The MAK Collection for Occupational Health and Safety

Benzyl butyl phthalate

MAK Value Documentation – Translation of the German version from 2018

A. Hartwig[1,*], MAK Commission[2,*]

[1] *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

[2] *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

* *email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

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Benzyl butyl phthalate / 2-O-Benzyl 1-O-butyl benzene-1,2-dicarboxylate

MAK Value Documentation

A. Hartwig[1, *], MAK Commission[2, *]

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Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has evaluated benzyl butyl phthalate [85-68-7] to derive a maximum concentration at the workplace (MAK value), considering all toxicological endpoints. Available publications and unpublished study reports are described in detail. Critical effect is the toxicity to kidneys and liver. In higher doses benzyl butyl phthalate acts adversely to male reproductive organs, fertility and fetal development. The NOAEC of 218 mg/m³ derived from a 13-week inhalation study in rats is used to set a MAK value of 20 mg/m³ for the respirable fraction (R), the MAK value is supported by the long-term feeding studies in rats and mice.

Since a systemic effect is critical, Peak Limitation Category II is assigned. The default excursion factor of 2 is set as no half-life in blood is known.

In prenatal toxicity studies in rats at 450 mg/kg body weight and day and above an increased number of resorptions and at 750 mg/kg body weight and day increased mortality and teratogenicity occurred, with a NOAEL of 350 mg/kg body weight and day. From a 2-generation study in rats a NOAEL for fetotoxicity of 100 mg/kg body weight and day was derived. In a prenatal study in mice the LOAEL for increased resorptions and malformations was 910 mg/kg body weight and day with a NOAEL of 182 mg/kg body weight and day. The NOAELs can be scaled to concentrations of 613 mg/m³ (rat, prenatal), 182 mg/m³ (mice, prenatal) and 245 mg/m³ (rat, pre- and postnatal), respectively, at the workplace. Thus, damage to the embryo or foetus is unlikely when the MAK value is observed, and benzyl butyl phthalate is classified in Pregnancy Risk Group C.

Available in vitro and in vivo data predominantly show no genotoxic effects. A contribution of cytotoxic effects for some positive test results cannot be excluded. A dominant lethal test with subcutaneous administration to rats was negative. Therefore, the substance is not regarded as a germ cell mutagen.

No increased tumour incidence was observed in chronic feeding studies in mice. In F344 rats at high doses an increased incidence of mononuclear cell leukemia is observed, which was within the range of the historical control. Furthermore, in F344 rats incidences of adenomas (pancreas tumours, adrenal phaeochromocytomas, urinary bladder tumours), but not of carcinomas are increased. The adenoma incidences were mainly within the range of historical controls. A carcinogenic effect in humans, therefore, is unlikely.

Skin contact is not expected to contribute significantly to systemic toxicity.

Data in humans and limited data in animals do not show a skin sensitizing potential.

Keywords

benzyl butyl phthalate; 1,2-benzenedicarboxylic acid butyl phenylmethyl ester; butyl benzyl phthalate; phthalic acid benzyl butyl ester; 2-O-benzyl 1-O-butyl benzene-1,2-dicarboxylate; mechanism of action; toxicokinetics; metabolism; (sub)acute toxicity; (sub)chronic toxicity; irritation; allergenic effects; reproductive toxicity; fertility; developmental toxicity; genotoxicity; carcinogenicity; peak limitation; prenatal toxicity; germ cell mutagenicity; absorption through the skin; sensitization; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

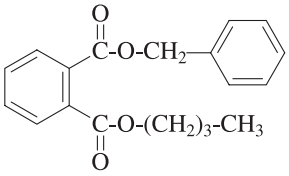
Author Information

[1] Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Department of Food Chemistry and Toxicology, Institute of Applied Biosciences, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany

[2] Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany

* Email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)

Benzyl butyl phthalate

MAK value (2017)	**20 mg/m³ I (inhalable fraction)**
Peak limitation (2017)	**Category II, excursion factor 2**
Absorption through the skin	**–**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (2017)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	1,2-benzenedicarboxylic acid butyl phenylmethyl ester butyl benzyl phthalate phthalic acid benzyl butyl ester
Chemical name	2-O-benzyl 1-O-butyl benzene-1,2-dicarboxylate
CAS number	85-68-7
Structural formula	
Molecular formula	$C_{19}H_{20}O_4$
Molar mass	312.35 g/mol
Melting point	< –35 °C (EU 2007)
Boiling point at 10.10 hPa	370 °C (EU 2007)
Density at 25 °C	1.1164 g/cm³ (EU 2007)
Vapour pressure at 20 °C	0.0000112 hPa (EU 2007)
log K_{OW}[1)]	4.84 (EU 2007)
Solubility	2.8 mg/l water (EU 2007)

1) octanol/water partition coefficient

This documentation is based primarily on the registration data publicly available through REACH (ECHA 2015) and the assessment of the Australian Government Department of Health (NICNAS 2015).

Benzyl butyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), di-*n*-butyl phthalate (DBP) and diisobutyl phthalate (DIBP) are included in Annex XIV of the REACH regulation and since February 2015 they may be sold on the market and utilised in the EU only if authorization has been granted for the specific use (EU 2011). Exempt from this is their use in certain types of packaging for medicinal products (in addition to research applications).

1 Toxic Effects and Mode of Action

Benzyl butyl phthalate is rapidly and almost completely absorbed by humans and animals after oral intake. Its metabolites are subject to enterohepatic circulation. Absorption through the skin is reported to be 5% in rats. There are no quantitative data available for absorption by inhalation; however, on the basis of the findings from inhalation studies, the substance is assumed to be readily absorbed. In humans, benzyl butyl phthalate is primarily metabolized to monobenzyl phthalate (MBeP), while monobutyl phthalate (MBuP) is the main metabolite in rats. Hydrolysis takes place in the intestines or liver. Urinary elimination is the primary route of elimination in rats, but shifts to faecal elimination at very high doses. The data do not indicate that either the substance or its metabolites accumulate in the organism.

In studies with exposure of the skin and eyes of rabbits and of human skin, benzyl butyl phthalate was not found to cause irritation of the skin but to cause slight irritation of the eyes.

Unlike after exposure to DEHP, the testes are not the most sensitive organ after exposure to benzyl butyl phthalate. In a 13-week inhalation study, the absolute and relative liver and kidney weights were significantly increased in female and male rats and the serum glucose level was reduced in males at concentrations of 789 mg/m^3 and above. In a 13-week feeding study in rats, the relative kidney weights were increased in both sexes after exposure to benzyl butyl phthalate doses of 381 mg/kg body weight and day and above. In mice given benzyl butyl phthalate with the diet for two years, body weights were decreased at doses of 900 mg/kg body weight and day and above.

In a 2-generation feeding study in rats, the first effects on fertility in the form of reduced mating and fertility indices were observed in the F1 generation at the dose of 750 mg/kg body weight and day. In prenatal developmental toxicity studies in rats, the number of resorptions was increased after exposure to benzyl butyl phthalate doses of 450 mg/kg body weight and day and above and increased mortality and malformations were found in foetuses at doses of 750 mg/kg body weight and day and above. These effects were observed in mice given 910 mg/kg body weight and day.

Most of the available in vitro and in vivo data do not indicate that benzyl butyl phthalate causes genotoxic effects. Cytotoxic effects may have contributed to the isolated positive findings at high doses or obtained with an indicator test (SCE).

The incidence of tumours was not increased in B6C3F1 mice. The incidence of mononuclear cell leukaemia was found to be increased in F344 rats at high doses;

however, it was within the range of the historical controls. Also the incidence of benign pancreatic tumours, which are considered to be of only little relevance for humans, was elevated. The incidence of hyperplasia and benign phaeochromocytomas in the adrenal medulla was increased in male F344 rats, but was within the range of the historical controls. Hyperplasia and papillomas of the bladder were observed in females; these were also within the range of the historical controls. The incidence of bladder carcinomas was slightly, but not significantly, increased after dietary restriction and lifetime exposure, but not after two years.

Benzyl butyl phthalate did not cause sensitizing effects on the skin or respiratory tract. The findings from epidemiological studies or animal studies did not establish a plausible association between exposure to benzyl butyl phthalate and an increased risk of developing allergic asthma induced by ubiquitous allergens.

2 Mechanism of Action

Peroxisome proliferation

Peroxisome proliferation was induced in rats after exposure to benzyl butyl phthalate, but to a lesser extent than after exposure to DEHP (EU 2007). The relevant studies are included in Section 5.2.2.

As far as is known today, the peroxisome proliferation that is activated in the liver of rodents by PPAR α (peroxisome proliferator-activated receptor) is not relevant for humans because, firstly, PPAR α levels are considerably lower in humans (1% to 10% of the amount found in the liver of rats and mice) and, secondly, the PPAR α-activated response is weaker (Klaunig et al. 2003). As regards the expression of other genes activated by PPAR α, for example those regulating proliferation or apoptosis (see also documentation "Di(2-ethylhexyl)phthalate (DEHP)" 2009 and supplement "Di(2-ethylhexyl) phthalate (DEHP)" 2015), not enough data are available to establish quantitative relationships (Klaunig et al. 2003).

Antiandrogenic effects

Benzyl butyl phthalate and its metabolite monobenzyl phthalate (MBeP) induce antiandrogenic effects in male rats (Ema et al. 2003; Ema and Miyawaki 2002; NICNAS 2015; Tyl et al. 2004). The same is true for DEHP and di-*n*-butyl phthalate (documentation "Di(2-ethylhexyl)phthalate (DEHP)" 2009; supplement "Di(2-ethylhexyl) phthalate (DEHP)" 2015; documentation "Di-*n*-butyl phthalate" 2013 and supplement "Di-*n*-butyl phthalate" 2015). The antiandrogenic effects are caused by a change in steroid metabolism as well as in the expression of genes that are critical for the development of the male reproductive organs. As a precaution, these effects are regarded as relevant for humans because the exact mechanism behind the effects on fertility, foetal hormone levels and the growth and development of rodents is not known (NICNAS 2015).

Pancreatic tumours

Apart from benzyl butyl phthalate, which induces pancreatic adenomas in rats, various peroxisome proliferators induce not only liver and Leydig cell tumours, but also

pancreatic tumours. One such example is perfluorooctanoic acid. Reduced secretion of bile acid with subsequent cholestasis and increased cholecystokinin (CCK) levels were suggested as a plausible mechanism of the tumorigenic effect of PPAR α agonists in the pancreas. A decrease in bile acid synthesis by inhibition of the transcription of cholesterol-7α-hydroxylase reduces the trypsin activity. This leads to the increased release of CCK from the duodenum, which in turn binds to the CCK_A receptor in the pancreas and thus stimulates acinar cell proliferation (documentation "Perfluorooctanoic acid and its inorganic salts" 2006). This mechanism might also apply for benzyl butyl phthalate, but was not tested with the substance itself. In humans, the CCK_A receptor in the pancreas is expressed to a much lesser extent than in mice and rats, so that even with increased CCK levels, the pancreatic tumours were considered to be of little relevance for humans (Klaunig et al. 2003).

Developmental toxicity

In view of the decrease in the progesterone concentration in plasma, the authors suggest the disruption of luteal function to be the mechanism for the induction of post-implantation losses in rats by benzyl butyl phthalate. However, the substance may have a direct effect on uterine functions such as decidualization, a process that results in changes to the mucous membrane (Ema et al. 1994). The decrease in the progesterone concentration might be caused by the reduced synthesis of the hormone that is produced from pregnenolone via 3β-hydroxysteroid dehydrogenase, or the diminished release of the hormone induced by the luteinising hormone (LH). Neither mechanism was investigated by this study.

In a follow-up study carried out by the same research group, reduced uterine decidualization was induced by benzyl butyl phthalate (Ema et al. 1998). Uterine decidualization is necessary for blastocyte implantation.

In a primary culture of isolated human luteal cells (large and small luteal cells in the midluteal phase) from 23 female patients who underwent surgery for non-endocrine gynaecological illnesses, benzyl butyl phthalate reduced the basal (at 10^{-5} mM and above) and hCG-stimulated (human chorionic gonadotropin) release of progesterone (at 10^{-4} mM and above). In addition, the release of prostaglandin E2 (at 10^{-3} mM benzyl butyl phthalate) and of VEGF (vascular endothelial growth factor, at 10^{-5} mM and above)—both of which are local luteotrophic (corpus luteum-stimulating) factors—was reduced. This demonstrated the impairment of luteal function by benzyl butyl phthalate in vitro (Romani et al. 2014). The relevance of the in vitro findings for humans remains unclear.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

Oral

Benzyl butyl phthalate is rapidly and almost completely absorbed by humans and animals after oral intake. Oral absorption is assumed to be 100% (NICNAS 2015).

After oral doses of up to 1600 mg/kg body weight, the radioactively labelled substance was detected primarily in the liver, kidneys, small intestine and intestinal contents of rats. There were no signs of accumulation in these or other tissues. More than 80% of the substance was eliminated with the urine within five days. The rest was eliminated with the faeces. As about 20% of the dose was eliminated with the faeces after intravenous injection, 100% oral absorption is assumed (EU 2007).

The main excretion pathway in rats, which is the urine (61% to 74% of the radioactivity) at lower doses, shifted to the faeces (57%; only 16% with the urine) at oral doses of 2000 mg/kg body weight and above. This is probably caused by the incomplete absorption of benzyl butyl phthalate or its metabolites during enterohepatic circulation. The kinetic of benzyl butyl phthalate after oral administration in rats is therefore dependent upon the dose (EU 2007; NICNAS 2015). Beagles absorbed only 10% of an oral dose of 5000 mg/kg body weight based on the recovery of unchanged benzyl butyl phthalate in the faeces (88% to 91%) and in the urine (4%). This indicates that toxicokinetics differs considerably between species. In humans, 67% to 84% of an administered dose of 0.253 to 0.506 mg/kg body weight and day was found in the urine (NICNAS 2015).

