600 or 2,100 mg/kg/day; 25/group in the 0, 450, 750 and 1,250 mg/kg/day dose group, 10/group in the 270, 350, 580, 970, 1,600 and 2,100 g/kg/day dose group	NOAEL maternal 450 mg/kg/day (exp. gd 6-20) and 580 mg/kg/day (exp gd 6-15) NOAEL offspring 270 mg/kg/day (exp gd 6-20) and 350 mg/kg/day (exp.gd 6-15)	Maternal; increased liver weight at 580 mg/kg/day (exp. gd 6-20) and at 750 mg/kg/day (exp. gd 6-15). Offspring; decreased relative testis weight at 270 mg/kg/day (exp. gd 6-20). Effects on testicular migration at 580 mg/kg/day (more pronounced after long exp.), reduced fetal weight from 350 mg/kg/day (exp. gd 6-20) and from 450 mg/kg/day (exp. gd 6-15).	Piersma et al. (2000)
CD Sprague-Dawley rats; 2-generation study; 30/sex/group; administration in feed; 0, 750, 3,750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day.	NOAEL for developmental effects: 50 mg/kg bw/day based on reduced anogenital distance from 250 mg/kg bw/day in F1 and F2 offspring. NOAEL for maternal toxicity: 250 mg/kg bw/day	Development: reduced anogenital distance (absolute and adjusted) from 250 mg/kg bw/day in F1 and F2 offspring. Weight changes in the reproductive organs in F1 and F2 male offspring, and macroscopic and microscopic lesions in the reproductive organs in male offspring at 750 mg/kg bw/day. Maternal toxicity: organ weight changes, and histopahological lesions in the liver graded as minimal in females at 750 mg/kg bw/day.	Tyl et al. (2004)
Pregnant Sprague-Dawley rats; (no information about number); administration by gavage; 0 or 750 mg/kg bw/day from gd 14 through pnd 3.		Maternal toxicity: no information. Offspring: on pnd 2 AGD and testis weight was decreased, and on pnd 13 the incidences of areolas were increased for pups exposed *in utero* toBBP.	Parks et al. (2000)
Sprague-Dawley rats; twogeneration study; 25/sex/group; administration by gavage; 0, 20, 100 and 500 mg/kg bw/day	NOAEL: 20 mg/kg bw/day for developmental effects based on decreased body weight in F1 offspring from 100 mg/kg bw/day.	FO; decrease i body weight gain in males at 500 mg/kg/day. A dosedependent increase in kidney weight of both sexes, (significant from 100 mg/kg/day in females and at 500 mg/kg/day in males), a significant increase in liver weight in males at 500 mg/kg/day, and a significant decrease in ovary weight in females at 500 mg/kg/day. A decrease	Nagao et al. (2000)

Study Design	Effect Level	Critical Effect	Reference
		in testosterone (significant at 500 mg/kg/day), and increase in FSH (significant from 100 mg/kg/day) in males. F1: significantly decreased body weight at birth from 100 mg/kg/day, and at 500 mg/kg/day throughout the study. AGD (absolute) was decreased and preputial separation delayed in males at 500 mg/kg/day. Macroscopic and microscopic changes of testis, and decreased testosterone levels at 500 mg/kg/day after puberty. Significantly decreased testis, epididymis, and seminal vesicle weight at 500 mg/kg/day. Decreased number of germ cells in the seminiferous tubules, and sperm in the epididymis at 500 mg/kg/day. BBP did not affect reproductive ability, including delivery and lactation. F2: no significant effects related to BBP exposure up to pnd 21.	
Pregnant Sprague-Dawley rats 5/group; administration by gavage; corn oil or 750 mg/kg/day from gd 14 through postnatal day 3.		Maternal toxicity: no information. Offspring; 84% showed malformations in the testis, epididymis, accessory reproductive organs and external genitalia at 3-4 month of age. Reduced anogenital distance, decreased testis, seminal vesicle, ventral prostate and epididymis weight at day 2 of age, and males with areolas at day 13 of age.	Gray et al. (2000)
Pregnant Wistar rats 16/group; administration by gavage; olive oil, 250, 500 or 1,000 mg/kg from gd 15 to 17.	NOAEL maternal: 250 mg/kg NOAEL offspring: 250 mg/kg	Maternal toxicity: Reduced body weight gain and food consumption from 500 mg/kg. No effect on adjusted body weight gain. Decrease in the number of live foetus/litter, and decreased foetal body weight at 1,000mg/kg. Decrease in AGD in male offspring, and increase in the incidence of undescended testis from 500 mg/kg.	Ema et al. (2002)
Wistar rats; 26-34 female/group; exposure 2 weeks before mating, throught gestation, and 22 days post partum; Administration in drinking water; 1mg/L (0.125 mg/kg bw/day first day - 0.370 mg/kg bw/day before weaning).		Small reduction in absolute and relative testes weight, reduced daily sperm production	Sharpe et al. (1995)
Alpk:ApfSD (AP-rats) 19 female/group; exposure through gestation and up to post natal day 90; Administration in drinking water; 1 mg/L (0.186 mg/kg bw/day).		No critical effects in pups on testicular weights and testicular sperm counts.	Ashby et al. (1997)

Study Design	Effect Level	Critical Effect	Reference
Wistar-King A rats; MBuP; Administration by gavage on gd 7-10 (2 pregnant rats), on gd 11-14 (2 preganant rats) on gd 15-18 (6 pregnant rats); Approx. 1,000 mg/kg bw/day, control rats (5 pregnant rats) received sesame oil from gd 7-18.		Maternal toxicity: No information. Offspring: on gd 20 the testis was located significantly higher in the abdominal cavity after exposure on gd 11-14 and 15-18, compared to controls. The testosterone levels were significantly lower in MBuP treated fetuses compared to control fetuses.	Shono et al. (2000)
Wistar-King A rats; MBuP; 7/group; Administration by gavage on gd 15-18; 0 and approx. 1,000 mg/kg bw/day		Maternal toxicity: no information. Offspring: on gd 20 testis were located higher in the abdominal cavity compared to control pups. On pnd 30-40 the incidence of cryptorchidism was 84.6% in exposed animals and 0% in the control group.	Imajima et al. (1997)
Wistar rats; MBeP; Gastric intubation on pregnancy day 15-17.; 167, 250 and 375 mg/kg bw/day. LOAEL maternal: 167 mg/kg bw/day NOAEL offspring: 250 mg/kg bw/day		Significantly decreased foetal weight gain at 375 mg/kg bw/day. Significant increase in the incidence of undescended testis from 250 mg/kg bw/day. Decreased AGD from 250 mg/kg bw/day. Significantly decreased maternal food consumption and weight gain from 167 mg/kg bw/day. Significantly decreased adjusted maternal weight gain from 250 mg/kg bw/day.	Ema et al. (2003)

a) Adverse effects indicative of endocrine disruption

Several studies on reproductive and endocrine effects of BBP *in vivo* have been published since data was collected for the EU risk assessment report. Key studies on endocrine effects of BBP are summarized in table 4.

The studies included in table 4 are generally evaluated as reliable (Klimisch score 1 or 2).The reliability of a few of these studies are evaluated as somewhat limited, because they use low number of animals and only one dose level (Kwack et al., 2009 and Wilson et al., 2004)), but these studies have anyway been included in the overview table because the findings of these studies in general are accordance with the more comprehensive studies shown in the table and hence can be used as supportive evidence. Overall, the dataset is evaluated as very reliable due to the consistency of the findings with regards to both the adverse effects and the mode of action.

Table 4. Summary of *in vivo* studies not included in the EU risk assessment report on BBP (EU RAR, 2007) showing adverse effects and/or showing an *in vivo* endocrine mode- and/or mechanism of action.

Study design	Effects	Reference
Pregnant rats, gavage from GD 14 to parturition	Rats were gavaged with 4, 20 or 100 mg/kg bw/day of BBP from GD 14 to parturition. Measurement of anogenital distance at PND 5 and 25 did not show any statistically significant effect of BBP (or DBP). Pup weight was reduced at PND 1 and 21 in all groups. Statistically significant reductions in epididymis weight, prostate weight, kidney weight, serum testosterone level, sperm count, sperm motility (%) and sperm abnormalities (%) were seen in adulthood at the highest dose of BBP. At 20 mg/kg of BBP a reduction in adult body weight was seen.	Ahmad et al., 2014
Pubertal	21-day old rats were exposed to 20 or 200 mg/kg BBP for three or 20 days	Ahmad et

Study design	Effects	Reference
rats, uterotrophic assay and pubertal onset assay	using DES as a positive control. BBP did not increase uterus or ovary weights in the uterotrophic assay, but rather reduced uterus weight at 200 mg/kg. Significant decreases in body weight were seen at both doses of BBP in the 20 day assay, and uterus, vagina and ovary weights were also significantly reduced after 20 days exposure. Data on age at vaginal opening were not presented and appeared flawed (only one control had vaginal opening on postnatal day 42).	al., 2013
Rats, two-generation study, gavage	Male and female Crj:CD Sprague Dawley IGS rats were exposed to BBP by gavage at doses of 0, 100, 200, or 400 mg/kg bw/day. BBP was administered starting at 5 weeks of age for the F0 parents and 3 weeks of age for the F1 parents for 10 weeks prior to mating, and continued through weaning. Effects in male offspring are summarized here: F1 males had significantly lower body weights from 100 mg/kg bw/day and lowered epididymal weights and increased liver weight from 200 mg/kg bw/day, and reduced seminal vesicle weights and increased thyroid weights at 400 mg/kg bw/day. Aplasia and/or dysplasia and small epididymes and testes were seen at 400 mg/kg. Softening testes and histological changes, including atrophy of testicular seminiferous tubules, decreased spermatozoa and residual germ cells in epididymal lumina, were seen starting at 100 mg/kg bw/day, but reached statistical significance ($p<0.05$) only at 400 mg/kg bw/day. The F2 male offspring had decreased AGD at all doses when corrected by division with the cube root of body weights. The NOAEL for decreased AGD was therefore <100 mg/kg bw/day, the NOAEL for testicular effects 200 mg/kg bw/day.	Aso et al., 2005
Pregnant rats, gavage from GD 8 to 18	BBP decreased fetal testosterone production in rats at doses from 300 mg/kg bw/day (NOAEL 100 mg/kg bw/day). In this study, pregnant Sprague-Dawley rats were exposed to 0, 100, 300, 600, or 900 mg/kg bw/day of BBP from GD 8 to 18 by gavage in corn oil (n=4 to 9 dams per group). Testicular testosterone production ex vivo was assessed by incubation of testes of 18 day old fetuses for 3 hours and testosterone measurement in the media. Dose-related decreases in testosterone production was seen for BBP and the other tested phthalates (DIBP, DBP, and DEHP) from 300 mg/kg bw/day and above, and for DPP (dipentyl phthalate) from 100 mg/kg bw/day	Howdeshell et al., 2008
Male rats, 4 weeks gavage	Groups of 5-week-old SD rats to different phthalates including BBP. Groups of 6 rats were exposed to 500 mg/kg bw/day of BBP (or other phthalates) daily for 4 weeks. Control rats received corn oil. Body weight gain was reduced and relative liver weights were increased with BBP (and other phthalates), but no changes in other organ weights were seen for BBP (testis weight decreased by DEHP and MEHP). Sperm counts were lowered to 70% by BBP, whereas e.g. DEHP decreased sperm counts to 34%. Sperm motility (%) was significantly reduced by BBP and other phthalates. Other measures of sperm motility were affected by other examined phthalates, but not by BBP. Overall, BBP appears to affect sperm quality in a similar manner as e.g. DEHP and DBP	Kwack et al., 2009
Pregnant rats, gavage GD 10 to delivery	Pregnant Sprague Dawley CD rats were gavaged with 120mg or 500mg BBP/kg/day from day 10 post-conception to delivery. Female litters were euthanized at 21, 35, 50 and 100 days. Prenatal exposure of BBP induced delayed vaginal opening and changes in the post-natal mammary gland long after the end of the treatment, mainly by 35 days of age. Exposure to the high dose resulted in modifications in architecture and proliferative index of the mammary gland, mostly affecting the undifferentiated terminal end buds. Moreover, the expression profiles of this gland in the exposed rats were modified in a dose-dependent fashion. Analysis of functional categories showed that modified genes were related to immune function, cell signaling, proliferation and differentiation, or metabolism. The authors concluded that in utero exposure to BBP induced a delayed pubertal onset and modified morphology of the mammary gland. These alterations were accompanied by modifications in gene expression previously associated with an increased	Moral et al., 2011

Study design	Effects	Reference
	susceptibility to carcinogenesis.	
Pregnant rats, gavage GD 14 to 18	Effects of DEHP, DBP and BBP on steroid hormone production and insl3 gene expression were examined in fetal rats exposed orally from gestation day 14 to 18. Reduced testosterone production and insl3 gene expression was seen in males at GD 18.	Wilson et al., 2004

In summary, several rodent studies have demonstrated adverse reproductive and developmental effects of BBP (Agarwal et al., 1985; Ahmad et al., 2013; Ahmad et al 2014; Aso et al., 2005; Ema et al., 2002; Ema et al., 2003; Gray et al., 2000; Hammond et al., 1987; Howdeshell et al., 2008; Imajima et al., 1997; Kwack et al., 2009; Lake et al., 1978; Moral et al., 2011; Nagao et al., 2000; NTP 1997; Parks et al., 2000; Piersma et al., 2000; Piersma et al., 1995; Sharpe et al., 1995; Shono et al., 2000; Tyl et al., 2004). Most well-described are the effects of BBP and the metabolite mono-n-butyl phthalate (MBuP) on the development of the male reproductive system, e.g. increased nipple retention, decreased anogenital distance, delayed preputial separation, cryptorchidism, reduced number of spermatocytes and sperm motility and testicular changes, including decreased number of germ cells (Tables 1 and 2). Based on the types of effects on male reproductive organs, an endocrine disrupting mode of action of DEHP is considered plausible.

Increasing attention to effects on the female reproductive system as well as improved methods for detection of effects in females has led to a still growing number of findings of adverse effects of phthalates in females. For BBP, only one study was found describing effects on developing females. Moral et al. (2011) found delayed vaginal opening and changes in mammary glands after prenatal exposure to BBP. Adverse effects on estrogen related endpoints *in vivo* have not been detected (Ahmad et al., 2013).

Moreover, one *in vivo* study showed increased thyroid weights in male F1 offspring following exposure to a high dose of BBP (Aso et al., 2005), and it is thus possible that BBP may act as an endocrine disrupter via a thyroid hormone disrupting mode of action. This has however not been thoroughly examined.

In conclusion, several rodent studies have demonstrated adverse effects in intact organisms, especially on male reproductive development and adult male reproductive organs.

4.2.3 Endocrine mode of action

The studies in tables 3 and 4 show adverse effects of BBP and the metabolite MBuP and/or an endocrine mode of action[1] *in vivo*. The *in vivo* mode of action data show effects on testosterone production, i.e. effects on steroidogenesis, substantiated by one study showing mechanistic[2] *in vivo* data reducing insl3 gene expression. Studies on testosterone production in fetal male rats show an endocrine disrupting mode of action of BBP and its monoester metabolite MBuP *in vivo* (Howdeshell et al. 2008; Shono et al. 2000; Wilson et al. 2004).

Signs of an estrogenic mode of action of BBP have been found as well as effects on the thyroid system. BBP has been found to interact with estrogen and thyroid hormone receptors *in vitro* (Ghisari and Bonefeld-Jorgensen 2009, Lee et al., 2012; Zacharewski et al., 1998), but adverse effects on estrogen related endpoints *in vivo* have not been detected and for thyroids there is only indications from increased thyroid weights (Ahmad et al., 2013). Studies on interaction of BBP with androgen receptors *in vitro* have shown conflicting results depending

[1] Mode of action defined as effects on organ/tissue/organism/physiological level.

[2] Mechanism of action defined as effects at the cellular/sub-cellular/organelle/biochemical level (genes, receptors, enzymes etc).

on the cell assay with some showing androgen receptor antagonistic activity and others showing no activity (Krüger et al., 2008; Takeuchi et al., 2005).

Phthalates are absorbed as monoesters and/or rapidly metabolized to monoesters and monoesters are transported across the placenta and reach the fetus (David 2006). Thus, it is the metabolites of phthalate diesters that are endocrine disrupting and mainly effects of metabolites such as MBuP are relevant. Thus, the adverse effects of BBP are considered to be primarily related to effects on steroidogenesis.

In conclusion, several rodent studies have demonstrated an endocrine mode of action *in vivo* and some studies showed an endocrine mode of action *in vitro*. Several of the studies showed decreased testosterone levels, indicating an anti-androgenic mode of action of BBP and the metabolite MBuP due to effects on steroidogenesis. It is biologically plausible that the suggested anti-androgenic mode of action give rise to the adverse reproductive effects of BBP reported in the previous section.

4.2.4 Plausible link between adverse effects and endocrine mode of action

Altered steroidogenesis is related to adverse effects in males as well as females, and the adverse effects of BBP may be attributed to decreased testosterone levels, i.e. an anti-androgenic mode of action (EU RAR 2007). Investigation of toxicological effects of BBP in rat studies have provided convincing evidence that exposure can cause changes in the developing endocrine system as well as irreversible adverse reproductive effects. It is biologically plausible that the observed adverse effects are linked to the endocrine mode disrupting mode of action of BBP and the metabolite MBuP.

Moreover, effects on weight of thyroid glands were described in one study. This effect may be linked to the effects on the interaction with the thyroid hormone receptor reported *in vitro*. This, however, may need to be further elucidated in the future.