Dermal

After continuous occlusive application of benzyl butyl phthalate to the skin of male F344 rats for 7 days (around 8 mg/cm^2), 34.8% of the applied dose was absorbed (86% total recovery minus 6.3% from the occlusive cap and another 44.9% from the skin). The daily absorption of 5% of the applied dose can be concluded from these data (Elsisi et al. 1989). The same study found the amount of DEHP absorbed through the skin after 7 days to be 7%. As it is a highly lipophilic substance (log K_{OW} 8), it is likely that DEHP forms a skin depot. In contrast, with a log K_{OW} of 4.84, benzyl butyl phthalate has much lower lipophilicity. Therefore, on the basis of the study of Elsisi et al. (1989), it cannot be assumed that the amount of benzyl butyl phthalate absorbed over a period of 24 hours would already have penetrated into the skin after only 1 hour, as was demonstrated for DEHP. This makes it difficult to compare the relative absorption of benzyl butyl phthalate and DEHP. A flux of 17 µg/cm^2 and hour is calculated from the amount of benzyl butyl phthalate absorbed in 24 hours.

In an in vitro study, dermatomed human epidermis was exposed to undiluted benzyl butyl phthalate in a static diffusion cell for 8 hours at a concentration of 100 µl/cm^2. The receptor fluid was a physiological saline solution that contained 6% polyethoxyoleate as a solubilizer. The skin was washed after 8 hours and the stratum corneum removed by adhesive tape stripping. Of the dose, 0.582% was found in the stratum corneum and 0.197% in the rest of the skin; only the latter was considered to have been absorbed. The substance was not found in the receptor fluid (ECHA 2015). On the basis of an absorbed fraction of about 0.2% of the dose and a density of 1.1 g/cm^3, 220 µg/cm^2 was absorbed after 8 hours. This is equivalent to an absorption rate of 27.5 µg/cm^2 and hour and the amount absorbed in 1 hour after exposure of a surface area of 2000 cm^2 of skin is 55 mg.

Inhalation

Quantitative investigations of absorption after exposure by inhalation are not available (EU 2007; NICNAS 2015). As inhaled phthalate esters do not undergo first-pass

metabolism in the liver, the inhaled fraction is expected to be systemically available (NICNAS 2015). This is confirmed by the systemic effects observed in inhalation studies (Section 5.2.1).

3.2 Metabolism

The primary metabolite in rats is monobutyl phthalate (MBuP); other metabolites are benzyl alcohol and monobenzyl phthalate (MBeP) and in lesser amounts *n*-butyl alcohol (see Figure 1; EU 2007; NICNAS 2015).

Benzyl butyl phthalate is hydrolysed in humans and animals, in part by intestinal esterases. Hydrolysis takes place not only in the intestines, but also in the liver. Subsequently, the primary metabolites are metabolized further and eliminated, in some cases after undergoing glucuronidation (EU 2007; NICNAS 2015).

In vitro studies found that benzyl butyl phthalate was hydrolysed 3 to 15 times faster in the intestines than di-*n*-butyl phthalate and DEHP (EU 2007; see also supplement "Di(2-ethylhexyl) phthalate" 2015; documentation "Di-*n*-butyl phthalate" 2013).

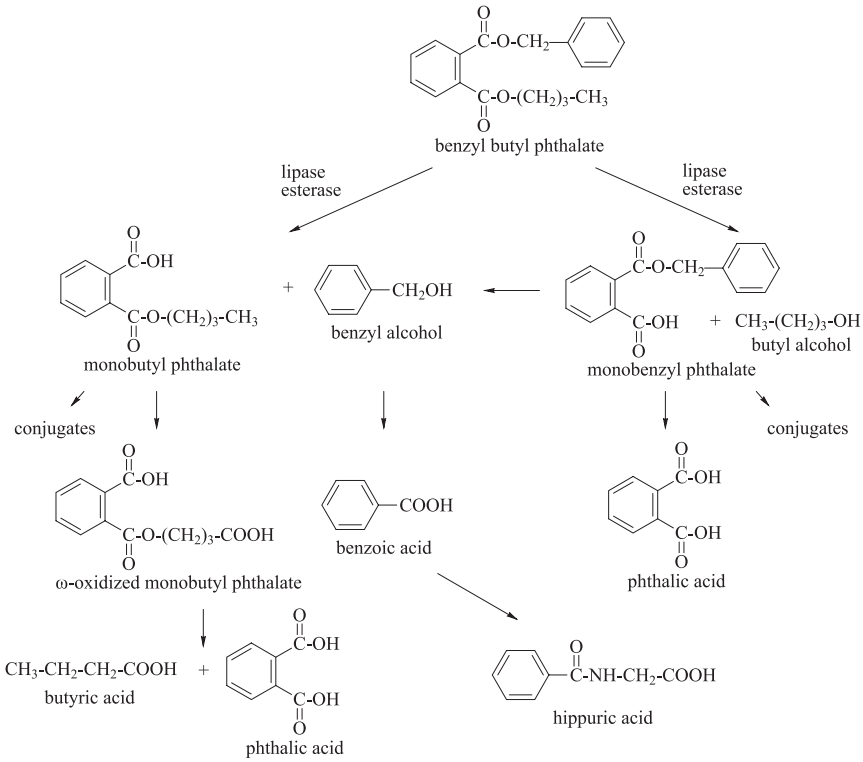


Figure 1 Suggested metabolism of benzyl butyl phthalate after oral administration to female Wistar rats (Cal EPA 2013).

The ratio of MBuP to MBeP in the urine of adult and sexually immature rats was 3:1. Both metabolites were found in the bile; therefore, re-uptake by the intestines can be assumed (enterohepatic circulation). The percentage of the metabolites MBuP and MBeP was higher in adult rats than in sexually immature animals (EU 2007).

Unlike in rats, MBeP is the main metabolite formed in humans; this metabolite is therefore best suited as a biomarker for human exposure (EU 2007; NICNAS 2015).

Correspondingly, an in vitro study found that benzyl butyl phthalate is metabolized primarily to MBeP, followed by MBuP, in the liver microsomes of humans and dogs while the opposite is true for monkeys, rats and mice (NICNAS 2015).

Like di-*n*-butyl phthalate (see documentation "Di-*n*-butyl phthalate" 2013), benzyl butyl phthalate forms the metabolite MBuP.

4 Effects in Humans

4.1 Single exposures

There are no data available.

4.2 Repeated exposure

Male workers (n = 54) in the PVC (polyvinyl chloride) industry with an average age of 38 years (21–64) who were exposed by inhalation to phthalic acid esters, primarily DEHP, DIDP or benzyl butyl phthalate, for 1 to 21 years (on average 8 years) excreted somewhat higher concentrations (not significant) of phthalic acid ester metabolites (18 to 25 µmol/l) with the urine in comparison with control persons (17 µmol/l). Changes in secretory immunoglobulin A levels (no other details) were found in workers who were exposed to high levels in the year preceding urinalysis. The exposure concentrations (2-hour values) at the different workplaces were 0.02 to 2 mg/m^3 (HPLC (high performance liquid chromatography); detection limit 0.01 mg/m^3). Effects on the peripheral nerves and on the respiratory tract were not observed in a clinical examination of the 54 male workers. With increasing length of employment, there was a slight reduction in haemoglobin levels and an increase in α-1-antitrypsin levels in the blood (Nielsen et al. 1985). As the exposure was to a mixture of substances, the study cannot be used to assess toxicity induced by the inhalation of benzyl butyl phthalate. The observed effects have neither been investigated nor observed in studies with exposure to benzyl butyl phthalate alone or in studies with exposure to one of the other phthalate esters; thus, it is not possible to draw conclusions regarding the substance responsible for the effects.

A study carried out in the 1970s determined the health status of 87 women and 60 men, 75% of whom were no older than 40 years of age, who were exposed to plasticizers, primarily di-*n*-butyl phthalate and higher alkylated phthalates and periodically to dioctyl phthalate (DOC), diisooctyl phthalate (DIOP) and benzyl butyl phthalate. The workers were exposed for 0.5 to 5 years (n = 54), 6 to 10 years (n = 28) or 10 to 19 years (n = 65). The concentration of the plasticizers (mixed phthalate esters) in air was 1.7 to 66 mg/m^3 in the work area. The workers exhibited polyneu-

ropathy and the affected number of persons and the severity increased with the exposure period (no other details). An investigation of the sensory functions revealed an early decrease in the excitability of the vestibular and olfactory receptors and of cutaneous sensitivity. The study did not include (or investigate) a control group of persons who were not exposed (Milkov et al. 1973). As exposure was to a mixture of substances, with only periodic exposure to benzyl butyl phthalate and exposure primarily to di-*n*-butyl phthalate and higher alkylated phthalates, the study cannot be used to assess toxicity induced by the inhalation of benzyl butyl phthalate. The observed effects have neither been investigated nor observed in studies with exposure to benzyl butyl phthalate alone or in studies with exposure to one of the other phthalate esters; thus, it is not possible to draw conclusions regarding the substance responsible for the effects.

In summary, a number of limitations can be identified in the studies with humans, including the lack of a control group, an insufficient number of exposed persons and inadequate documentation of the study procedure and findings. In addition, exposure was to a mixture of substances. The observed effects cannot be attributed to individual substances. In all, no conclusions can be drawn from the studies about the cause of the haematological findings and neurological symptoms.

Epidemiological studies of the association of frequency of respiratory symptoms in children and adults and exposure to benzyl butyl phthalate (and other phthalates)

A Swedish cross-sectional study of 10 851 children was not able to establish a relationship between the presence of PVC flooring material and the prevalence of asthma, rhinitis, dyspnoea or coughing during the preceding twelve months. Adjusted for personal and familial attributes and living environment (living with animals, eating habits, family size, etc.), the odds ratio (OR) was 0.90–1.15. A stronger association was identified, however, between asthma and the combination of (intermediately) increased indoor air/room humidity and the use of PVC as a flooring material (adjusted OR: 1.48; 95% confidence interval CI: 1.11–1.98) (Bornehag et al. 2005).

A nested case–control study was carried out with a subgroup of the subjects of the cross-sectional study described above after a follow-up period of 1.5 years. The subgroup was made up of 198 persons with persistent allergic respiratory symptoms both at the beginning and after the follow-up examination. The control group was composed of 202 persons who did not have these symptoms. All of the subjects underwent a medical examination and their living environment was inspected by a person trained for this purpose. The case status was associated with the presence of PVC flooring in the bedroom (OR adjusted for age, sex, living environment and tobacco smoke: 1.59; 95% CI: 1.05–2.41). The dust concentrations of the six phthalates benzyl butyl phthalate, di-*n*-butyl phthalate, DEHP, diethyl phthalate, diisobutyl phthalate and diisononyl phthalate were determined. The risk for developing asthma correlated with the DEHP concentration. However, the mean concentration of benzyl butyl phthalate in dust was only slightly increased at 0.18 mg/g dust in 79 children with rhinitis and 115 children with eczema in comparison with 0.12 mg/g dust in 177 asymptomatic children (Bornehag et al. 2004). This study has a number of limitations: it is a cross-sectional study that established a relationship between current exposure and an (earlier) onset of disease. The large number of models applied

in the study could lead to an increased number of statistically significant findings, which needs to be taken into account when evaluating the findings. The exposure is not described in detail. The authors themselves noted that bias could not be ruled out because a questionnaire was used for data collection. The study did not include biomonitoring, which means that no data are available for the body burden of benzyl butyl phthalate in symptomatic or healthy persons. However, this would only be relevant if the findings had indicated that the effects on the airways (asthma) were caused by a systemic and not local burden of benzyl butyl phthalate. In summary, a causal relationship between asthma and phthalate exposure cannot be derived from this study. No relationship was established by the nested case–control study between asthma and phthalate exposure when dose–response relationships were analysed taking other influencing factors into consideration.

In a follow-up study carried out 5 years later with children who had been 1 to 3 years old at the time of the initial study, no correlation was found between the incidence of respiratory symptoms and increased room and indoor air humidity, but a positive association was determined for the presence of PVC flooring in the home (Larsson et al. 2010).

Another case–control study of Bulgarian children established an association between the DEHP concentration, but not the benzyl butyl phthalate concentration, in the dust from the bedroom and the risk of dyspnoea, rhinitis and asthma. The study investigated 102 children aged 2 to 7 years who had had symptoms of dyspnoea, rhinitis and/or eczema during the preceding 12 months and 82 children who did not have any symptoms. The data for the symptoms of dyspnoea were provided by the parents. The dust samples were analysed for the levels of benzyl butyl phthalate, DEHP, dimethyl phthalate, diethyl phthalate, di-*n*-butyl phthalate and di-*n*-octyl phthalate. The samples of house dust collected in the homes of the symptomatic children were not found to contain higher concentrations of benzyl butyl phthalate than the samples taken from the homes of asymptomatic children (Kolarik et al. 2008). The same methodological limitations that were described in detail for the study of Bornehag et al. (2004) also apply in this case.

A National Health and Nutrition Examination Survey (NHANES) was carried out in the United States to assess anamnestic findings from the years 2005 to 2006 and the incidence of sensitization to at least one of 19 tested ubiquitous respiratory allergens in relation to the concentrations of phthalates and their metabolites in the urine of 2325 persons. The risk of sensitization was assessed in 1546 adults also in terms of the relationship between the urinary concentrations of the primary metabolite monobenzyl phthalate (MBeP) and the symptoms wheezing (OR: 1.78, 95% CI: 1.22–2.6), asthma (OR: 1.46; 95% CI: 1.01–2.11), hay fever (OR: 1.68; 95% CI: 1.09–2.59) and rhinitis (OR: 1.24; 95% CI: 1.01–1.52). In children and adolescents between 6 and 17 years of age (n = 779), urinary concentrations of MBeP were not or were more likely to be inversely correlated with the frequency of the named symptoms and were not associated with an increased OR for the presence of specific immunoglobulin E (Hoppin et al. 2013). A tenfold increase in the urinary concentrations of MBeP was associated with an increase in the nitrogen monoxide concentration in the exhaled air of around 6.8% to 8.7% in another study using 3 models to analyse the data for 274 or 313 children (Just et al. 2012 b).

A cross-sectional/case–control study investigated a total of 500 preschool children aged 3 to 5 years. After a medical examination, 440 children remained for further examination, of which 269 were assigned to the group of healthy children and 171 were assigned to the group of children with allergic diseases (asthma, rhinoconjunctivitis or atopic dermatitis). IgE levels, allergens from indoor dust, phthalates (including benzyl butyl phthalate), polycylic aromatic hydrocarbons and nicotine were determined in the samples of indoor dust. The frequency of airing was also determined. The authors concluded that there was no clear association of the clinically determined disease status with the phthalate concentrations in the indoor dust or the concentrations of phthalate metabolites in the urine (Beko et al. 2015; Callesen et al. 2014 a, b).

A study of 101 southern Taiwanese children aged 3 to 9 years found that children with allergic rhinitis or eczema had a significantly increased level of potential exposure to benzyl butyl phthalate in indoor dust. However, in comparison with the concentrations determined for DEHP (and di-*n*-butyl phthalate), the benzyl butyl phthalate fractions were very small (Hsu et al. 2012).

Comparing 146 asymptomatic North American children with 154 children aged 5 to 11 years with a history of asthma or with a current diagnosis of asthma (94 of the 154 examined children), increased concentrations (background exposure) of MBeP and MBuP in the urine of the 300 mothers during pregnancy were associated with the risk of asthma (determined by anamnesis or a current diagnosis). However, again in this study, the concentrations of the two metabolites were much lower than the concentration determined for mono(2-ethyl-5-hydroxyhexyl) phthalate and in particular for monoethyl phthalate (Whyatt et al. 2014). The authors established an association also between the prenatal concentration of MBeP in the urine of the mothers and the incidence of eczema developing during the first two years of life (Just et al. 2012 a).

A study of 361 Catalan children established an association between the MBeP concentration in the urine of the mothers during pregnancy and an increased risk for the development of asthma symptoms at the age of 7. However, a corresponding association was found also for the concentrations of DEHP metabolites (Gascon et al. 2015). In comparison with the concentrations for the other phthalate metabolites investigated, the mean concentration determined for MBeP in this study was again (very) small.

A study of 623 Norwegian children aged 10 years did not find an association between the MBeP concentration in urine and an increased risk of asthma (Bertelsen et al. 2013).

In the NHANES mentioned above (1999 to 2006), a multivariable linear regression model was applied to assess the data from about 5000 to 8000 participants, respectively. Positive associations were found for the concentrations in the urine of MBeP and the C-reactive protein, alkaline phosphatase and ferritin. An inverse association was found for the bilirubin levels (Ferguson et al. 2011, 2012).

In summary, epidemiological studies with children and adults came to different conclusions with regard to the risk of asthma, asthma-like symptoms, rhinitis or eczema after exposure to benzyl butyl phthalate and other phthalates. Other factors, such as the socio-economic (domestic) environment, may also have caused the effects determined in these studies. Most of the studies did not analyse other sub-

stances that may also have induced the effects. In addition, the benzyl butyl phthalate concentrations in the air were not determined before the symptoms developed, which means that a correlation cannot be made between the exposure and findings. The studies provide no evidence of a relationship between exposure to benzyl butyl phthalate by inhalation and the development of allergic respiratory diseases. As the methods used are not suitable for causal analysis and the levels of exposure were not sufficiently quantified, the studies are of limited use. The data provided by the studies are therefore not reliable and cannot be used for assessment. For this reason, the findings of these studies cannot be used to answer the question whether the increased incidence of allergic respiratory diseases, in particular asthma, is promoted also by the presence of phthalates such as benzyl butyl phthalate in house dust.

4.3 Local effects on skin and mucous membranes

Undiluted benzyl butyl phthalate was applied to the skin of 200 test persons in a repeated insult patch test (RIPT) for 24 hours, 3 times a week, for 5 weeks (no details of whether application was occlusive or open). No primary effects of irritation were found on the skin (EU 2007).

4.4 Allergenic effects

A RIPT carried out in 200 volunteers with 24-hour occlusive application of undiluted benzyl butyl phthalate 3 times a week over a period of 5 weeks found no evidence of sensitization. Challenge treatment with the undiluted substance 14 days after the last induction treatment did not induce any reactions that were assessed as irritant or allergic (EU 2007).

In an earlier investigation, 15–30 volunteers (no other details) were subjected to a 48-hour occlusive application of a 10% formulation of benzyl butyl phthalate (no other details). No data was provided for the development of irritation during the induction treatment. After the substance was applied again 14 days later, slight irritation, but no allergic reactions, were observed in 12% of the test persons (Mallette and von Haam 1952). As the findings were not described in sufficient detail, the study is not included in the evaluation.

4.5 Reproductive toxicity

Two studies were included in the EU Risk Assessment Report (EU 2007).

One study found an association between maternal exposure to benzyl butyl phthalate and other phthalates and the anogenital distance in boys. The maternal burden was determined on the basis of the concentrations of the different phthalates in the urine of the mothers during pregnancy. For MBeP, which reflects exposure to benzyl butyl phthalate, the OR for a shorter anogenital index (AGI) was 3.8. The AGI was determined based on the anogenital distance taking into account body weight and age. The following ORs were determined for the same end point given exposure to other phthalate monoesters: 10.2 for MBuP (exposure to dibutyl phthalate), 4.7 for

MEP (monoethyl phthalate, exposure to diethyl phthalate) and 9.1 for MiNP (monoisononyl phthalate, exposure to diisononyl phthalate) (all p-values < 0.05) (EU 2007). The US Consumer Product Safety Commission was critical of the study findings, noting that there is no standardized range for a "normal" anogenital distance or anogenital index. Several scientists were unable to replicate the statistical methods used or reproduce the significance (US CPSC 2010).

The other study did not find an association between phthalate monoester concentrations (MEP, MMP: monomethyl phthalate, MBuP, MBeP, MINP, MEHP: monoethylhexyl phthalate) in breast milk and cryptorchidism in newborn boys. However, a significant association was found between the concentrations of various phthalates (MEP, MBuP, MMP, MINP) in breast milk and the postnatal surge of various hormones (SHBG: sex hormone binding globulin, LH: luteinizing hormone, testosterone, inhibin B) in newborn boys. The tendencies were similar for MBeP, but not statistically significant. Both studies were criticized for the small group sizes of only 85 and 130 examined boys, respectively (EU 2007).

Three 24-hour urine samples were collected from 33 young and healthy Danish men over a 3-month observation period and analysed for the metabolites of phthalates. The daily intake of the sum of di-*n*-butyl phthalate, diisobutyl phthalate, DEHP, diisononyl phthalate and benzyl butyl phthalate was estimated based on the excretion of the metabolites. Based on the hazard quotient for the antiandrogenic effects of the individual phthalates, that is, the ratio between the daily intake and an acceptable level of exposure according to the data of the EFSA (European Food and Drug Administration), a hazard index was calculated for each man as the sum of the hazard quotients for each individual phthalate. The median hazard indices were in a range from 0.11 to 0.17 and thus all below 1, the "acceptable" cumulative threshold. Two of the 33 men had hazard indices above 1.0 in one of their 3 urine samples, indicating that the combined exposure to phthalates reached a level that may no longer be considered safe (Kranich et al. 2014). Benzyl butyl phthalate was not assessed on the basis of the determined metabolites MBeP and MBuP or by means of a questionnaire or examinations of the test persons.

A daily intake of benzyl butyl phthalate of 0.0021 µg/kg body weight was calculated from the analysed concentrations of benzyl butyl phthalate in water that had been filled into PET (polyethylene terephthalate) bottles. The temperature of the water was 40 °C as benzyl butyl phthalate could not be detected at room temperature (detection limit: 0.01 to 0.025 µg/l for the determined phthalates). Similar to the study above, hazard indices for antiandrogenic effects were calculated, but these were below 0.004. The authors therefore consider it very unlikely that benzyl butyl phthalate released from PET bottles induces adverse effects in pregnant and lactating women (Jeddi et al. 2016). Again, the study analysed the data for the different phthalates together. For this reason, no conclusions can be drawn for benzyl butyl phthalate. In addition, no other investigations were carried out in pregnant and lactating women.

4.6 Genotoxicity

There are no data available.

4.7 Carcinogenicity

There are no data available.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

Exposure of 4 male rats to an atmosphere saturated with benzyl butyl phthalate vapour (no other details) for 6 hours was not lethal. The observation period after exposure was 3 days (NICNAS 2015).

5.1.2 Oral administration

The oral LD_{50} of benzyl butyl phthalate was between 2330 and 20 400 mg/kg body weight in rats and 4170 and 6160 mg/kg body weight in female and male mice, respectively (see Table 1).

5.1.3 Dermal application

The dermal LD_{50} exceeded 10 000 mg/kg body weight in rabbits and was 6700 mg/kg body weight in rats (see Table 1).

5.1.4 Intraperitoneal injection

Intraperitoneal doses of benzyl butyl phthalate above 1800 mg/kg body weight were lethal in rats. LD_{50} values of 3160 mg/kg and between 4000 and 5000 mg/kg body weight were determined in 2 studies with mice (see Table 1).

5.2 Subacute, subchronic and chronic toxicity

Studies of the toxicity of benzyl butyl phthalate after repeated exposure have been assessed by a number of international bodies and the key studies selected from among them (ECHA 2015; EU 2007; NICNAS 2008, 2015).

For this reason, only the studies that are relevant to the evaluation are discussed below.

5.2.1 Inhalation

Two 4-week inhalation studies and one 13-week inhalation study with exposure of Sprague Dawley rats to aerosol/vapour mixtures are available; however, they do not include data for the percentage of vapour in the mixtures. The studies are summarized in Table 2. A vapour saturation concentration of 0.14 mg/m^3 can be calculated

Table 1 Studies of the acute toxicity of benzyl butyl phthalate

Species, strain, number per group	Dose (mg/kg body weight)	End point	References
oral			
rat, Sprague Dawley, groups of 2–3 ♂, 2–3 ♀	12 600, 15 300, 20 000 and 25 100	LD_{50}: 20 400 mg/kg body weight	Hammond et al. 1987; EU 2007
rat, 16–18, no other data		LD_{Lo}: > 4000 mg/kg body weight	EU 2007
rat, F344, no other data		LD_{50}: 2330 mg/kg body weight	NTP 1982
mouse, B6C3F1, ♂, ♀, no other data		LD_{50}: ♂: 6160 mg/kg body weight LD_{50}: ♀: 4170 mg/kg body weight	NTP 1982
dermal			
rat, no other data	no other data	LD_{50}: 6700 mg/kg body weight	EU 2007
rabbit, New Zealand White, 1–2, no other data	3980, 6310, 10 000	LD_{50}: > 10 000 mg/kg body weight	Hammond et al. 1987
intraperitoneal			
rat, 16–18, no other data		LD_{Lo}: > 1800 mg/kg body weight	EU 2007
mouse, AKR/JL, 5, no other data		4000 mg/kg body weight < LD_{50} < 5000 mg/kg body weight	EU 2007
mouse, Swiss Webster, no other data	500–16 000	LD_{50}: 3160 mg/kg body weight	EU 2007

from the vapour pressure of benzyl butyl phthalate. The vapour fraction was therefore negligible in the studies.

In a 4-week inhalation study carried out according to Good Laboratory Practice (GLP) guidelines with whole-body exposure to benzyl butyl phthalate in the form of an aerosol/vapour mixture, groups of 20 male and 20 female Sprague Dawley rats were exposed to concentrations of 0, 360, 1000 or 2100 mg/m^3 for 6 hours a day on 5 days a week. The particle sizes were approximately: 15% 4.7–9 µm, 70% 1.1–3.3 µm, 15% < 0.4–0.7 µm. In the high concentration group, mortality (♂: 3/20; ♀: 4/20) and a statistically significant decrease in body weight gains (♂: 33%; ♀: 13%) were observed and, in males, atrophy of the spleen and the reproductive organs. The NOAEC (no observed adverse effect concentration) of this study was 1000 mg/m^3

(Monsanto Co 1981). As the organ weights were not determined and a histopathological examination was not carried out, the study is of only limited relevance for the evaluation.

In another 4-week inhalation study, groups of 5 male and 5 female Sprague Dawley rats were exposed whole-body to benzyl butyl phthalate in the form of an aerosol/vapour mixture in a 500-litre exposure chamber for 6 hours a day on 5 days a week. The analysed exposure concentrations were 0, 49, 144 and 526 mg/m^3. In the high concentration group, the body weight gains in both sexes were reduced by 17% to 19% in comparison with the values for the control animals. The clinical parameters and organ weights remained unchanged and no unusual findings were observed in the histopathological examination. The NOAEC was 144 mg/m^3 in this study (Hammond et al. 1987). No data were provided as to which organs were examined histopathologically; this study is thus of only limited relevance for the evaluation, even though the findings correspond with those of the 13-week study described below.