4.2.5 Further work substantiating the plausible link between adverse effects and endocrine mode of action

In addition to the above studies showing an endocrine disrupting mode of action of BBP, a review paper by David, 2006, describes the mechanisms of action that may be involved in the effects observed for phthalates, including BBP. Three adverse outcome pathways were presented by David, 2006, to describe cascades of events that could lead to the adverse health effects observed for BBP and other phthalate esters with a straight-chain backbone of 4-6 carbons.

Path A describes how altered gene expression for cholesterol transport and steroidogenesis in Leydig cells (Lehmann et al., 2004, Schultz et al., 2001, Barlow et al., 2003, Lee et al., 2004; Liu et al., 2005) can lead to decreased cholesterol transport (Schultz et al., 2001, Gazouli et al., 2002, Barlow et al., 2003) and subsequent decreased T synthesis (Bell et al., 1978, Foster et al., 1983, Parks et al., 2000, Akingbemi et al., 2001; Zhu et al., 2005). In turn, this can lead to the adverse health effects of hypospadias and underdeveloped secondary sex organs (Wine et al., 1997, Mylchreest et al., 1998, 1999, 2000, Gray et al., 1999, 2000, Parks et al., 2000).

Path B describes how altered gene expression of insl3 protein in Leydig cells (Lehmann et al., 2004; Liu et al., 2005) can lead to decreased levels of insl3 (Wilson et al., 2004; Liu et al., 2005) and failure of gubernacular ligament to develop (Nef and Parada, 1999). In turn, this can lead to the adverse health effect of cryptorchidism (Gray et al., 1999, 2000, Parks et al., 2000).

Path C describes effects on Sertoli cells and gonocytes including presence of multinucleated gonocytes in the seminiferous tubules. Influences on Sertoli cells are not clear but include decreased expression of cyclin D2 in neonatal Sertoli cells, decreased gene expression for cell

junctions, decrease in Sertoli cell proliferation, interference with cytoskeleton, decreased intercellular communication, and inhibition of gap junctional intercellular communication (Liu et al., 2005, Li and Kim, 2003, Li et al., 1998, 2000, Kleymenova et al. 2005, Yu et al., 2005, Kang et al 2002). Additionally, decreased T production in Leydig cells may lead to inhibition of Sertoli cell numbers (Atanassova et al., 2005). Gonocyte effects may be related to Sertoli cell changes, but this has not been clarified.

Overall, the suggested mechanisms of action described above support the conclusion that an endocrine disrupting mode of action of BBP is highly plausibly linked to the adverse effects observed in rodent studies including the adverse effects reviewed in the EU risk assessment report from 2007 (EU RAR, 2007) giving rise to the classification of BBP as toxic to reproduction. The current body of evidence points to an endocrine disrupting mode of action mainly related to altered steroidogenesis following exposure to BBP.

4.2.6 Human relevance

Human relevance of the experimental data will be addressed also using read across to other phthalates when relevant, as data on human relevance of the effects of BBP are sparse. This approach is considered justified, as many similarities have been found between phthalate esters containing a straight-chain backbone of approximately 4-6 carbons. DEHP is branched with straight C6 backbones. DBP is linear with straight C4 backbones. BBP has one benzyl side chain and a straight C4 backbone. DBP and BBP share the same metabolite, mono-butyl phthalate. DIBP is the branched isoform of DBP with straight C3 backbones. For these phthalates many similarities also have been found between (1) adverse effects in endocrine related organs, (2) in *in vivo* endocrine modes of action and (3) a plausible link between the adverse effects and the endocrine modes of action.

For example, several studies have shown similar adverse effects and endocrine mode of action for phthalates containing a straight chain backbone of 3-7 carbons. Adverse effects on reproductive organs, genital development and nipple retention were observed in males exposed to DEHP, BBP, DINP or DBP (Gray et al. 1999; Gray et al. 2000). Moreover, DEHP, DBP, BBP, DPP, DIBP and DINP reduced testosterone production, indicating an anti-androgenic mode of action of these phthalates (Borch et al. 2004; Howdeshell et al. 2008; Liu et al. 2005). As the adverse effects of the phthalates plausibly are linked to their anti-androgenic mode of action, a read-across between phthalates is considered relevant, for example when evaluating human relevance.

Due to recent studies showing differences in male reproductive effects of these phthalates between different species (rats, mice and marmosets), the issue of human relevance has been debated. Current knowledge indicates that phthalate induced effects on fetal testosterone production are not consistently found in mice, marmoset or human testis (ex vivo), but that changes in germ cell development can be induced by phthalates in different species.

Several studies are indicative of species differences in the reproductive effects of phthalates. In a study by Tomonari et al. (2006), no reproductive effects were seen in male marmosets (n=5-6 per dose group) exposed to DEHP by oral gavage at 100, 500 and 2500 mg/kg bw/day from 3 months of age until sexual maturity (18 months). Similarly, no reproductive effects were seen in a study by Kurata et al., 1998, in which male marmosets (n=4 per dose group) were dosed with 100, 500 and 2500 mg/kg bw/day of DEHP during 12-15 months of age. However, in another study on 4-day-old marmosets (5 co-twins and 4 non-twins, total n=14) treated for 14 days with 500 mg/kg bw/day of MBP, an increased Leydig cell volume was observed (Hallmark et al., 2007). A second study from the same authors revealed suppressed blood testosterone levels in male marmosets (n = 9) exposed at 2-7 days of age to a single dose of 500 mg/kg bw/day of MBP (measurement 5h after dose). In 4 day old co-twin marmosets (5 co-twins, n=10) were exposed to MBP neonatally during 14 days, and no effects on germ cell number or differentiation were apparent (McKinnell et al., 2009). It has been argued that the critical programming window for reproductive effects in marmosets is exposure during week 7 to 15 of gestation, but MBP did not alter the male reproductive system in the

one study using this exposure period (McKinnell et al., 2009). In that study, no effects on testicular morphology, reproductive tract, testosterone levels at birth, germ cell number nor germ cell proliferation were observed in male offspring (n=6) of pregnant marmosets exposed to 500 mg/kg bw/day MBP from GD 49-105 (McKinnell et al., 2009). However, unusual clusters of undifferentiated germ cells were found in two of six males examined at birth, and the biological significance of this observation is unclear. Overall, data from marmoset studies are weakened by a low number of animals, and results appear to depend on the timing of exposure.

In mice it has proved difficult to find comparable effects of phthalates on testosterone production to those seen in rats. A study in fetal mice exposed to DBP did reveal changes in several immediate genes, but no decreases were observed in testosterone levels or in genes related to cholesterol homeostasis or steroidogenesis as would be expected for rats (Gaido et al., 2007). The study in fetal DBP-exposed mice showing no influence on steroidogenesis did reveal comparable changes in germ cells to those seen in fetal rats, i.e. increased seminiferous cord diameter, and increased numbers of multinucleated gonocytes (Gaido et al., 2007). *In vitro* studies on cultured rat, but not human, fetal testes have shown the ability of phthalates to reduce testosterone production, indicating species differences in sensitivity to the testosterone suppressing effect of phthalates (Hallmark et al., 2007; Lambrot et al., 2009, Chauvigné et al., 2009). In these *in vitro* studies human testis samples were from first or second trimester fetuses, but it is not clear whether these ages correspond to the sensitive window for phthalate exposure in rats (Lambrot et al., 2009, Hallmark et al., 2007). Data from *in vitro* studies are not consistent, as an vitro study on adult human testes has shown that exposure to DEHP and MEHP impaired testosterone production, and that the measured concentrations of phthalate metabolites in the incubated testes were as low as the phthalate metabolite levels measured in humans (Desdoits-Lethimonier et al., 2012).

In contrast to the possible differences seen between species regarding phthalate-induced changes in testosterone production, there appears to be similarities between rats, mice, marmosets and humans regarding influence of phthalate exposure on germ cell proliferation and differentiation. *In vitro* studies on phthalate exposure of fetal testis tissue have been able to show comparable changes in germ cells whether using testes from rats, mice or humans (Lambrot et al., 2009, Lehraiki et al., 2009, Chauvigné et al., 2009, Habert et al., 2009). This clearly supports the possibility that reproductive effects of phthalates are relevant to humans.

Another experimental model has been applied for species comparisons, i.e. transplantation of testicular tissue from fetal rats or humans to a (transgenic) castrated mouse. A study using this model was able to demonstrate a testosterone inhibiting effect of DBP when using rat fetal testis explants, but not when using human fetal testis explants (Mitchell et al., 2012). However, there were several differences in study design between the fetal rat testis graft and the fetal human testis graft study, including duration of grafting before exposure and timing of exposure and age of the testis explant at the time of exposure. In the fetal human graft study, mice were supplied with hCG to promote testosterone production, whereas no LH (luteinizing hormone) stimulation was necessary for the rat graft to produce testosterone, and absolute testosterone levels therefore greatly differed in the two experimental setups (Mitchell et al., 2012). The differences in study design between the fetal rat testis graft study and the fetal human testis graft study thus complicate conclusions, and no firm conclusions regarding human relevance can be made on the basis of this study.