The 13-week inhalation study likewise investigated benzyl butyl phthalate in the form of an aerosol/vapour mixture. Groups of 25 male and 25 female Sprague Dawley rats per concentration were exposed to benzyl butyl phthalate in a 10 000-litre exposure chamber for 6 hours a day on 5 days a week. The analysed exposure concentrations were 0, 51, 218 and 789 mg/m^3 (more than 90% < 10 µm, 80% 1.1–4.7 µm, geometric standard deviation 1.9). No unusual findings with regard to body weight gains were observed in comparison with the control values. The absolute and relative kidney and liver weights were increased in males and females at 789 mg/m^3. In addition, a marked decrease in serum glucose levels was observed in the males of the high concentration group. There were no substance-induced gross-pathological or microscopic findings. The NOAEC of this study was 218 mg/m^3 (Monsanto Co 1982). A histopathological examination of the larynx was not performed and, unlike the four planes of section that are standard today, only one plane of section was taken of the nose. The plane of section would correspond approximately to today's plane of section III. With regard to the study method, an investigation of the seromucous glands was described, which are probably the goblet cells. Histopathological examination of the lungs and trachea was performed; no details were provided of the number and types of sections.

Conclusion: The inhalation study relevant to the evaluation is the 13-week study in rats because, with the exception of the larynx, a full histopathological examination was performed. The NOAEC of this study was 218 mg/m^3. At a concentration of 789 mg/m^3 and above, an increase in the absolute and relative liver and kidney weights was observed and a decrease in the serum glucose levels was detected in male animals. The increase in liver weights was caused by peroxisome proliferation and is not considered to be relevant for humans (see Section 2). The 28-day study of Hammond et al. (1987), which did not report which organs underwent histopathological examination, does not contradict the results of the 13-week study. A NOAEC of 144 mg/m^3 was determined in this study; decreased body weights were observed at 526 mg/m^3 and above. Organ weights remained unchanged at this concentration. The effects induced in the kidneys did not increase over time. However, this cannot be ruled out because the highest concentration tested in the 28-day study was 526 mg/m^3 and effects on the kidneys were observed in the 13-week study at 789 mg/m^3.

Table 2 Effects of benzyl butyl phthalate after repeated inhalation exposure

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, groups of 20 ♂, 20 ♀	**28 days**, 0, 360, 1000, 2100 mg/m³, vapour + aerosol (70% 1.1–3.3 µm), 6 hours/day, 5 days/week, whole-body	**1000 mg/m³: NOAEC**; **2100 mg/m³**: ♂/♀: mortality, body weights ↓, ♂: splenic and testicular atrophy; organ weights not determined, no histopathological examination	Monsanto Co 1981
rat, Sprague Dawley, groups of 5 ♂, 5 ♀	**28 days**, 0, 49, 144, 526 mg/m³, vapour + aerosol (no other data), 6 hours/day, 5 days/week, whole-body	**144 mg/m³: NOAEC**; **526 mg/m³**: ♂/♀: body weight gains (17%–19%) ↓, no changes in clinical parameters and organ weights (brain, heart, liver, kidneys, gonads, lungs, spleen), no unusual histopathological findings; no data as to which organs underwent histopathological examination	Hammond et al. 1987
rat, Sprague Dawley, groups of 25 ♂, 25 ♀	**13 weeks**, 0, 51, 218, 789 mg/m³, vapour + aerosol (above 90% < 10 µm, 80% = 1.1–4.7 µm, GSD 1.9), 6 hours/day, 5 days/week, whole-body	**218 mg/m³: NOAEC**; **789 mg/m³**: ♂/♀: absolute (24%/11% in ♂/♀) and relative (21%/12%) liver weights ↑, absolute (18%/13% in ♂/♀) and relative (15%/14%) kidney weights ↑, ♂: concentration-dependent decrease in serum glucose levels (102, 94, 92, 76 mg/dl at 0, 51, 218 and 789 mg/m³, respectively; 9%–26%); no substance-induced gross-pathological or microscopic findings; no histopathological examination of the larynx, only 1 plane of section of the nose examined	Monsanto Co 1982

GSD: geometric standard deviation

The NOAEC of 218 mg/m^3 can be used as the starting point for the derivation of a MAK value. However, it needs to be taken into account that the larynx was not investigated in the 13-week study, but is the target organ of di-*n*-butyl phthalate (documentation "Di-*n*-butyl phthalate" 2013 and supplement "Di-*n*-butyl phthalate" 2015). As the squamous epithelial-like metaplasia of the larynx observed for di-*n*-butyl phthalate is to be regarded as an adaptive effect, the lack of an examination of the larynx does not pose a problem. Also, only one plane of section was taken from the nose in the 13-week study of benzyl butyl phthalate, which today would correspond to plane of section III. The number of sections taken from the lungs was not documented. Unusual findings such as goblet cell hyperplasia, which was observed after exposure to di-*n*-butyl phthalate, or changes in the lungs, as reported after exposure to di-*n*-butyl phthalate (documentation "Di-*n*-butyl phthalate" 2013 and supplement "Di-*n*-butyl phthalate" 2015), DEHP (documentation "Di(2-ethylhexyl) phthalate (DEHP)" 2009; supplement "Di(2-ethylhexyl) phthalate (DEHP)" 2015), DIDP (documentation "Diisodecyl phthalate, mixture of isomers" 2011) and DPHP (documentation "Di(2-propylheptyl) phthalate" 2015), were already documented by pathologists in the 1980s. For this reason, the Commission considers the study to be reliable. With regard to the study method, an investigation of the seromucous glands was described, which are probably the goblet cells. The study is very well documented and, with only slight methodological uncertainties, yielded no evidence of effects on the respiratory tract induced by exposure up to the high concentration. In summary, the study can be used for the derivation of a MAK value.

5.2.2 Oral administration

The reviews EU (2007) and NICNAS (2015) described in detail studies with repeated oral administration of benzyl butyl phthalate. For this reason, only those studies that are important for the derivation of the MAK value are discussed in detail below (Table 3).

Peroxisome proliferation

A 21-day feeding study with groups of 5 F344 rats per sex and dose investigated the effects on the liver and liver fats induced by dietary concentrations of benzyl butyl phthalate of 0%, 0.6%, 1.2% or 2.5% (doses of around 720, 1440 and 3000 mg/kg body weight and day, respectively, conversion factor 0.12 for subacute studies according to EFSA 2012). The absolute and relative liver weights were increased in all treated animals; at the highest dose level, exposure to benzyl butyl phthalate caused marked testicular atrophy and significantly reduced testis weights. The cyanide-insensitive palmitoyl-CoA oxidase activity and lauric acid 11 and 12-hydroxylation were increased in a dose-dependent manner. The examination of 2 animals per sex of the high dose group by electron microscope revealed a moderate increase in the number and size of peroxisomes in the liver. The LOAEL (lowest observed adverse effect level) was thus 720 mg/kg body weight and day (EU 2007).

Table 3 Effects of benzyl butyl phthalate after repeated oral administration

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, groups of 10 ♂, 10 ♀	**90 days**, 0, 2500 to 12 000 mg/kg feed (♂: 0, 151, 381, 960 mg/kg body weight and day, ♀: 0, 171, 422, 1069 mg/kg body weight and day)	**151/171 mg/kg body weight: NOAEL** ♀: relative caecum weights ↑ (12%, 19%, 27% at 171, 422, 1069 mg/kg body weight); **381/422 mg/kg body weight and above**: ♂/♀: relative kidney weights ↑ (8/8%, 13/19% at 381/422, 960/1069 mg/kg body weight), ♂: liver: red foci ↑, pancreas: vacuolization and enlargement of endocrine cells, congestion and infiltration of inflammatory cells, in some cases with slight fibrosis, pyknotic nuclei, acinar atrophy; **960/1069 mg/kg body weight**: ♂/♀: body weight gains ↓ (5/7%) with reduced feed consumption, relative liver weights ↑ (28/21%), ♂: urinary pH ↓, slight anaemia, liver: foci with cellular necrosis	Hammond et al. 1987
rat, Sprague Dawley, groups of 10 ♂, 10 ♀	**90 days**, 0, 2500 to 20 000 mg/kg feed (0, 188, 375, 750, 1125, 1500 mg/kg body weight and day)	**375 mg/kg body weight: NOAEL**; **750 mg/kg body weight and above**: ♂: relative kidney weights ↑ (6%, 7%, 11% at 750, 1125, 1500 mg/kg body weight), ♀: relative liver weights ↑ (16%, 25%, 31% at 750, 1125, 1500 mg/kg body weight); **1125 mg/kg body weight and above**: ♂: body weight gains (10%), feed consumption ↓, relative liver weights ↑ (19%); **1500 mg/kg body weight**: ♂: relative liver weights ↑ (29%), ♀: body weight gains ↓ (10%), feed consumption ↓; no substance-induced histopathological findings	Hammond et al. 1987
rat, F344, groups of 10 ♂, 10 ♀	**90 days**, 0, 1600 to 25 000 mg/kg feed (0, 232, 473, 938, 1875 mg/kg body weight and day)	**938 mg/kg body weight: NOAEL**; **1875 mg/kg body weight**: ♂: body weight gains ↓ (28%), testicular degeneration (no other data); no data for organ weights	Hammond et al. 1987; NTP 1982

Table 3 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, F344, 15 ♂	**26 weeks**, 0, 300, 900, 2800, 8300, 25 000 mg/kg feed (0, 30, 60, 180, 550, no data for highest dose, about 550 × 3 = 1650 mg/kg body weight and day)	**180 mg/kg body weight: NOAEL**; **550 mg/kg body weight and above**: MCH ↑, relative liver weights ↑, ♂: relative kidney weights ↑ (8% and 18% at 550 and 1650 mg/kg body weight, respectively); **1650 mg/kg body weight**: ♂: body weights and body weight gains ↓, feed consumption ↓, macrocytic anaemia, absolute weights of epididymis, cauda epididymis, testes ↓, sperm concentration ↓, atrophy of seminiferous tubules, degeneration of the testes and epididymis	NTP 1997 a
rat, F344, groups of 50 ♂, 50 ♀	**2 years**, 0, 6000, 12 000 mg/kg feed (about 0, 300, 600 mg/kg body weight and day[1]) interim necropsy after 28 weeks	**no NOAEL**; **300 mg/kg body weight: LOAEL**; **300 mg/kg body weight and above**: ♂: from week 14: mortality (internal bleeding without histopathological correlate) ↑, testing with remaining animals aborted after 29–30 weeks, ♀: body weight gains ↓, feed consumption ↓ (70%–80%), neoplastic findings see Section 5.7.2	NTP 1982

Table 3 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, F344/N, groups of 60 ♂, 60 ♀	**2 years**, ♂: 0, 3000, 6000, 12 000 mg/kg feed (0, 120, 240, 500 mg/kg body weight and day), ♀: 0, 6000, 12 000, 24 000 mg/kg feed (0, 300, 600, 1200 mg/kg body weight and day), interim necropsy after 6 and 15 months	**120 mg/kg body weight: LOAEL**; **120 mg/kg body weight and above**: ♂: relative kidney weights ↑ (9%, 10%, 16% at 120, 240, 500 mg/kg body weight after 15 months, no determination of organ weights after 24 months), ♀: no females investigated at this dose; **240/300 mg/kg body weight and above**: ♂: slight increase in focal acinar hyperplasia, significant only at next dose level, ♀: **LOAEL**; relative kidney weights ↑ (8%, 7%, 21% at 300, 600, 1200 mg/kg body weight after 15 months – no determination of organ weights after 24 months), nephropathy ↑ (34/50, 47/50, 43/50, 45/50 at 0, 300, 600, 1200 mg/kg body weight), hyperplasia of the transitional epithelium ↑ (0/50, 3/50, 7/50, 4/50), mineralisation of the kidneys ↓ (43/50, 34/50, 37/50, 35/50); **500/600 mg/kg body weight and above**: ♂: body weights ↓, relative kidney weights ↑, pigmentation of the renal tubules ↑ (after 15 and 24 months), neoplastic findings see Section 5.7.2, ♀: absolute kidney weights ↑ (after 15 months); **1200 mg/kg body weight**: ♀: body weights ↓, relative kidney weights ↑ (after 15 months), haematocrit ↓ (after 15 months), triiodothyronine ↓ (from 6 months), pigmentation of the renal tubules ↑ (after 15 and 24 months), acanthosis, hyperkeratosis of the skin, neoplastic findings see Section 5.7.2, ♂: no males investigated at this dose	NTP 1997 a

Table 3 (continued)

Species, strain, number per group	Exposure	Findings	References
mouse, B6C3F1, groups of 10 ♂, 10 ♀	**90 days**, 0, 1600 to 25 000 mg/kg feed (0, 240, 464, 946, 1875, 3750 mg/kg body weight and day)	**no NOAEL for ♂;** **240 mg/kg body weight and above**: ♂: **LOAEL**: body weight gains ↓ (14%, 22%, 23%, 25%, 35% at 240, 464, 946, 1875, 3750 mg/kg body weight), no data for feed consumption; **946 mg/kg body weight:** ♀: **NOAEL**; **1875 mg/kg body weight and above**: ♀: body weight gains ↓ (22%, 19% at 1875, 3750 mg/kg body weight); no other findings	Hammond et al. 1987; NTP 1982
mouse, B6C3F1, groups of 50 ♂, 50 ♀	**2 years**, 0, 6000, 12 000 mg/kg feed (about 0, 900, 1800 mg/kg body weight and day[2)])	**900 mg/kg body weight and above**: ♂/♀: body weights ↓; no other findings; no data for organ weights	NTP 1982
dog, beagle, groups of 3 ♂, 3 ♀	**90 days**, 0, 10 000 to 50 000 mg/kg feed (♂: 0, 400, 1000, 1875 mg/kg body weight and day, ♀: 0, 700, 1270, 1973 mg/kg body weight and day) due to palatability problems, substance administered in capsule form from day 39 of study	**no NOAEL for ♂;** **400 mg/kg body weight**: ♂: body weight gains ↓ (60%); **1000/1270 mg/kg body weight and above**: ♂: body weight gains ↓ (♂: 260%; ♀: 130%–266%); no other findings	Hammond et al. 1987

[1)] conversion factor 0.05 (long-term) according to EFSA (2012)
[2)] conversion factor 0.15 (long-term) according to EFSA (2012)
MCH: mean corpuscular haemoglobin

Groups of 5 male F344 rats per dose were exposed to dietary concentrations of 0%, 0.01%, 0.05%, 0.1%, 0.5% or 1.0% benzyl butyl phthalate (doses of about 12, 60, 120, 600 and 1200 mg/kg body weight and day, respectively, conversion factor 0.12 for subacute studies according to EFSA 2012) for 28 days. The study included a positive control group exposed to DEHP at a dose of 900 mg/kg body weight and day. No histopathological changes were determined after exposure to benzyl butyl phthalate. Changes in body weights and testis weights were not observed, but the increases in absolute and relative liver weights were statistically significant after exposure to both benzyl butyl phthalate and DEHP at a dose of 900 mg/kg body weight and day. The increase in hepatic palmitoyl-CoA oxidase activity was statistically significant in the high dose groups after exposure to the two phthalates. An increase in hepatic eosinophils was observed only in the group exposed to DEHP. The NOAEL (no observed adverse effect level) for benzyl butyl phthalate was 600 mg/kg body weight and day in this study (EU 2007).

Female F344 rats were given 0%, 0.6%, 1.2% or 2.4% benzyl butyl phthalate with the diet for up to 12 months. Signs of peroxisome proliferation in the form of increased carnitine acetyltransferase activity were reported only 1 month after exposure to the low dose (720 mg/kg body weight and day, conversion factor 0.12 for subacute studies according to EFSA 2012) and above, and increased palmitoyl-CoA oxidase activity was observed at 1440 mg/kg body weight and day and above. Cell proliferation was not found in the liver after 1, 3 or 12 months. The LOAEL of this study was therefore 720 mg/kg body weight and day (EU 2007).

Summary

Benzyl butyl phthalate induces the proliferation of peroxisomes in rats, but to a smaller extent than DEHP (see documentation "Di(2-ethylhexyl)phthalate (DEHP)" 2009). In studies of peroxisome proliferation in the rat liver, the LOAEL for increased carnitine acetyltransferase activity was 720 mg/kg body weight and day in rats given oral doses of benzyl butyl phthalate for 1 month. Increased palmitoyl-CoA oxidase activity was observed at 1200 mg/kg body weight and day and above (EU 2007).

No histopathological findings were observed in subchronic and chronic studies with dietary administration of benzyl butyl phthalate to mice and dogs (Hammond et al. 1987; NTP 1982). Palatability issues were probably the cause of the very severe body weight losses in dogs observed at 400 mg/kg body weight and day and above (no NOAEL). A 90-day study in mice reported that body weight gains were reduced by 14% in males exposed to the low dose of 240 mg/kg body weight and day (Hammond et al. 1987; NTP 1982). As this effect was not observed in the chronic study after 13 weeks, the LOAEL for mice is 900 mg/kg body weight and day. A dose-dependent reduction in body weights was found at the end of the chronic study at this dose and above. A NOAEL was not determined (NTP 1982).

Rats were found to be the more sensitive species with histopathological findings in the kidneys, liver, testes and pancreas and increased organ weights in the liver and kidneys. A NOAEL of 151 mg/kg body weight and day was determined in Wistar rats given benzyl butyl phthalate in the diet for 90 days (Hammond et al. 1987). After 26 weeks, a NOAEL of 180 mg/kg body weight and day was established for F344 rats given benzyl butyl phthalate in the diet. The relative kidney weights were increased by 8% at 550 mg/kg body weight and day; the Commission considers this to be the

LOAEL because a statistically significant increase in kidney weights of 9% was observed in the chronic study (NTP 1997 a). Therefore, on the basis of the increased kidney weights, the LOAEL of the chronic feeding study in F344 rats was 120 mg/kg body weight and day (NTP 1997 a). A NOAEL was not determined.

5.2.3 Dermal application

A study described the application of benzyl butyl phthalate to the skin for 5 months; however, the study is insufficiently documented and does not provide data for the species used and the frequency and type of application. The doses applied were 1, 5, 10 or 100 mg/kg body weight. Benzyl butyl phthalate had a local irritant effect. The doses were not lethal (EU 2007).

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

In patch tests in groups of 2 to 4 white rabbits with undiluted benzyl butyl phthalate, moderate irritation was observed (no other details; EU 2007).

Skin irritation was not induced after 24-hour occlusive application of 0.5 ml undiluted benzyl butyl phthalate to the shaven and, in half of the animals, the abraded skin of groups of 6 New Zealand White rabbits (Hammond et al. 1987).

5.3.2 Eyes

Acute irritation of the eyes was tested in two separate groups of 6 New Zealand White rabbits and evaluated according to Draize. Amounts of 0.1 ml undiluted benzyl butyl phthalate were instilled into the conjunctival sac of the eyes of the animals. This led to slight irritation after 1 and 24 hours that was reversible after 48 hours (no other details; Hammond et al. 1987).

5.4 Allergenic effects

5.4.1 Sensitizing effects on the skin

Several studies, which were not carried out according to the guidelines and are not relevant for the evaluation, did not find evidence of skin sensitization induced by benzyl butyl phthalate.

Groups of 5 AKR/JL mice treated with open application of 1.125% or 11.25% benzyl butyl phthalate (no other details) for induction did not react to open challenge treatment at the same concentrations after 5, 10 and 15 days (EU 2007).

In a similar study in BALB/c mice, induction treatment was carried out with 0.01% or 11.25% benzyl butyl phthalate, followed by challenge treatment 7 days later with 11.25% benzyl butyl phthalate. An increase in ear thickness was not observed in any of the groups (EU 2007).

Sensitizing effects were not observed in AKR and BALB/c mice after intraperitoneal injection of 0.1, 1, 10 or 100 µmol benzyl butyl phthalate and challenge treatment with 11.25% benzyl butyl phthalate (EU 2007).

Groups of 4 guinea pigs or 5 AKR mice were given 0.2 µmol (guinea pigs) or 0.022, 0.22, 2.15 or 21.5 µmol benzyl butyl phthalate for induction by injection into the paws. Signs of sensitization were not observed after open challenge treatment with 11.25% benzyl butyl phthalate (mice) or about 0.1%, 1.125% or 11.25% benzyl butyl phthalate (guinea pigs) after 7 and 15 days on the ears of mice or after 14 days on the belly of guinea pigs (EU 2007).

In addition, in studies with induction by intraperitoneal and intradermal injection of benzyl butyl phthalate, no reaction was observed after challenge with phthalic anhydride (no other details) at the same respective concentration (EU 2007).

In an invalid study, undiluted benzyl butyl phthalate caused moderate irritation after 48-hour occlusive application to rabbits (no other details). Although the authors interpreted the weak reactions (no other details) observed after renewed application 2 weeks later as evidence of a slight sensitizing effect (Mallette and von Haam 1952), this assessment is not plausible in view of the irritation observed after the initial application.

5.4.2 Sensitizing effects on the airways

Exploratory studies used a test for passive cutaneous anaphylaxis in rats to investigate the generation of antibodies in groups of 5 AKR mice given 2 or 20 µmol benzyl butyl phthalate by intraperitoneal injection (EU 2007); the studies yielded negative or questionable findings (NICNAS 2015), which, however, cannot be included in the evaluation because of a lack of standardization and methodological limitations.

No conjugates were found in an exploratory study after the incubation of serum albumin with benzyl butyl phthalate (EU 2007).

5.5 Reproductive toxicity

5.5.1 Fertility

Studies of the toxic effects on the reproductive organs and fertility and generation studies are shown in Table 4.

Effects on the reproductive organs

In a large number of studies in rats, benzyl butyl phthalate induced effects on the male reproductive organs.

A decrease in the absolute and relative testis weights and testicular atrophy were found in Sprague Dawley rats given gavage doses of benzyl butyl phthalate of 1600 mg/kg body weight and day for 4 days. The NOAEL was 800 mg/kg body weight and day (EU 2007).

Table 4 Studies of the reproductive organs, fertility studies and generation studies after administration of benzyl butyl phthalate

Species, strain, number per group	Exposure	Findings	References
Studies of the reproductive organs			
rat, Sprague Dawley, groups of 6 ♂	**4 days**, gavage, 0, 800, 1600 mg/kg body weight and day	**800 mg/kg body weight: NOAEL effects ♂ reproductive organs**; **1600 mg/kg body weight**: absolute and relative testis weights ↓, testicular atrophy	EU 2007
rat, Wistar and Sprague Dawley, groups of 6 ♂	**14 days**, gavage, 0, 480, 1600 mg/kg body weight and day	Sprague Dawley: **480 mg/kg body weight: LOAEL effects ♂ reproductive organs**; **480 mg/kg body weight**: testicular atrophy (1/6); **1600 mg/kg body weight**: absolute and relative testis weights ↓, testicular atrophy (6/6); Wistar: **480 mg/kg body weight: NOAEL effects ♂ reproductive organs**; **1600 mg/kg body weight**: absolute and relative testis weights ↓, testicular atrophy in all animals	EU 2007
rat, Sprague Dawley, groups of 6 ♂	**14 days**, gavage, 0, 160, 480, 1600 mg/kg body weight and day	**160 mg/kg body weight: NOAEL effects ♂ reproductive organs**; **480 mg/kg body weight and above**: testicular atrophy (1/3); **1600 mg/kg body weight**: testis weights ↓ (no data whether absolute or relative)	EU 2007
rat, F344, groups of 10 ♂	**14 days**, diet, 0%, 0.625%, 1.25%, 2.5%, 5.0% in the diet (0, 312, 625, 1250, 2500 mg/kg body weight and day)	**625 mg/kg body weight: NOAEL effects ♂ reproductive organs**; **1250 mg/kg body weight and above**: body weights ↓; absolute and relative weights of the testes and seminal vesicles ↓; atrophy of the testes, seminal vesicles, prostate gland; epididymis: immature sperm in the tubular lumen, necrosis of the tubular epithelium in the caput ↑; plasma FSH and LH ↑; **2500 mg/kg body weight**: absolute and relative testis weights ↓; epididymal atrophy ↑; plasma testosterone ↓	EU 2007

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Cpb-WU, groups of 3 ♂	**28 days**, gavage, 0, 270, 350, 450, 580, 750, 970, 1250, 1600, 2100 mg/kg body weight and day	**450 mg/kg body weight and above**: plasma testosterone ↓; **750 mg/kg body weight: NOAEL effects ♂ reproductive organs**; **970 mg/kg body weight and above**: testicular atrophy ↑; **1250 mg/kg body weight and above**: relative testis weights ↓; feed consumption unchanged	EU 2007
rat, Sprague Dawley, groups of 5–10 ♂, 5–10 ♀	**28 days**, diet, 0, 500, 1000, 1500, 2000, 3000, 4000 mg/kg body weight and day	**1000 mg/kg body weight: NOAEL effects ♂ reproductive organs**; **1500 mg/kg body weight and above**: testicular atrophy; **2000 mg/kg body weight and above**: body weights ↓	Hammond et al. 1987
rat, F344, groups of 10 ♂, 10 ♀	**90 days**, diet, 0, 1600 to 25 000 mg/kg feed ♂/♀: 0, 232, 473, 938, 1875 mg/kg body weight and day	**938 mg/kg body weight: NOAEL effects ♂ reproductive organs**; **1875 mg/kg body weight:** ♂: body weight gains ↓ (28%), testicular degeneration (no other data); no data for organ weights	Hammond et al. 1987; NTP 1982
rat, F344/N, groups of 60 ♂, 60 ♀	**2 years**, diet, ♂: 0, 3000, 6000, 12 000 mg/kg feed (0, 120, 240, 500 mg/kg body weight and day); ♀: 0, 6000, 12 000, 24 000 mg/kg feed (0, 300, 600, 1200 mg/kg body weight and day)	interim examination after 15 months: **240 mg/kg body weight and above**: body weights ↓; relative epididymis weights ↑; no unusual findings after histological examination of epididymis, preputial gland, prostate gland, testes	NTP 1997 a

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, groups of 6 ♂	**28 days**, gavage, BBP, DEHP, DBP, DnOP, DEP, DMP, DIDP, DUP or DINP: 500 mg/kg body weight and day; or MEHP, MBuP, MBeP, MEP, MMP or PA: 250 mg/kg body weight and day	sperm motility in the epididymis ↓: DEHP > DBP > DnOP > DUP > DIDP > **BBP** and MBuP > MEP > MEHP; BBP: no effects on testis weights	Kwack et al. 2009
rat, Wistar, no data for number of ♀	**2 weeks before mating, throughout gestation and lactation up to PND 22**, 0, 1 mg/l drinking water (PND 1–2: about 0.126 mg/kg body weight and day, PND 10–12: about 0.274 mg/kg body weight and day, PND 20–21: about 0.366 mg/kg body weight and day (NTP-CERHR 2003)), vehicle: ethanol (0.5 ml/5 l drinking water), at birth, reduction of litter size to 8 offspring, examination: PND 22, PND 90–95	**0.126–0.366 mg/kg body weight**: offspring: ♂: body weights ↑ (PND 22), absolute and relative testis weights ↓ (PND 90–95), daily sperm production ↓ (PND 90–95); NTP notes that the same research group observed unexplained fluctuations in the testis weights in the control animals (NTP-CERHR 2003); contradicts the study of Ashby et al. 1997 and tested only one dose, therefore not included in the evaluation	Sharpe et al. 1995