Another recent study comparing phthalate effects on rat, mouse and human testis in xenotransplant studies revealed similar effects as those described by Mitchell et al 2012 (Heger et al., 2012). Fetal testis xenotransplant studies revealed that effects on steroidogenic gene expression and ex vivo testosterone production were only seen with fetal rat testis, whereas multinuclear gonocytes were seen with rat, mouse and human fetal testis tissue (Heger et al 2012). Another study on fetal human testis xenografts showed that DBP did not affect testosterone levels or weights of androgen-sensitive host organs, whereas a CYP17A1 inhibitor, abiraterone acetate, did (Spade et al., 2013). DBP increased the number of multinucleated germ cells and altered the expression of oxidative stress response genes and actin

cytoskeleton genes (Spade et al., 2013). These gene expression changes may reflect possible mechanistic targets that are suggested as subjects for further studies. Changes in the seminiferous chords may be important to germ cell development and may be related to persistent effects on testes as seen in the testicular dysgenesis syndrome (Toppari et al., 2010).

Human epidemiological studies are difficult to interpret due to the effects being delayed relative to the time of exposure. Interestingly, a study comparing phthalate exposure in mother's milk and testosterone levels in their infant sons revealed correlations between exposure to certain phthalate monoesters and the ratio of LH to testosterone (Main et al., 2006). This is in good agreement with the marmoset study showing that neonatal phthalate exposure impaired testosterone production and induced testicular effects characteristic for high LH levels (Hallmark et al., 2007), and may indicate that the neonatal period may be a sensitive window of exposure for humans/primates. As described by Welsh et al., 2008, testosterone levels peak in late gestation in rats, but earlier (week 14-18) in humans, and this coincides with important periods of differentiation of reproductive organs. However, reproductive development continues postnatally in humans and may also be sensitive to exposure to endocrine disrupting compounds during early development (den Hond and Schoeters, 2006, Jacobson-Dickman and Lee, 2009).

In a recent review, data on phthalate toxicity to the fetal rat testis were compared with data from studies using mice or human testicular tissue (Johnson et al., 2012). The overall conclusions were that species-specific differences in testicular response following in utero phthalate exposure between mice and rats were observed, and that the response of human fetal testis to phthalate exposure may be more comparable to the response of a mouse than a rat. This review recognized two different pathways of phthalate effect on the fetal testes, namely a) suppression of steroidogenic gene expression and suppressed testosterone secretion and b) increase in multinucleated gonocyte number. A better understanding of molecular mechanisms responsible for the differences in sensitivity or resistance to developmental phthalate exposure and more insight into the molecular pathways controlling steroidogenesis in the human fetal testis is warranted. In relation to risk assessment Johnson et al. (2012) conclude that "molecular mechanistic understanding will be needed for risk assessment to progress beyond the default protective assumption that humans respond similarly to the most sensitive species" (Johnson et al 2012).

In their assessment of this Background document to the Opinion on the Annex XV dossier proposing restrictions on four phthalates (ECHA 2012), RAC concluded regarding human relevance of reproductive effects of these four phthalates: "For marmosets, however, limited data are available for in utero, peri- and neonatal exposure. There is no study with exposure during the entire life cycle such as the multigeneration studies in rats. In fact, there is only one developmental toxicity study (using a single high dose of MBP) with a period of exposure that covers the sensitive window for the programming of the male reproductive system, demonstrating some effects on the testes of neonatal marmosets of which the toxicological significance is unclear. This, combined with the relatively low number of (non-inbred) animals tested in the marmoset studies, makes it difficult to compare the results with those found in (inbred) rats. All in all, RAC concluded that there is too much uncertainty in the data available to allow a conclusion on humans being less, equally or more sensitive than rats, and thus suggested not to deviate from the default interspecies factor of 10" (ECHA 2012).

Overall, there are clear indications of species differences in metabolism and possibly in effects on fetal steroidogenesis, but there are also important differences in timing and duration of exposure in the experimental studies showing these species differences. Thus, the current knowledge on species differences is not sufficient to disregard the human relevance of phthalate effects. There are clear indications that changes in germ cell development can be induced by phthalates in several species including rats, mice, marmosets and xenotransplanted human fetal testis tissue. The implications or importance of these germ cell changes on long term effects on male reproduction are not fully elucidated, but it is evident from the current knowledge on the human testicular dysgenesis syndrome that early changes in the

seminiferous chords may be important to germ cell development and related to persistent effects on testes (Toppari et al., 2010).

For thyroid disrupting effects of BBP the issue of human relevance has not been addressed. It is, however plausible that the endocrine disrupting effects of BBP may be of relevance to humans whether related to steroidogenesis interference or to thyroid disruption. It is therefore assumed that these effects may also be relevant to humans, as no data demonstrate non-relevance.

4.2.7 Summary

Based on the definition of endocrine disrupters by WHO/IPCS in 2002 and the recommendation from the European Commission's Endocrine Disrupter Expert Advisory Group in 2013, the following four topics are covered to clarify how BBP fulfills the definition of being an endocrine disrupter:

1) Adverse health effects
2) Mode of action
3) Causality / plausible link between adverse effects and mode of action
4) Human relevance of experimental data

The EU risk assessment report from 2007 (EU RAR, 2007) acknowledges that "BBP was shown in one *in vitro* study to be a potent anti-androgen (…). Nine *in vivo* studies are available which indicate an anti-androgen-like activity of BBP or its major metabolites in rats, MBuP and MBeP" based on the studies available at the time. More recent studies published after data was collected for the EU risk assessment report confirm this hypothesis.

Rodent studies have demonstrated adverse reproductive effects, especially in male reproductive organs, such as testicular changes, reduced number of spermatocytes and sperm motility, cryptorchidism, delayed preputial separation, decreased anogenital distance and increased nipple retention. It is considered as highly plausible that these effects are induced by an endocrine mode of action of BBP and one of its major metabolites, MBuP. Further, studies on BBP and MBuP also showed decreased levels of testosterone and other effects on steroidogenesis such as reduced insl3 gene expression in the steroid biosynthesis pathway, confirming an endocrine disrupting mode of action of BBP. There is convincing evidence of a biologically plausible link between the adverse effects observed in males and the anti-androgenic mode of action of BBP and its metabolite MBuP.

Effects on female reproduction have also been reported as well as effects on the thyroid system. An estrogenic and a thyroid mode of action of BBP cannot be excluded. The anti-androgenic related effects of BBP that are evaluated to be relevant in humans include congenital malformations of the male reproductive organs, reduced semen quality, reduced male reproductive hormone levels and changes in pubertal timing. It has been hypothesized that these disorders may comprise a testicular dysgenesis syndrome with a common origin in fetal life. Testicular cancer may also be part of this syndrome.

In conclusion, BBP is classified as toxic to reproduction based on evidence of adverse effects on the reproductive organs in adult and developing male rodents, and these adverse effects are attributed to the anti-androgenic mode of action of BBP. Thus, BBP is considered as an endocrine disrupter that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism and its progeny.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Other effects: Endocrine disruption

5.1.1 General approach - Environment

To clarify how BBP fulfil the definition of being endocrine disrupters, the topics described in chapter 4 will be covered in relation to the environment.

Endocrine disruptive effects in mammalian species are analysed in chapter 4 and will not be repeated here. It is important to emphasize that the results on mammalian species are generally considered of direct relevance for mammalian wildlife, especially to wildlife species with low reproductive output including top predators, primates and other larger mammals (including endangered species), because any negative effect on development or reproduction has a high likelihood of leading to serious effects at the population level for such species. In addition, in relation to the environment, adverse effects concerning development and reproduction are generally regarded as endpoints of particular regulatory relevance because such effects are likely to manifest themselves at the population level.

However, cross-species extrapolation seems relevant, even if apical responses vary across phyla, since the key molecular initiating events (in this case inhibition of enzymes involved in steroid synthesis) are similar between species even though sensitivity to adverse effects have been observed, e.g. between rats and fish (Ankley and Gray (2013)).

In addition to cross species extrapolation, as mentioned above in the start of section 4.2.6. BBP has a strong structural similarity of main features of the molecule to other phthalates (such as DBP and DEHP) with a more extensive experimental data, including data in fish, concerning endocrine activity and adverse effects. Hence read across for hazard identification of the endocrine disruptive properties between BBP and DBP and DEHP seems appropriate.

As described for human health, in this report it is assumed that a substance should fulfil the recommendations from the European Commission's Endocrine Disrupters Expert Advisory group in order to be identified as an endocrine disruptor, and the available information is assessed based on the following topics:

1) Adverse effects

2) Endocrine mode of action

3) Plausible link between adverse effects and endocrine mode of action

For considering endocrine disrupting effects in the environment, data from both terrestrial and aquatic species should be analyzed. This is in conformity with the agreement of the European Commission's Endocrine Disrupters Expert Advisory group that "In relation to ecotoxicology, data on all species, including mammalian data generated to assess human toxicity, are generally considered relevant for the assessment of effects on ecosystems. In addition, since ecotoxicological assessment relates to impact at the population level rather than the individual level, relevance is applied in the context of identified adverse effects being relevant for the population" (JRC 2013).