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Alpk:AP$_f$SD (AP), 19 ♀	**2 weeks before mating, throughout gestation and lactation up to PND 22**, replication of the study of Sharpe et al. 1995, 0, 1 mg/l drinking water, about 0.183 mg/kg body weight and day, vehicle: ethanol (0.5 ml/5 l drinking water), at birth no reduction in litter size, examination: up to PND 90	**0.183 mg/kg body weight**: offspring: ♂: body weights ↑ (birth, returned to normal on PND 90); no unusual findings in: offspring ♂ and ♀: sexual development, relative incidence of FSH-positive cells in the pituitary gland, ♂: testis weights, daily sperm production, ♀: uterine weights; contradicts the study of Sharpe et al. 1995 and tested only one dose, therefore not included in the evaluation	Ashby et al. 1997
mouse, B6C3F1, groups of 50 ♂, 50 ♀	**2 years**, 0, 6000, 12 000 mg/kg feed (about 0, 900, 1800 mg/kg body weight and day)	**900 mg/kg body weight and above**: ♂/♀: body weights ↓; histological examination of male and female reproductive organs without substance-induced findings; no data for organ weights	NTP 1982

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
Fertility studies			
rat, F344/N, groups of 15 ♂	**10 weeks**, modified mating protocol, diet, 0, 300, 2800, 25 000 mg/kg feed (0, 20, 200, 2200 mg/kg body weight and day), mated with untreated ♀	**200 mg/kg body weight: NOAEL effects ♂ reproductive organs and fertility**; **200 mg/kg body weight and above**: epididymal sperm concentration ↓, interval between mating and counting of sperm varied, counting therefore invalid (EU 2007), no automated sperm analysis, instead analysis performed on a slide using a haemacytometer (counting chamber on slide), preparation problems in the 26-week study (see study); **2200 mg/kg body weight**: body weights and body weight gains ↓; feed consumption ↓; absolute and relative weights of the prostate gland ↓; absolute weights of right cauda epididymis, right epididymis and right testis ↓; degeneration of germ cell epithelium (15/15), fertility index ↓; litter parameters unchanged	NTP 1997 a
rat, F344/N, groups of 15 ♂	**26 weeks**, diet, 0, 300, 900, 2800, 8300, 25 000 mg/kg feed (0, 30, 60, 180, 550 mg/kg body weight and day), highest dose not calculated because of considerable feed waste, feed consumption assumed to be the same as at other doses: 1650 mg/kg body weight and day (NTP-CERHR 2003)	**550 mg/kg body weight: NOAEL effects ♂ reproductive organs and fertility**; **1650 mg/kg body weight**: incidence of pregnancy ↓; absolute weights of right epididymis, cauda epididymis and right testis ↓, sperm concentration in the epididymis ↓, however: preparation problems (see below), hypospermia and atrophy of the seminiferous tubules; sperm concentrations in the epididymis of control animals lower than in the historical controls of the laboratory, caused by improper mincing of the epididymis, no data whether this applies also to the organs of the treated animals (EU 2007)	NTP 1997 a

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, RIVM WU, groups of 10 ♂, 10 ♀	**14 days before and during mating, up to PND 6**, OECD Test Guideline 421 (BBP test substance for validation of test guideline), gavage, 0, 250, 500, 1000 mg/kg body weight and day	**500 mg/kg body weight: NOAEL effects ♂ reproductive organs, fertility and foetotoxicity**; **500 mg/kg body weight**: dams: feed consumption ↑ (during gestation); offspring: body weights ↓ (PND 1, returned to normal on PND 6); **1000 mg/kg body weight**: dams: body weight gains ↓, post-implantation losses ↑, number of living offspring ↓ (PND 2, PND 6), body weights ↓ (PND 6); sires: absolute epididymis weights ↓; testicular degeneration and Leydig cell hyperplasia	Piersma et al. 1995; see also Section "Developmental toxicity"
rat, Wistar Crl:(WI) WU BR, 28 ♀	**2 weeks before mating, throughout gestation and lactation up to PND 22**, replication of study of Sharpe et al. 1995, 0, 0.1, 1, 3 mg/l drinking water, estimated uptake of dams from consumption of drinking water: 0.012, 0.14, 0.385 mg/kg body weight and day, examination: no data	**0.385 mg/kg body weight**: postnatal mortality ↑ (second experiment confirmed effect, no statistical significance when evaluated on a per litter basis, but higher than for the historical controls of the laboratory; during the study period, similar high incidence of postnatal mortality in vehicle control groups); no unusual findings with respect to the mating index, fertility, post-implantation losses or the offspring (organ weights, daily sperm production); contradicts study of Sharpe et al. 1995; original study not available	NTP-CERHR 2003

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 21–25 ♀	**2 weeks before mating, throughout gestation and lactation up to PND 21**, 0, 1, 3 mg/kg in feed or mg/l drinking water (exposure during mating/gestation/lactation: 1 mg/kg in the diet: 0.08–0.09/0.06–0.07/0.11–0.06; 1 mg/l in the drinking water: 0.10–0.12/0.11–0.11/0.17–0.24; 3 mg/kg in the diet: 0.27–0.28/0.19–0.25/0.34–0.49; 3 mg/l in the drinking water: 0.34–0.35/0.35–0.35/0.54–0.80 mg/kg body weight and day)	**up to 0.80 mg/kg body weight**: no unusual findings with respect to: fertility, body weight gains, feed and water consumption, number of resorptions, litter size, survival and body weights of the offspring; original study not available	NTP-CERHR 2003
Generation studies			
rat, Wistar, as specified by Test Guideline 20 pregnant ♀	**one-generation study**, OECD Test Guideline 415, diet, 0%, 0.2%, 0.4%, 0.8% in the diet (♂: 0, 103, 206, 418 mg/kg body weight and day, ♀: 0, 108, 217, 446 mg/kg body weight and day), production of 2 litters	**206/217 mg/kg body weight: NOAEL parental toxicity**; **418/446 mg/kg body weight: NOAEL reproductive behaviour and foetotoxicity;** **418/446 mg/kg body weight**: dams: body weight gains and feed consumption ↓; absolute and relative liver weights ↑; no unusual findings with respect to mortality, clinical signs, viability index and index of live births, gross-pathological and microscopic examination of the reproductive organs; original study not available	EU 2007

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, CD® (SD), groups of 30 ♂, 30 ♀	**two-generation study**, F0: treatment from 10 weeks before mating to weaning (PND 21); F1: treatment for 10 weeks after weaning (from PND 21); diet, 0, 750, 3750, 11 250 mg/kg feed (0, 50, 250, 750 mg/kg body weight and day)	**50 mg/kg body weight: NOAEL ♀ parental toxicity**; **250 mg/kg body weight: NOAEL foetotoxicity; NOAEL ♂ parental toxicity**; **250 mg/kg body weight and above**: F0 ♂ adults: absolute kidney weights ↑ (250 mg/kg body weight: 7%, 750 mg/kg body weight: 8%), no unusual histological findings; F0 ♀ adults: relative kidney weights ↑ (250 mg/kg body weight: 6%, 750 mg/kg body weight: 9%), no unusual histological findings, absolute kidney weights ↑; F1 ♂ pups: AGD/litter ↓; F2 ♂ pups: AGD/litter ↓; AGD: very sensitive parameter. Shortened AGD in ♂, no change in ♀: indicator for antiandrogenic effect, biological significance and relevance unknown (US EPA 2005), shortening of AGD without other effects on the reproductive organs in ♂ F1 and F2 offspring, not regarded as adverse; **750 mg/kg body weight**: F0 ♂ adults: body weights and body weight gains ↓; absolute and relative liver weights ↑; relative kidney weights ↑; liver: minimal to slight diffuse cytoplasmic granules, number of peroxisomes ↑; F0 ♀ adults: body weights and body weight gains ↓; absolute and relative liver weights ↑; absolute and relative ovarian and uterine weights ↓; histological changes in liver (as described above);	Tyl et al. 2004; see also Section "Developmental toxicity"

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
		F1 ♂ pups: body weights/litter ↓ (PND 0); feed consumption and body weights ↓; percentage of animals with at least 1 nipple ↑ (PND 11–13); number of nipples/animal ↑ (PND 11–13); percentage of animals with >1 areola ↑ (PND 11–13); number of areolae/animal ↑ (PND 11–13); delayed preputial separation; body weights ↓ (PND 21); absolute thymus weights ↓ (PND 21); absolute and relative spleen weights ↓ (PND 21); absolute and relative testis weights ↓ (PND 21; at 250 mg/kg body weight ↑); absolute and relative epididymis weights ↓ (PND 21); percentage with malformations in reproductive tract ↑ (partial or complete loss of epididymis, including reduced epididymal or testicular size, missing testes including undescended testes; PND 21); F1 ♀ pups: body weights/litter ↓ (PND 0); delayed vaginal opening; absolute thymus weights ↓ (PND 21); absolute and relative spleen weights (PND 21); absolute ovarian and uterine weights ↓ (PND 21); F1 adults: mating index and fertility index ↓; number of implantations/litter ↓; number of living offspring/litter ↓; F1 ♂ adults: body weights and body weight gains ↓; absolute and relative liver and pancreas weights ↑; relative weights of adrenal gland ↑; absolute testis and epididymis weights ↓; absolute and relative weights of prostate gland ↓; absolute weights of seminal vesicles with coagulating gland ↓; sperm concentration in epididymis ↓; number of motile sperm ↓; histological changes in liver (as described above); F1 ♀ adults: body weights and body weight gains ↓; relative ovarian weights ↓; absolute and relative uterine weights ↑; histological changes in the liver (as described above); F2 ♂ pups: percentage of animals with at least 1 nipple ↑ (PND 11–13); number of nipples/animal ↑ (PND 11–13); percentage of animals with >1 areola ↑ (PND 11–13); number of areolae/animal ↑ (PND 11–13); body weights ↓ (PND 21); absolute thymus weights ↓ (PND 21); absolute and relative spleen weights ↓ (PND 21); relative brain weights ↑ (PND 21); absolute and relative testis weights ↓ (PND 21); percentage with malformations in reproductive tract (as described above) ↑; F2 ♀ pups: body weights ↓ (PND 21); relative brain weights ↑ (PND 21); absolute and relative spleen weights ↓ (PND 21); absolute thymus and ovarian weights ↓ (PND 21);	

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Crj:CD(SD)IGS, groups of 35 ♂, 35 ♀	**two-generation study**, F0: treatment from 12 weeks before mating up to necropsy (age: 23 weeks); F1: treatment after weaning (from PND 22) up to necropsy (age: 10 weeks); gavage, in corn oil, 0, 20, 100, 500 mg/kg body weight and day	**100 mg/kg body weight: NOAEL foetotoxicity and parental toxicity**; **100 mg/kg body weight and above**: F0 ♂ adults: salivation; serum FSH ↑; F0 ♀ adults: salivation; absolute and relative kidney weights ↑ (absolute: 100 mg/kg body weight: 7%, 500 mg/kg body weight: 7%; relative: 100 mg/kg body weight: 8%, 500 mg/kg body weight: 6%; no dose dependency, no unusual histological findings); F1 ♂ pups: body weights ↓ (PND 0, 6%, at 500 mg/kg body weight: 7%, effects reversible after 4 days); TSH ↓ (PND 22); F1 ♀ pups: body weights ↓ (PND 0, 6%, at 500 mg/kg body weight: 6%, effects reversible after 4 days); T3 ↓ (PND 22); F1 ♂ adults: body weights ↓ (10 weeks); absolute heart weights ↓ (10 weeks); absolute kidney weights ↑ (10 weeks); **500 mg/kg body weight: NOAEL fertility**; **500 mg/kg body weight**: F0 ♂ adults: body weights ↓; relative brain weights ↑; relative lung weights ↑; absolute and relative liver weights ↑; absolute kidney weights ↑; serum testosterone ↓; serum T3 and T4 ↓; F0 ♀ adults: absolute and relative ovarian weights ↓; serum prolactin ↑; serum T4 ↓; F1 ♂ pups: survival (PND 0–4) ↓; AGD (PND 14, 21) ↓; body weights ↓ (PND 22); absolute and relative testis weights ↓ (PND 22); absolute epididymis weights ↓ (PND 22); serum FSH ↓ (PND 22); number of spermatocytes in the seminiferous tubules ↓ (9/10; PND 22); F1 ♀ pups: survival (PND 0–4) ↓; body weights ↓ (PND 22); absolute ovarian weights ↓ (PND 22); relative uterine weights ↑ (PND 22);	Nagao et al. 2000; see also Section "Developmental toxicity"