Hence the fourth issue that should be considered as regards endocrine disrupters in relation to the environment is – not as for human health, human relevance – but rather environmental relevance, i.e. whether the adverse effects observed are also likely to cause effects at the population level.

Generally in regulatory ecotoxicology effects on survival, growth, but in particular development and reproduction are considered relevant endpoints for effects on populations and as such these endpoints are used to derive regulatory hazard and risk assessment decisions. It is noted that effects after longer time exposure relating to development and reproduction are generally preferred types of data for such decision.

5.1.2 Effects in the aquatic compartment (including sediment)

Overviews of the key studies on effects of BBP on wildlife were given in the EU risk assessment report for BBP (EU RAR 2007). *In vivo* studies from the report which include endpoints relevant for the assessment of endocrine disrupting effects are presented in table 5 below. Detailed study summaries can be found in the EU risk assessment report.

Table 5. Key studies on effects of BBP on wildlife which include endpoints relevant for the assessment of endocrine disrupting effects as given in the EU risk assessment report for BBP (2007).

Species	Vehicle	Exp. period	Endpoint	Effect conc. (mg/l)		Comment	Reference and estimated reliability score (Klimisch)
Fathead minnow (Pimephales promelas) (21 d reproduction test)	Methanol	21 days	Fecundity, GSI, vitellogenin, secondary sex characteristics	NOEC	0.082	No effects at the highest exposure concentration of 0.082 mg/l except for a decline in spawning frequency. Weak estrogenic activity in the Yeast estrogen screen.	Harries et al. (2000) (Score 2)[3]
Rainbow trout (Oncorhynchus mykiss) Juvenile	Peanut oil intraperetonial injection	18 days	Vitellogenin, HSI	LOEC	500 mg/kg	Vitellogenin induction at 500 and 1000 mg/kg. Injection on day 0, 6 and 12.	Christiansen et al. (2000) (Score 2)
Japanese medaka (Oryzias latipes)		21 d	Vitellogenin, HSI	NOEC LOEC	0.34 1.05	Study by Japanese EPA. Slight increase in Vtg after 14 days exposure to 1.05 mg/l. No effect after 21 days	Japanese EPA (Score 2)
Oncorhynchus mykiss ELS test		124	Hatchability, growth, mortality	NOEC	>0.2	An early life stage test in a flow through system with rainbow trout covering a 124-day period (109-day post hatch) was performed according to US EPA-TSCA guidelines and according to GLP. Mean measured test concentrations were 0.012, 0.021, 0.044, 0.095 and 0.2 mg/l. No effects were reported at any tested concentrationl.	Rhodes et al. (1995) (Score 1)[4]
Fathead minnow (*Pimephales*		30 days	hatching, length of fish and survival	NOEC LOEC	0.14 0.36	Five concentrations were tested; 0.02, 0.03, 0.07, 0.14 and 0.36 mg/l (mean	Gledhill et al. (1980) (Score 2)[5]

[3] The study is comparable to OECD TG 229 but differs by exposing two pairs of fathead minnow in two replicates instead of 2 males and 4 females in each of four replicates.

[4] No endocrine specific endpoints were included in the study.

[5] No endocrine specific endpoints were included in the study.

promelas) ELS						measured concentrations) in a flow through test system. A total of 120 embryos were tested at each concentration. A 14% reduced weight at the highest concentration resulted in a NOEC of 0.14 mg/l.	

Beside the studies described above, some *in vitro* studies were also discussed in the EU RAR (2007): Sohoni and Sumpter (1998) tested estrogenicity, anti-estrogenicity, androgenicity and anti-androgenicity of BBP in human estrogen/androgen receptor yeast assays. BBP was shown to have weak estrogenic activity which was also the conclusion by Harries et al (2000) described in the table above and by Knudsen and Pottinger (1998) who tested the displacement of estradiol from rainbow trout hepatic estrogen receptor by BBP. These *in vitro* results supports the *in vivo* vitellogenin induction in rainbow trout (*Oncorhynchus mykiss*) reported by Christiansen et al. (2000) and in an earlier study by Christiansen et al. (1998) where intraperitoneally injection of 500 mg/kg BBP in immature rainbow trout caused a slight increase in plasma vitellogenin. The Japanese EPA study (2002) with Japanese medaka (*Oryzias latipes*) also showed a slightly increased Vtg concentration after 14 days exposure to 1.05 mg/l. The effect disappeared after 21 days. In the study of Sohoni and Sumpter (1998) BBP was also shown to have strong anti-androgenic activity – inhibiting the DHT induction of the androgen receptor as strong as the model anti-androgenic compound flutamid. The anti-androgenic effects of BBP are confirmed in the mammalian studies presented in chapter 4. From the data presented in the EU RAR (2007) it is possible to conclude that BBP is weakly estrogenic but no studies confirm adversity.

Several studies have been performed with endocrine relevant endpoints included since the EU 2007 RAR.

5.1.2.1 Studies conducted after the EU RAR (2007) – Fish and amphibians

5.1.2.1.1 Short-term toxicity to fish

No endocrine relevant endpoints are included in the short-term toxicity tests to fish and therefore these studies are not discussed in this part of the dossier.

5.1.2.1.2 Long-term toxicity to fish and amphibians

At the end of each study summary an estimated reliability score (Klimisch 1997) is given. In this respect is should be noted that significant effects from studies with only nominal exposure concentrations are also seen as reliable. One reason is that exposure above the water solubility level still may expose the organisms to the substance, but then as well as to truly dissolved BBP also to BBP micelles. Even in case much higher concentrations than also the critical micelle concentration was used and effects observed reliably, it can be concluded that BBP had these effects even though an effect concentration cannot be established (besides referring to the nominal concentrations employed, meaning that the likely true exposure concentration was probably to both fully dissolved BBP and BBP micelles). Finally use of also test data with only nominal concentrations are regarded as acceptable for hazard identification and hence because the conclusions regarding whether BBP fulfills article 57 f of REACH may be reached without necessarily also being able referring to a LOEC, ECx or NOEC value in each of the references used as the scientific background information.

BBP registration Dossier (2008) (Available from ECHA web page). A Fathead minnow (*Pimephales promelas*) study covering 126 days and two generations was performed from

2006-2008 according to multiple guidelines and was conducted in accordance with GLP. At both the low (18.1 µg/L) and high (67.5 µg/L) treatment levels of benzyl butyl phthalate there were impacts on the gonadal histology of the fish: Increased incidence and severity of spermatogonia in testes of males in both treatment groups, increased incidence of oocyte atresia in females in both treatment groups. Altered gonadal stage scores in both males and females at high dose level. No effects seen at either high or low treatment rates for any of the biological parameters measured. (ECHA 2008). (Klimisch Score 1 (GLP guideline study).

Kaplan et al. (2013): Mummichog (*Fundulus heteroclitus)* preference for association with familiar conspecifics of similar body length was impacted by benzyl butyl phthalate (BBP); this was found to be a statically significant result with a $p < 0.0001$. When presented with equally sized shoals consisting of either large or small fish, the majority of unexposed (84% of N=29) and acetone exposed control (82% of N=47) fish selected the shoal of large fish. A small number of control fish chose either the shoal of small fish (6% and 10%) or the neutral zone (10% and 8%) where they were clear morphological outliers. Fish exposed to nominal 0.1 mg/L BBP (N=53) daily for four weeks selected the shoal of small fish more often than unexposed or acetone controls (7.5- and 4.5-fold respectively). They also remained in the neutral zone and displayed agitation at levels more than twice that of control. (Klimisch Score 2).

Wibe et al. (2002): The effects on fish behavior caused by BBP were investigated. The authors showed that shoaling behavior and bottom-dwelling behavior in threespine stickleback, *Gasterosteus aculeatus*, were altered as a result of exposure to 0.1 mg/L BBP (nominal concentration). Threespine sticklebacks, collected from a freshwater population in central Norway, were exposed to BBP for 26 days. BBP was administered daily through the water. It was found that exposed fish aggregated more into one single shoal than control fish (30-32 trials). Further, the exposed fish spent more time at the bottom of the test aquarium than the control fish. (Klimisch Score 2).

Wibe et al. (2004): A laboratory experiment documented effects of sublethal concentrations of p,p0-2,2-bis(p-chlorophenyl)-1,1-dichloroethylene (DDE) and butylbenzylphthalate (BBP) on feeding behavior in threespine stickleback *Gasterosteus aculeatus*. The fish were exposed for 31 days to either BBP (10 or 100 µg/L) or DDE (5 or 50 µg/L) or to a mixture of BBP and DDE in the corresponding concentrations. Two replicates of 20 fish were used for each exposure scenario and control. Five weeks after exposure termination, the authors showed that fish that had been exposed to the higher concentrations of DDE and/or BBP initiated feeding more often than control fish. The latency time to feeding (ranging from 0.25 to 5.0 min) differed between control fish and fish exposed to mixtures of DDE and BBP. Concentrations of BBP and DDE were analyzed in fish samples. (Klimisch Score 2).