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
		F1 ♂ adults: delayed preputial separation; absolute brain weights ↑ (10 weeks); relative liver weights ↑ (10 weeks); absolute spleen weights ↓ (10 weeks); absolute testis weights ↓ (10 weeks); absolute epididymis weights ↓ (10 weeks); absolute weights of the ventral prostate gland ↓ (10 weeks); relative thyroid weights ↑ (10 weeks); relative pituitary gland weights ↑ (10 weeks); serum testosterone ↓ (10 weeks); serum LH ↓ (10 weeks); serum T4 ↓ (10 weeks); atrophy of the seminiferous tubules (6/10; 10 weeks); number of germ cells in the seminiferous tubules ↓ (4/10; 10 weeks); interstitial oedema (4/10; 10 weeks); number of sperm in the lumen of the epididymis, bilateral and cell debris (5/10; 10 weeks); F0 adults: no effects on mortality, feed consumption, histology; F0 ♂ adults: no effects on sperm parameters; F0, F1 ♀ adults: no effects on oestrus cycle, gestation period, mating index, fertility index; F2 pups: no effects on survival, development	

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Crj:CD®(SD) IGS, groups of 24 ♂, 24 ♀	**two-generation study**, validation of a custom protocol for the recognition of non-steroidal chemicals with endocrine activity, gavage, 7 days/week, 0, 100, 200, 400 mg/kg body weight and day	**100 mg/kg body weight: NOAEL parental toxicity**; **100 mg/kg body weight and above**: F1 ♀ adults: AGD ↑ (no dose dependency); F2 ♂ adults: AGD ↓ (no dose dependency); **200 mg/kg body weight: NOAEL effects ♂ reproductive organs of F1 adults**; **200 mg/kg body weight and above**: F0 ♂ adults: absolute weights of left kidney ↑; F0 ♀ adults: absolute and relative liver weights ↑; absolute weights of left and right kidney ↑; F1 ♂ adults: relative liver weights ↑; relative thyroid weights ↑; absolute weights of left epididymis ↓; **400 mg/kg body weight: NOAEL fertility**; **400 mg/kg body weight**: F0 ♂ adults: relative liver weights ↑; relative weights of left kidney ↑; absolute weights of left epididymis ↓; F0 ♀ adults: relative weights of left and right kidney ↑; F1 ♂ adults: fraction of animals with preputial separation ↓; absolute epididymis weights ↓; small testes (6/24); soft testes (4/24, not statistically significant); epididymis: aplasia, hypoplasia, small epididymis (1/24, 4/24, 3/24; not statistically significant); testes: diffuse atrophy of the seminiferous tubules (9/24), Leydig cell hyperplasia (5/24); F1 ♀ adults: relative liver weights ↑; F1 ♂ pups: absolute and relative spleen weights ↓ (PND 25–27); F2 ♂ pups: absolute and relative spleen weights ↓ (PND 21); F0 adults: no changes in the mating index, fertility index, hormone analysis (LH, FSH, testosterone, oestradiol); F0 ♂ adults: no changes in the number of sperm in the testes, number of sperm in the cauda epididymis, motility and abnormality of the sperm in the epididymis; F0 ♀ adults: no changes in the oestrus cycle, gestation index, number of days necessary for copulation, gestation period, number of implantations, number of offspring	Aso et al. 2005; see also Section "Developmental toxicity"

AGD: anogenital distance; BBP: benzyl butyl phthalate; DBP: di-*n*-butyl phthalate; DEHP: di(2-ethylhexyl) phthalate; DEP: diethyl phthalate; DIDP: diisodecyl phthalate; DINP: diisononyl phthalate; DMP: dimethyl phthalate; DnOP: di-*n*-octyl phthalate; DUP: diundecyl phthalate; FSH: follicle-stimulating hormone; LH: luteinizing hormone; MBuP: monobutyl phthalate, MBeP: monobenzyl phthalate; MEHP: mono(2-ethylhexyl) phthalate; MEP: monoethyl phthalate; MMP: monomethyl phthalate; PA: phthalic acid; TSH: thyroid-stimulating hormone; T3: triiodothyronine; T4: thyroxine

The same research group observed that Sprague Dawley rats were more sensitive as regards testicular atrophy than Wistar rats. These histological changes were thus found in Sprague Dawley rats at the dose level of 480 mg/kg body weight and day and above but not until the dose of 1600 mg/kg body weight and day in Wistar rats. The corresponding NOAEL was 160 mg/kg body weight and day for Sprague Dawley rats and 480 mg/kg body weight and day for Wistar rats (EU 2007).

Testicular atrophy was observed in Sprague Dawley rats given gavage doses of 480 mg/kg body weight and day and above for 14 days. This effect was not induced at a benzyl butyl phthalate dose of 160 mg/kg body weight and day (EU 2007).

In a 14-day feeding study in F344 rats, a decrease in the absolute and relative weights of the testes and the seminal vesicles, atrophy of the testes, seminal vesicles and prostate gland, immature sperm in the tubular lumen and necrosis of the tubular epithelium in the caput epididymis were found after exposure to benzyl butyl phthalate doses of 1250 mg/kg body weight and day and above. A NOAEL of 625 mg/kg body weight and day was derived for effects on the male reproductive organs (EU 2007).

Testicular atrophy was observed in Cpb-WU rats given gavage doses of benzyl butyl phthalate of 970 mg/kg body weight and day and above for 28 days. The NOAEL for this effect was 750 mg/kg body weight and day. The testosterone concentration in plasma was reduced at doses of 450 mg/kg body weight and day and above, but not at the dose of 350 mg/kg body weight and day (EU 2007).

Testicular atrophy was observed in Sprague Dawley rats fed a diet containing benzyl butyl phthalate doses of 1500 mg/kg body weight and day and above for 28 days. The NOAEL for effects on the male reproductive organs was 1000 mg/kg body weight and day (Hammond et al. 1987).

In a 90-day feeding study in F344 rats, testicular degeneration (no other details) was observed at the dose of 1875 mg/kg body weight and day (Hammond et al. 1987; NTP 1982).

In a 2-year feeding study, decreased relative epididymis weights were found with a simultaneous decrease in body weights in the interim examination after 15 months at doses of 240 mg/kg body weight and day and above. The absolute weights remained unchanged and no unusual histological findings were reported (NTP 1997 a).

In a comparative study in Sprague Dawley rats given gavage doses of 500 mg/kg body weight and day, the lowest decrease in the epididymal sperm concentration was observed with benzyl butyl phthalate compared with DEHP, di-*n*-butyl phthalate, diethyl phthalate, diisodecyl phthalate, diisononyl phthalate, dimethyl phthalate, di-*n*-octyl phthalate and diundecyl phthalate (Kwack et al. 2009).

Two drinking water studies in rats reported contradictory findings with respect to sperm production; each of the studies tested only one dose (Ashby et al. 1997; Sharpe et al. 1995). According to the NTP, one study (Sharpe et al. 1995) found unexplained fluctuations in the testis weights of control animals (NTP-CERHR 2003). Neither study is included in the evaluation.

In a 2-year feeding study in B6C3F1 mice, no substance-induced histological findings were observed in male and female reproductive organs up to the highest dose tested of 1800 mg/kg body weight and day (NTP 1982).

Fertility studies

In a modified mating protocol with dietary administration of benzyl butyl phthalate for 10 weeks, a dose of 2200 mg/kg body weight and day caused germ cell degeneration in all male F344 rats and a reduced fertility index. Epididymal sperm concentrations were reduced at 200 mg/kg body weight and day and above. The NOAEL of benzyl butyl phthalate for fertility was 200 mg/kg body weight and day (NTP 1997 a). It was noted in the EU Risk Assessment Report that the interval between mating and the counting of the sperm varied (EU 2007). In addition, an automated system was not used to analyse the sperm and there may have been preparation problems such as those encountered in the 26-week study. For this reason, the determination of the sperm concentration in the epididymis is not considered valid.

In a 26-week feeding study, F344/N rats were given doses of benzyl butyl phthalate of 0, 300, 900, 2800, 8300, 25 000 mg/kg feed (0, 30, 60, 180, 550, 1650 mg/kg body weight and day). The absolute weights of the epididymis, cauda epididymis and testes and the sperm concentrations were reduced at the highest dose tested of 1650 mg/kg body weight and day. In addition, hypospermia and atrophy of the seminiferous tubules were observed at this dose. The NOAEL for effects on the male reproductive organs and for fertility was 550 mg/kg body weight and day (NTP 1997 a). The EU Risk Assessment Report noted that problems had occurred while mincing the epididymis (EU 2007), which means that the reduced sperm concentration is questionable.

Benzyl butyl phthalate was used as a test substance for the validation of OECD Test Guideline 421 in rats. The body weights of the offspring were reduced on postnatal day 1 at a dose of 500 mg/kg body weight and day; this effect was reversible. An increase in post-implantation losses, fewer surviving offspring and effects on the reproductive organs of males were observed at a dose of 1000 mg/kg body weight and day. The NOAEL for effects on the male reproductive organs, fertility and foetotoxicity was 500 mg/kg body weight and day (Piersma et al. 1995; see also Section "Developmental toxicity").

In 2 drinking water studies in rats, neither effects on fertility nor foetotoxic effects were observed up to a dose of 0.385 mg/kg body weight and day and up to 0.80 mg/kg body weight and day, respectively; the original studies are not available (NTP-CERHR 2003).

Generation studies

In a 1-generation study carried out according to OECD Test Guideline 415 in Wistar rats with administration via the diet, no effects on reproductive behaviour and development were observed up to the highest dose tested of 418/446 mg/kg body weight and day (♂/♀); the original study is not available. The NOAEL for fertility and foetotoxicity was thus greater than 418/446 mg/kg body weight and day. At this dose, the body weights and body weight gains of the dams were decreased and the absolute and relative liver weights were increased. The NOAEL for parental toxicity was 206/217 mg/kg body weight and day (EU 2007).

In a 2-generation study in Sprague Dawley rats with administration via the diet, mating and fertility indices were found to be decreased in the F1 generation at 750 mg/kg body weight and day. At this dose, also the absolute and relative liver

weights were increased in the adult animals of the F0 and F1 generations; these effects were accompanied by histological changes. Reduced birth weights per litter and delayed onset of puberty in male and female F1 pups were observed at this dose. In addition, nipple and areolae retention were found in male F1 pups at this dose. At 750 and 250 mg/kg body weight and day the anogenital distance (AGD) in male F1 and F2 pups was shortened; the decrease in length was statistically significant. However, the AGD in F2 offspring was still within the range of the historical controls of the laboratory at 250 mg/kg body weight and day. It is questionable whether the finding at 250 mg/kg body weight and day is biologically relevant for F1 and F2 offspring; although a decreased AGD is an indicator of an antiandrogenic effect, without further accompanying toxic effects on the development, structure or function of the reproductive organs, this effect alone is not sufficient to be considered adverse. The absolute and relative kidney weights were increased in female F0 adults at doses of 250 mg/kg body weight and day and above; the NOAEL was thus 50 mg/kg body weight and day for parental toxicity in females and 250 mg/kg body weight and day for parental toxicity in males. The dose of 250 mg/kg body weight and day was the NOAEL for fertility and foetotoxicity (Tyl et al. 2004; see also Section "Developmental toxicity"). The US EPA considers the AGD to be a very sensitive parameter. A shortened AGD in males, with no effects on the female AGD, is an indicator of an antiandrogenic effect. An increased AGD in females, with no effects on males, is an indicator of an androgenic effect. The biological significance of the AGD and the relevance of changes to this parameter are unknown (US EPA 2005).

In a 2-generation study in Crj:CD(SD)IGS rats given gavage doses of benzyl butyl phthalate, no effects on the oestrous cycle, gestation period and mating and fertility indices were found in F0 and F1 adult animals up to the highest dose tested of 500 mg/kg body weight. A decrease in birth weights was observed in F1 pups at doses of 100 mg/kg body weight and day and above; however, this effect was reversible. The kidney weights were increased by about 7% without dose dependency at doses of 100 mg/kg body weight and day and above; no unusual histological findings were reported. In addition, salivation and increased serum concentrations of luteinizing hormone (LH) were found at this dose. The NOAEL for fertility was 500 mg/kg body weight and day, the highest dose tested. The NOAEL for foetotoxicity was 100 mg/kg body weight and day (Nagao et al. 2000; see also Section "Developmental toxicity"). The effects induced at the lowest dose tested of 100 mg/kg body weight and day were assessed as follows: the increased salivation was not observed in any other study and was probably caused by the gavage administration. Increased serum concentrations of follicle-stimulating hormone in males are not considered adverse if they are not accompanied by other effects on fertility or reproductive behaviour. The increase in kidney weights observed in females at doses of 100 mg/kg body weight and day and above was not dependent upon the dose. The NOAEL for parental toxicity was therefore in the range of 100 mg/kg body weight and day.

A 2-generation study in Crj:CD®(SD)IGS rats used benzyl butyl phthalate as a test substance to validate a protocol for the recognition of the endocrine activity of non-steroidal chemicals. No effects on fertility were observed up to the highest dose tested of 400 mg/kg body weight and day. Therefore, in this study the NOAEL for fertility was 400 mg/kg body weight and day, but may also be higher. At this dose, the effects induced in the reproductive organs of male F1 adults were aplasia and

hyperplasia of the testes, atrophy of the seminiferous tubules and Leydig cell hyperplasia. For this reason, the NOAEL for effects on the male reproductive organs was 200 mg/kg body weight and day. The NOAEL for parental toxicity was 100 mg/kg body weight and day because liver and kidney weights were increased at doses of 200 mg/kg body weight and day and above. The relative anogenital distance in female F1 pups and male F2 pups was increased at doses of 100 mg/kg body weight and day and above (Aso et al. 2005; see also Section "Developmental toxicity"). These changes are not plausible because the effects were not observed in the same sex in the two other generations.

5.5.2 Developmental toxicity

A large number of studies have investigated the developmental toxicity of benzyl butyl phthalate and its metabolites MBeP and MBuP; the data from the studies are shown in Table 5.