Sugiyama et al. (2005): The authors developed a thyroid hormone (TH) inducible primary screening assay for the identification and assessment of chemicals that interfere with the TH-signalling pathway within target cells. The assay was developed in a *Xenopus laevis* cell line that was transduced with a self-inactivating (SIN) lentivirus vector (LV) containing a luciferase gene. The luciferase activation in this cell line was TH-specific: 3,3´,5-L-triiodothyronine (T3) > 3,3´5-L-triiodothyroacetic acid (Triac) > 3,3´,5-D-triiodothyronine (D-T3), > L-thyroxine (T4) > 3,3´,5´-L-triiodothyronine (rT3). The application of the ligand-dependent luciferase assay for screening for thyroid system-disrupting chemicals revealed that three phthalates (dicyclohexyl phthalate, n-butylbenzyl phthalate, and di-n-butyl phthalate), two herbicides (ioxynil and pentachlorophenol) and a miticide (dicofol) had 3,3´,5-L-triiodothyronine- T3-antagonist activity at concentrations ranging from 10^{-6} to 10^{-5} M. These chemicals also inhibited the expression of the endogenous primary T3-response TH nuclear receptor b (TRb) gene. The inhibitory characteristics of these chemicals were similar for both assays performed, although the assay for T3-dependent activation of TRb gene was more sensitive than the luciferase assay. Of the six chemicals, only n-butylbenzyl phthalate and pentachlorophenol exhibited T3- antagonist activity in an *in vivo* metamorphosis-based assay after 5 days exposure (T3-dependent activation of TRb gene in T3- induced metamorphosing tadpoles). It should be noted that chemicals elicited thyroid system-disrupting activity in the luciferase assay did not always interfere with the thyroid system *in vivo*. (Klimisch Score 2).

Shimada & Yamauchi. (2004): The authors characterized the 3,5,3′-$_L$-triiodothyronine (T3)-uptake system on the plasma membrane of *Rana catesbeiana* tadpole red blood cells (RBCs) in the presence of a variety of inhibitors and potentially competing amino acids. To investigate the effect of endocrine-disrupting chemicals (EDCs) on [125^I]T3 uptake, RBCs were incubated with [125^I]T3 in the presence of each chemical. Among the test chemicals, di-n-butyl phthalate, n-butylbenzyl phthalate and the miticide, dicofol, were the most powerful inhibitors of [125^I]T3 uptake, with an IC50 of 2·2 μM, which was one order of magnitude greater than that for T3 (IC50, 0·14 μM). (Klimisch Score 2).

Mankidy et al. (2013): The study investigated cytotoxicity, endocrine disruption, effects mediated via AhR, lipid peroxidation and effects on expression of enzymes of xenobiotic metabolism caused by di-(2-ethy hexyl) phthalate (DEHP), diethyl phthalate (DEP), dibutyl phthalate (DBP) and benzyl butyl phthalate (BBP) in developing fish embryos (*Pimephales promelas*). One exposure replicate consisted of 10–15 eggs in each well of a 6-well plate containing 2 ml of control water or containing phthalates. All exposures were repeated 5 times. Oxidative stress was identified as the critical mechanism of toxicity (CMTA) in the case of DEHP and DEP, while the efficient removal of DBP and BBP by phase 1 enzymes resulted in lesser toxicity. DEHP and DEP did not mimic estradiol (E2) in transactivation studies, but at concentrations of nominal 10 mg/L synthesis of sex steroid hormones was affected. Exposure to 10 mg BBP/L resulted in weak transactivation of the estrogen receptor (ER). All phthalates exhibited weak potency as agonists of the aryl hydrocarbon receptor (AhR). The order of potency of the 4 phthalates studied was; DEHP > DEP > BBP » DBP. The study highlights the need for simultaneous assessment of: (1) multiple cellular targets affected by phthalates and (2) phthalate mixtures to account for additive effects when multiple phthalates modulate the same pathway. Such cumulative assessment of multiple biological parameters is more realistic, and offers the possibility of more accurately identifying the CMTA. In summary exposure to 10 mg BBP/L resulted in weak transactivation of the estrogen receptor (ER) in a MVLN transactivation reporter assay. Exposure of fertilized fathead minnow eggs to 1 mg/l BBP for 96 h increased the androgen receptor mRNA significantly. (Klimisch Score 2).

5.1.2.2 Studies conducted after the EU RAR (2007) - Aquatic invertebrates

5.1.2.2.1 Short-term toxicity to aquatic invertebrates

Planelló et al. (2011) investigated the effects of DEHP and BBP in the larvae of *Chironomus riparius* under acute short-term treatments (24 h). Three independent experiments were carried out in each concentration for each phthalate, using 10 larvae arising from three different egg masses (same age or days after hatching), and each sample consisted of at least three replicates (n = 9). The potential effect of DEHP and BBP on the ecdysone endocrine system was studied by analysing the two genes, EcR and usp, of the heterodimeric ecdysone receptor complex. It was found that BBP provoked the overexpression of the EcR gene, with significant increases from exposures of nominal 0.1 mg/L and above, while DEHP significantly decreased the activity of this gene at the highest concentration. (Klimisch Score 2).

Otherwise, no endocrine relevant endpoints are included in the short-term toxicity tests to aquatic invertebrates and therefore these studies are not discussed in this part of the dossier!

5.1.2.2.2 Long-term toxicity to aquatic invertebrates

No endocrine specific endpoints are included in the long-term toxicity tests to aquatic invertebrates. Endpoints as reproduction rate could though inform about adverse effects without describing the causality of an endocrine derived mode of action. In the EU RAR (2007) 4 long term toxicity tests to invertebrates exposed via water (3 *Daphnia magna*, 1 *Mysidopsis bahia*) were presented and it can be concluded that the NOECs on reproduction are between 75 and 280 μg/l BBP. The 75 μg/l NOEC was used in the risk assessment to derive a $PNEC_{aquatic}$.

5.1.2.3 Adverse effects related to endocrine disruption

Below, the endocrine disruptive effects in non-mammalian aquatic (vertebrate) species are summarized from the above mentioned studies.

None of the reported fish studies describe endocrine specific adverse effects. Vitellogenin induction as seen in the Japanese EPA study and Christiansen et al. (1998 and 2000) is endocrine specific at concentrations below systemic toxicity but not regarded as adverse. The anti-thyroidal effects seen in the amphibian *in vivo* study (Sugiyama et al. (2005)) could be adverse but a longer developmental study will be needed to confirm this. The gonadal changes observed in the fathead minnow dossier study are interesting and could potentially affect reproduction if higher concentrations were tested. Also the behavioral effects observed in several studies could potentially cause adversity but again more studies are needed to confirm/reject this. The clear *in vitro* anti-androgenic effects seen in Sohoni and Sumpter (1998) could cause adverse effect in wildlife but no studies have investigated this to date.

In conclusion, no adverse effects were observed in the available studies. However, read across for hazard identification of the endocrine disruptive properties from DBP and DEHP seems appropriate

5.1.2.4 Endocrine mode of action

Below, the influence of BBP on the endocrine system in non-mammalian aquatic (vertebrate) species are summarized from the above mentioned studies.

Fish - effects on vitellogenin (Vtg) concentration in fish after waterborne and/or injection exposure to BBP has been investigated in a few studies. When combining the results of the Japanese EPA study (2002), and the two studies by Christiansen et al. (1998 and 2000), it can be concluded that BBP is a very weak estrogen.

Fish - effects on steroidogenesis: Not tested in fish but planelló et al. (2011) observed changed expression of genes related to the ecdysone endocrine system of *Chironomus* larvae.

Fish - phenotypic sex: Not tested in fish

Fish - thyroidal effects: Reduced weight was seen in fathead minnow after 30 days exposure in a developmental test with NOEC of 140 µg/l and LOEC of 360 µg/l (Gledhill et al., 1980). It is not possible to conclude whether or not the effect was thyroid mediated.

Fish - reproduction: Tests including reproductive endpoints have tested BBP concentration up to 82 µg/l only (Harries et al., 2000; ECHA dossier study 2008). No reproductive effects were seen up to this concentration. Histopatological changes in the gonads of both male and female fish were though seen in the fathead minnow study (ECHA 2008) at both tested concentrations (18.1 and 67.5 µg/l). In conclusion, one study found with histopathological changes in the gonads. Effects on reproduction was not observed at concentrations up to 82 µg/l.

Fish behavioral effects: Three studies reported behavioral changes in fish after waterborne exposure to BBP. Wibe et al. (2002 and 2004) observed changed aggregation and bottom-dwelling activity as well as feeding behavior in three spined stickleback and Kaplan et al. (2013) observed changed preference for association with familiar conspecifics of similar body length was impacted by benzyl butyl phthalate (BBP) in mummichog. Effects in all studies were seen at 50-100 µg/l. In cconclusion, effects on behavior was observed, but MoA is not revealed.

In vitro: Beside the studies mentioned in the EU RAR (2007), exposure to 10 mg BBP/L resulted in weak transactivation of the estrogen receptor (ER) in a MVLN transactivation reporter assay and exposure of fertilized fathead minnow eggs to 1 mg/l BBP for 96 h

increased the androgen receptor mRNA significantly (Mankidy et al., 2013). In conclusion, BBP can lead to both estrogen and androgen activation.

Amphibians: Two *in vitro* and one *in vivo* study revealed anti-thyroidal (T3-antagonist) activity of BBP in amphibians (Shimada & Yamauchi. (2004); Sugiyama et al. (2005)). The *in vivo* study was a 5 days study on T3-dependent activation of TRb gene in T3- induced metamorphosing tadpoles. In conclusion, BBP is anti-thyroidal (T3-antagonist) in amphibians.

Conclusion: BBP is weakly estrogenic in fish and anti-thyroidal in amphibians but no adversity can be confirmed at this point in time.

5.1.2.5 Plausible link between adverse effects and endocrine mode of action

As seen from the ecotoxicological studies described above, several endocrine pathways could be affected by BBP. Anti-thyroid effects were confirmed in amphibians and weak estrogenicity in fish, however adversity cannnot be confirmed at this point in time. Read across for hazard identification of the endocrine disruptive properties from DBP and DEHP seems appropriate

5.1.3 Summary - Environment

Fish: None of the reported fish studies describe endocrine specific adverse effects. Vitellogenin induction as seen in two studies is endocrine specific at concentrations below systemic toxicity but is not regarded as adverse. The gonadal changes observed in the fathead minnow dossier study are interesting and could potentially affect reproduction if higher concentrations were tested. Also the behavioral effects observed in several studies could potentially cause adversity but again more studies are needed to confirm/reject this. The clear *in vitro* anti-androgenic effects seen in one study could cause adverse effect in wildlife but no studies have investigated this to date. Overall, adverse effects of BBP has currently not been observed in non-mammalian wildlife species, but observations of endocrine disruptive activity *in vitro* combined with *in vivo* induction of vitellogenin show that BBP has endocrine disruptive properties in fish.

Amphibians: Two *in vitro* and one *in vivo* study revealed anti-thyroidal (T3-antagonist) activity of BBP in amphibians. The *in vivo* study was a 5 days study on T3-dependent activation of TRb gene in T3-induced metamorphosing tadpoles. The anti-thyroidal effects seen in this vivo study could be adverse but a longer developmental study will be needed to confirm this. Overall, BBP is considered anti-thyroidal (T3-antagonist) in amphibians but no adversity can be confirmed.

Mammals: The 3 first topics for identifying endocrine disrupters including the severity of the observed adverse effects of BBP on rodents (impact on development and reproduction) as presented in chapter 4 are generally accepted as endpoints of concern for mammalian wildlife and as such accepted for reaching conclusions in regulatory hazard (and risk) assessment. Furthermore developmental and reproductive effects such as those of BBP are particular concern in relation to mammalian wildlife including top predator species, primates and large mammals (inclusive endangered species), where the described reproductive effects are expected to cause serious effects at population level because of a natural low reproductive output of such taxa.

However, as mentioned above, cross-species extrapolation for hazard identification of endocrine disruptive properties, seems relevant, e.g. between rodents and fish, even though apical responses vary across phyla, since the key molecular initiating events (in this case inhibition of enzymes involved in steroid synthesis) are similar between species even though some differences in sensitivity to adverse effects have been observed, e.g. between rodents and fish (Ankley and Gray (2013)).

In addition read across between structural analogs for hazard identification of the endocrine disruptive properties of BBP from other phthalates, such as DEHP and DBP, with sufficient

experimental data in fish and rodents supports that BBP has endocrine properties for which there is evidence of probable serious effects to wildlife species.

6 CONCLUSIONS ON THE SVHC PROPERTIES

6.1 Substances of equivalent level of concern assessment

In rodents, BBP has adverse effects, which with a very high probability are caused by induction of an anti-androgenic adverse outcome pathway including changes in steroidogenic gene expression and decreased fetal testosterone production. Adverse effects of BBP has currently not been observed in non-mammalian wildlife species, but observations of endocrine disruptive activity *in vitro* combined with *in vivo* induction of vitellogenin show that BBP has endocrine disruptive properties in fish, which have the potential to lead to serious effects in the environment. Further, cross-species extrapolation of chemical effects seem relevant, even if apical responses vary across phyla, since the key molecular initiating events (in this case inhibition of enzymes involved in steroid synthesis) are conserved between species.

To conclude on the endocrine disrupting properties of BBP, a summary of the findings in chapters 4 and 5 with regard to whether this substance fulfils the definition of an endocrine disrupter as given by WHO/IPCS, further elaborated by the European Commission's Endocrine Disrupters Expert Advisory Group (JRC 2013) on elements for identification of an endocrine disrupter, is provided below.

The basis for the criteria is envisaged to be the widely accepted definition of an endocrine disruptor by the WHO/IPCS:

An endocrine disruptor is an exogenous substance or mixture that

1) alters function(s) of the endocrine system and 2) consequently causes 3) adverse health effects in an intact organism, or its progeny, or (sub)populations.

In order to fulfil the recommendations from the European Commission's Endocrine Disrupters Expert Advisory Group for a substance to be identified as an endocrine disruptor, available information is assessed based on the following topics:

1) Adverse effects 2) Endocrine mode of action 3) Plausible link between adverse effects and endocrine mode of action 4) Human relevance (for human health only)

In relation to effects on wildlife the above mentioned topic 4) human relevance should be replaced with environmental relevance (see section "5.1.1 General approach – Environment").

Conclusion

Benzyl butyl phthalate (BBP) is proposed to be identified as a substance of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) because it is an endocrine disruptor, i.e. it has endocrine disrupting properties for which there is scientific evidence of probable serious effects to human health and to the environment, and this gives rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 REACH.

BBP has been shown to adversely affect the endocrine system of mammals primarily through *in vivo* findings on reduced fetal testosterone. These findings are further substantiated by mechanistic findings, also *in vivo*, of down-regulation of genes in the steroidogenic biosynthesis pathway. The spectrum of adverse effects observed in rats include increased nipple retention, decreased anogenital distance, genital malformations, reduced number of spermatocytes and testicular changes including multinucleated gonocytes, tubular atrophy and Leydig cell hyperplasia.

In relation to the environment, adverse effects concerning development and reproduction are generally regarded as endpoints of particular relevance because such effects are likely to manifest themselves at the population level. The effects observed in rats are of particular concern for wildlife species with a natural low reproductive output, including top predators, primates and other larger mammals (including endangered species) as negative effects on reproduction has an even higher potential for causing long term negative effect at the population level for such taxa.

Adverse effects caused by exposure to BBP has not been observed in non-mammalian wildlife as no fish, amphibian or invertebrate studies including endocrine relevant endpoints has been found for BBP. However, cross-species extrapolation for hazard identification of endocrine disruptive properties seems relevant, e.g. between rodents and fish, since the key molecular initiating events (in this case inhibition of enzymes involved in steroid synthesis) are similar between species (even though apical responses vary across phyla and some differences in sensitivity to adverse effects have been observed). In addition, read across between structural analogues for hazard identification of the endocrine disruptive properties of BBP from other phthalates, such as DEHP and DBP, with sufficient experimental data in fish and rodents, supports that BBP has endocrine properties for which there is evidence of probable serious effects to wildlife species.

In conclusion, when available information from toxicological and ecotoxicological studies are combined, BBP can be considered an endocrine disruptor for both the environment and for human health as it fulfils the WHO/IPCS definition of an endocrine disruptor and the recommendations from the European Commission’s Endocrine Disrupters Expert Advisory Group for a substance to be identified as an endocrine disruptor.

BBP is considered as a substance giving rise to an equivalent level of concern because scientific evidence shows that exposure during sensitive time windows of development may cause irreversible developmental programming effects leading to severe effects on development and reproduction, regarded as particularly serious in relation to human health and wild life species, also because these adverse effects may first manifest themselves in later life stages as a consequence of exposure during early life stages.

PART II

7 INFORMATION ON USE, EXPOSURE, ALTERNATIVES AND RISKS

Information on use, exposure, alternatives and risks is provided in the original Annex XV report prepared for this substance:

http://echa.europa.eu/documents/10162/1c5023db-5a6c-464b-bfb6-b5881f0c5c72

Use and exposure information is also provided in the registration dossiers (authorities with access rights only) or on ECHA's dissemination website[6]:

http://echa.europa.eu/information-on-chemicals

[6] Information published by ECHA on the substance can be searched at this site (field "Search for Chemicals" at upper right) by EC number, CAS number or substance name.

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Annex 1- Overview of key studies on effects of BBP on fertility and reproductive organs given in tables in the EU risk assessment report for BBP (2007).

Study design	Effect Level	Critical Effect	Ref.
CD Sprague-Dawley rats; 2-generation study; 30/sex/group; Administration in feed; 0, 750, 3750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day of BBP.	NOAEL for fertility: 250 mg/kg bw/day of BBP based on reduced mating and fertility indices in F1 parents to make F2 offspring at 750 mg/kg bw/day. NOAEL for developmental effects: 50 mg/kg bw/day of BBP based on reduced anogenital distance from 250 mg/kg bw/day in F1 and F2 offspring	Fertility: reduced mating and fertility indices in F1 parents to make F2 offspring at 750 mg/kg bw/day. Development: reduced anogenital distance from 250 mg/kg bw/day in F1 and F2 offspring. Weight changes in the reproductive organs in F1 and F2 male offspring, and macroscopic and microscopic lesions in the reproductive organs in male offspring at 750 mg/kg bw/day	Tyl et al. (2004)
Sprague-Dawley rats; two-generation study; 25/sex/group; Administration by gavage; 0, 20, 100 and 500 mg/kg bw/day BBP	NOAEL: 20 mg/kg bw/day BBP for developmental effects based on decreased body weight in offspring from 100 mg/kg bw/day. No NOAEL value could be derived for effects on fertility. NOAEL for effects on the reproductive organs: 100 mg/kg bw/day	FO; decrease i body weight gain in males at 500 mg/kg/day. A dose dependent increase in kidney weight of both sexes, (significant from 100 mg/kg/day in females and at 500 mg/kg/day in males), a significant increase in liver weight in males at 500 mg/kg/day, and a significant decrease in ovarie weight in females at 500 mg/kg/day. A decrease in testosterone (significant at 500 mg/kg/day), and increase in FSH (significant from 100 mg/kg/day) in males. F1: significantly decreased body weight at birth from 100 mg/kg/day, and at 500 mg/kg/day throughout the study. AGD was decreased and preputial separation delayed in males at 500 mg/kg/day. Macroscopic and microscopic changes of testis, and decreased testosterone levels at 500 mg/kg/day after puberty. Significantly decreased testis, epididymis, and seminal vesicle weight at 500 mg/kg/day in F1 postweaning. Decreased number of germ cells in the seminiferous tubules, and sperm in the epididymis at 500 mg/kg/day as well. BBP did not affect reproductive ability, including delivery and lactation. F2: no significant effects related to BBP exposure up to pnd 21.	Nagao et al. (2000)
Fisher 344 rats male; 14 days; Administration in diet; 0.625, 1.25, 2.5 and 5% (312, 625, 1,250 and 2,500 mg/kg bw/day BBP)	NOAEL: 625 mg/kg bw/day BBP for effects on reproductive organs LOAEL: 312 mg/kg/day BBP for effects on liver and kidney	At doses ≥1,250 mg/kg bw/day body, thymus, testes, epididymis and prostate weight decrease, histopathologic changes in testes, prostate and seminal vesicle with the presence of immature sperm and necrosis in tubular epithelium,	Agarwal et al. (1985)

Study design	Effect Level	Critical Effect	Ref.
		increased levels of LH and FSH. At 2,500 mg/kg bw/day decreased progesterone levels, general toxicosis.	
Cpb-WU male rats, 4 weeks of age; Administration of BBP by gavage 28 days; 3/group; 0, 270, 350, 450, 580, 750, 970, 1,250, 1,600, 2,100 mg/kg bw/day BBP	NOAEL for effects on the reproductive organs (reduced testis weight): 750 mg bw/kg/day BBP NOAEL for systemic toxicity (increased liver weight): 580 mg/kg bw/day	Liver weight increase from 750 mg/kg bw/day. A dose related decrease in testis weight from 750 mg/kg bw/day, however, statistically significant from 1,250 mg/kg bw/day. Decreased testosterone levels from 450 mg/kg bw/day. Severe testicular atrophy from 970 mg/kg bw/day.	Piersma et al. (2000)
Fisher 344 rats; 15/male/group; 10 weeks; Administration in diet; 300, 2,800 and 25,000 ppm (20, 200 and 2,200 mg/kg bw/day BBP)	NOAEL: 300 ppm (20 mg/kg bw/day BBP) for sperm effects. NOAEL: 2,800 ppm (200 mg/kg bw/day BBP) for fertility	At doses ≥ 200 mg/kg bw/day decreased epididymal spermatozoa concentration. At 2,200 mg/kg bw/day alterations in haematological values, decreased body, prostate and testes weight, degeneration in seminiferous tubules, no pregnancy after mating.	NTP (1997)
Fisher-344 male rats; 15 male/group; 26 weeks; Administration in diet; 0, 2,800, 8,300, and 25,000 ppm (0, 180, 550, 1,660 mg/kg bw/day BBP.)	NOAEL: 8,300 ppm (550 mg/kg bw/day BBP) for fertility and sperm effects.	Fertility; At 25,000 ppm decreased fertility, testis, epididymis weight, and epididymal spermatozoa conc. Degenerative changes in testis and epididymis. Other toxic effects see Table 4.24.	NTP (1997)
RIVM-bred WU-rats; 10/sex/group; 14 days prior to and throughout mating; gastric intubation; 250, 500 and 1,000 mg/kg bw/day BBP.	NOEL: 250 mg/kg bw/day BBP based on reduced pup weight at 500 mg/kg bw/day; NOAEL 500 mg/kg bw/day for effects on reproductive organs	At 1,000 mg/kg bw/day decreased body weight, pregnancy rate, live pups, pup weight, and epididymis weight, testicular degeneration. At 500 mg/kg bw/day slightly reduced pup weight.	Piersma et al. (1995)
Wistar rats; Administration in diet over one generation producing two litters; 0.2, 0.4 and 0.8% BBP	NOAEL parental: 0.4% (206 mg/kg bw/day BBP male and 217 mg/kg/day BBP female) based on increased liver and kidney weight NOAEL reproductive performence and developmental effects: 0.8% (418 mg/kg bw/day BBP male and 446 mg/kg bw/day BBP female). Based on reduced reproductive performance.	At 0.8% reduced body weight gain and food intake in dams. Slight increase in absolute and relative liver weight in female.	Monsanto (1993)
Sprague-Dawley rats; 6 male/group; 14 days; gastric intubation; 160, 480 and 1,600 mg/kg bw/day BBP.	NOEL: 160 mg/kg bw/day BBP	At 480 mg/kg bw/day histopathologic changes in testis in one of three rats examined, at 1,600 mg/kg bw/day decreased testes weight with testicular atrophy.	Lake et al. (1978)
Wistar rats and Sprague-Dawley rats; 6/male/group; 14 days; gastric	NOAEL Wistar rat: 480 mg/kg bw/day BBP LOAEL Sprague-Dawley rats:	At 480 mg/kg bw/day testicular atrophy in one Sprague-Dawley rat. At 1,600 mg/kg bw/day decreased testes weight with testicular atrophy	Lake et al. (1978)

Study design	Effect Level	Critical Effect	Ref.
intubation; 480 and 1,600 mg/kg bw/day BBP.	480 mg/kg bw/day BBP	in all rats. Sprague-Dawley rats were more severely affected than Wistar rats.	
Sprague-Dawley rats; 6/male/group; 4 days; gastric intubation; 800 and 1,600 mg/kg bw/day of BBP, 855 mg/kg bw/day of MBuP and 985 mg/kg bw/day of MBeP		At doses ≥ 800 (BBP), 855 (MBuP) and 985 MBeP) mg/kg bw/day reduced testes weight and testicular atrophy.	Lake et al. (1978)
Sprague-Dawley rats; 5-10/sex/group; 4 weeks; Administration in diet; 500, 1,000, 1,500 2,000, 3,000 And 4,000 mg/kg bw/day BBP.	NOAEL: 1,000 mg/kg bw/day BBP	At doses ≥ 1,500 mg/kg bw/day body weight decrease. From 1,500 mg/kg bw/day testicular atrophy. From 2,000 mg/kg/day stiffness while walking and bleeding around nares.	Hammond et al. (1987)
Sprague-Dawley rats; 10/sex/group; 3 month; Administration in diet; 2,500 – 20,000 ppm (corresp. To approx. 188, 375, 750, 1,125, 1,500 mg/kg bw/day BBP)	NOAEL female: 375 mg/kg bw/day BBP NOAEL male: 750 mg/kg bw/day BBP	At doses ≥ 750 mg/kg bw/day kidney and liver weight increase in females, at doses ≥ 1,125 mg/kg bw/day liver weight increase in males.	Hammond et al. (1987)
Wistar rats; 10/sex/group; 3 month; Administration in diet; 2,500 – 12,000 ppm (corresp. to approx. 151, 381, 960 mg/kg bw/day BBP)	NOAEL male and female: 151 mg/kg bw/day BBP	At doses ≥ 381 mg/kg bw/day kidney weight increase, urinary pH decrease. At 960 mg/kg bw/day body weight decrease, liver weight increase, slight anaemia, and histopathologic changes in liver and pancreas.	Hammond et al. (1987)