Prenatal exposure

A review of developmental toxicity studies similar to OECD Test Guideline 414 with exposure during organogenesis yielded NOAELs for developmental toxicity in rats of 420 mg/kg body weight and day (Ema et al. 1992 a) and 500 mg/kg body weight and day (NTP 1989). In Sprague Dawley rats, skeletal and visceral variations were induced concurrently with maternal toxicity at doses of 1100 mg/kg body weight and day and above and an increased number of resorptions, a reduced number of living foetuses per litter and teratogenicity were observed at 1640 mg/kg body weight and day (NTP 1989). In Wistar rats, an increase in post-implantation losses and teratogenic effects were found concurrently with maternal toxicity at doses of 750 mg/kg body weight and day and above (Ema et al. 1992 a). External malformations such as anophthalmia and cleft palates, skeletal malformations such as fused vertebrae, ribs or sternebrae (sternum) and visceral malformations such as a lack of kidneys were observed (Ema et al. 1992 a; NTP 1989).

In Wistar rats, prolonging exposure to gestation days 0 to 20 led to a decreased number of living foetuses per litter at doses of 375 mg/kg body weight and day and above; the decrease was not dose-dependent. The complete resorption of litters was observed in all dams at the highest dose tested of 974 mg/kg body weight and day. The adjusted maternal body weight gains (excluding the gravid uterus) were decreased at doses of 654 mg/kg body weight and above. The authors derived a NOAEL for embryo/foetotoxic effects of 654 mg/kg body weight and day and a NOAEL for maternal toxicity of 375 mg/kg body weight and day. There was no effect on pre-implantation losses, the number of corpora lutea per litter and the number of implantations per litter up to 654 mg/kg body weight. Teratogenic effects were not found up to this dose (Ema et al. 1990). The animals were treated up to the end of gestation, that is, up to birth. Occupational safety laws prohibit women from working at the workplace during the third trimester. For this reason, animal studies with exposure up to the day of birth are not considered relevant for the assessment of chemical substances at the workplace.

Table 5 Studies with prenatal and postnatal exposure to benzyl butyl phthalate or its metabolites

Species	Exposure	Findings	References
prenatal treatment with benzyl butyl phthalate			
rat, Sprague Dawley, 30 ♀	**GD 6–15**, 0%, 0.5%, 1.15%, 2.0% in the diet, 0, 420, 1100, 1640 mg/kg body weight and day, examination: GD 20	**420 mg/kg body weight: NOAEL for developmental and maternal toxicity**; **1100 mg/kg body weight and above**: dams: body weights and body weight gains ↓, absolute feed consumption ↓, relative feed consumption (related to body weight) ↑, relative liver weights ↑ (no histological changes), relative water consumption ↑; foetuses: skeletal and visceral variations ↑ (misaligned sternebrae, rudimentary ribs; dilated ureter); **1640 mg/kg body weight**: dams: relative kidney weights ↑ (no histological examination), ataxia, gait changes; foetuses: resorptions ↑, number of living foetuses ↓, body weights ↓, external, visceral and skeletal variations and malformations ↑ (hydroureter, hydronephrosis, missing kidneys, anophthalmia, fused or misaligned vertebrae, fused ribs)	NTP 1989
rat, Wistar, 10 ♀	**GD 7–15**, 0, 500, 750, 1000 mg/kg body weight and day, gavage, vehicle: olive oil, examination: GD 20	**500 mg/kg body weight: NOAEL for developmental and maternal toxicity**; **500 mg/kg body weight and above**: dams: feed consumption ↓; **750 mg/kg body weight and above**: dams: body weight gains ↓, 3 animals: complete resorption of litters; foetuses: number of resorptions and dead foetuses ↑, post-implantation losses ↑, body weights ↓, malformations ↑ (cleft palates, fused sternebrae) and variations ↑ (dilated renal pelvis); **1000 mg/kg body weight**: dams: 4 animals died, 6 animals: complete resorption of litters, adjusted body weight gains (without gravid uterus) ↓	Ema et al. 1992 a
rat, Wistar, 15–19 ♀	**GD 0–20**, 0%, 0.25%, 0.5%, 1.0%, 2.0% in the diet, 0, 185, 375, 654, 974 mg/kg body weight and day, examination: GD 20	**375 mg/kg body weight: NOAEL for developmental and maternal toxicity**; **375 mg/kg body weight and above**: foetuses: number of living foetuses/litter ↓ (185, 375, 654 mg/kg body weight: 13.4 ± 1.5, 11.3 ± 3.8, 12.3 ± 1.4, control group: 13.9 ± 1.6; not statistically significant at 654 mg/kg body weight, no dose dependency); **654 mg/kg body weight and above**: dams: feed consumption ↓, adjusted body weight gains ↓ (without gravid uterus); foetuses: body weights ↓ (♂: 6.7%; ♀: 6.8%); **974 mg/kg body weight**: dams: complete resorption of litters by all animals; no substance-induced pre-implantation losses, number of corpora lutea/litter and number of implantations/litter unchanged, no teratogenic effects exposure up to day of birth: not relevant for workplace exposure	Ema et al. 1990

Table 5 (continued)

Species	Exposure	Findings	References
rat, Harlan Cpb-WU, 10 ♀	**GD 6–15, GD 6–20**, 0, 270, 350, 450, 580, 750, 970, 1250, 1600, 2100 mg/kg body weight and day, gavage, vehicle: corn oil, examination: GD 21	**GD 6–15**: **< 270 mg/kg body weight: NOAEL maternal toxicity**; **270 mg/kg body weight and above**: dams: extramedullary haematopoiesis; foetuses: incidence of additional (13th) lumbar rib ↑ (rat strain with a high spontaneous incidence of lumbar ribs); **350 mg/kg body weight: NOAEL developmental toxicity**; **450 mg/kg body weight and above**: dams: number of resorptions ↑; **580 mg/kg body weight and above**: dams: relative liver and kidney weights ↑; foetuses: body weights ↓; **750 mg/kg body weight and above**: dams: ASAT ↑; **1600 mg/kg body weight and above**: dams: mortality ↑ (several animals sacrificed in extremis), body weights ↓ **GD 6–20**: slightly more severe effects, except: number of resorptions: no difference between GD 6–15 and GD 6–20; **580 mg/kg body weight and above**: foetuses: incidence of delayed testicular descent ↑	Piersma et al. 2000
rat, Sprague Dawley, 10 ♀	**GD 10–21**, 0, 120, 500 mg/kg body weight and day, gavage, vehicle: sesame oil, examination of ♀ offspring: PND 21, 35, 50, 100; ♂ not examined	**120 mg/kg body weight: NOAEL for delayed puberty**; **120 mg/kg body weight and above**: ♀ offspring: mammary glands: dose-dependent changes in the expression of genes that are related to immune function, cell signals, proliferation, differentiation, metabolism; **500 mg/kg body weight**: ♀ offspring: delayed vaginal opening (that is, delayed onset of puberty), TEB fraction on PND 35 ↑ (TEB: epithelial structures least differentiated, highly proliferative, susceptible to malignant degeneration), fraction of proliferative cells in Lob1 on PND 100 ↑ (about 12%, control: about 5%); no data for maternal toxicity; exposure up to day of birth: not relevant for workplace exposure	Moral et al. 2011

Table 5 (continued)

Species	Exposure	Findings	References
rat, albino, no other data, at least 6 ♀	**GD 14–birth**, 0, 4, 20, 100 mg/kg body weight and day, gavage, vehicle: corn oil, positive control: 6 µg DES/kg body weight and day, examination only ♂ offspring: PND 0, PND 75	**4 mg/kg body weight and above**: dams: length of gestation ↑; offspring: body weights on PND 0 and 21 ↓ (PND 0: 3.3%; PND 21: 12.7%); **20 mg/kg body weight and above**: dams: body weight gains (GD 21) ↓; **100 mg/kg body weight**: offspring: absolute epididymis, prostate gland and kidney weights ↓ (caused by reduced body weights), number of sperm ↓ (9.7%), percentage of motile sperm ↓ (6.0%), percentage of sperm abnormalities ↑ (53.6%); no noticeable changes: litter size, number of living offspring, foetal mortality, sex ratio, developmental milestones; reduced body weights of offspring 21 days after end of exposure not substance-induced as no accumulation of test substance; increase in percentage of sperm abnormalities questionable: no data for the number of investigated animals, age of animals only 11 weeks (PND 75) instead of 13–14 weeks as specified by OECD Test Guidelines 443 and 416; study therefore not suitable for assessment of developmental toxicity	Ahmad et al. 2014
rat, Wistar, 13–15 ♀	**GD 0–20, GD 0–11 or GD 11–20**, 0%, 2.0% in the feed, 0, 974 mg/kg body weight and day (Ema et al. 1991), examination: GD 20, pair-fed control groups	**974 mg/kg body weight**: GD 0–20: dams: complete resorption of litters by all animals, body weight gains with or without adjustment for gravid uterus ↓, feed consumption ↓; pair-fed control group: body weight gains as in BBP group without substance-induced malformations or resorptions: conclusion: increased number of resorptions not caused by reduced feed consumption, but induced by substance; GD 0–11: dams: complete resorption of litters by all animals; GD 11–20: dams: no post-implantation losses, foetuses: malformations ↑ (cleft palates, fused sternebrae)	Ema et al. 1991, 1992 b
rat, Wistar, 11–12 ♀	**GD 0–20, GD 0–7, GD 7–16 or GD 16–20**, 0%, 2.0% in the feed, 0, 974 mg/kg body weight and day (Ema et al. 1991), examination: GD 20	**974 mg/kg body weight**: dams of all groups: body weight gains and feed consumption ↓; GD 0–20: dams: complete resorption of litters by all animals, GD 0–7: dams: post-implantation losses ↑; GD 7–16: dams: post-implantation losses ↑; GD 16–20: dams: no increase in post-implantation losses, malformations ↑ (cleft palates, fused sternebrae)	Ema et al. 1992 c

Table 5 (continued)

Species	Exposure	Findings	References
rat, Wistar, 10 ♀	**GD 7–9, GD 10–12 or GD 13–15**, 0, 600, 750, 1000 mg/kg body weight and day, gavage, vehicle: olive oil, examination: GD 20	**GD 7–9**: **750 mg/kg body weight and above**: dams: number of resorptions and dead foetuses/litter ↑, post-implantation losses ↑, number of living foetuses/litter ↓; foetuses: body weights ↓, skeletal malformations ↑ (fused or missing cervical vertebral arches, fused or missing thoracic vertebral arches and bodies); **GD 10–12**: **750 mg/kg body weight and above**: dams: number of resorptions and dead foetuses/litter ↑, post-implantation losses ↑; **1000 mg/kg body weight**: dams: number of living foetuses/litter ↓; foetuses: body weights ↓, changes in sex ratio (fewer ♀); **GD 13–15**: **750 mg/kg body weight and above**: dams: number of resorptions and dead foetuses/litter ↑, post-implantation losses ↑, number of living foetuses/litter ↓; foetuses: skeletal malformations ↑ (fused or missing cervical vertebral arches), external malformations ↑ (cleft palates)	Ema et al. 1993
rat, Wistar, 6 ♀	**GD 0–7, GD 0–9 or GD 0–11**, 0%, 2.0% in the feed, 0, 974 mg/kg body weight and day (Ema et al. 1991), examination: GD 20, pair-fed control groups	**974 mg/kg body weight**: dams of all groups: body weight gains and feed consumption ↓, highest ↑ in post-implantation losses on GD 0–11, absolute uterine and ovarian weights ↓, plasma progesterone GD 7, 9, 11 ↓; no changes in number of corpora lutea, implantations; conclusion of authors: post-implantation losses could be caused by a decrease in the progesterone concentration in plasma, that is, a disruption in luteal function	Ema et al. 1994
rat, Wistar, 11–13 ♀	**GD 7–9, GD 10–12 or GD 13–15**, 0, 750, 1000, 1250 mg/kg body weight and day, BBP or DBP, gavage, vehicle: olive oil, examination: GD 20	**750 mg/kg body weight and above**: dams: post-implantation losses ↑; GD 7–9: foetuses: skeletal malformations ↑ (vertebral column and ribs); GD 10–12: foetuses: no malformations up to 1250 mg/kg body weight; GD 13–15: foetuses: external and skeletal malformations ↑ (cleft palates, fused sternebrae); BBP and DBP: similar dependency on treatment days and similar spectrum of malformations; the authors therefore suggest that both substances may use the same mechanism of action, possibly via a mutual metabolite	Ema et al. 1995 a

Table 5 (continued)

Species	Exposure	Findings	References
rat, Wistar, 10–14 ♀	**GD 0–8**, 0, 250, 500, 750, 1000 mg/kg body weight and day, gavage, vehicle: olive oil, examination: GD 20, group with pseudo-pregnant rats: mated with vasectomized ♂	**250 mg/kg body weight and above**: dams: body weight gains ↓, feed consumption ↓; **500 mg/kg body weight and above**: foetuses: body weights ↓; **750 mg/kg body weight and above**: dams: post-implantation losses ↑, **1000 mg/kg body weight**: dams: percentage of pregnant dams ↓, number of implantations ↓, pre-implantation losses ↑, foetuses: changes in sex ratio (fewer ♂) dams: no changes in number of corpora lutea; **pseudo-pregnant rats**: **500 mg/kg body weight and above**: dams: serum: progesterone concentration ↓, **750 mg/kg body weight and above**: dams: absolute uterine weights ↓ (indicator for reduced responsiveness of decidualization), absolute ovarian weights ↓, dams: no changes in number of corpora lutea, indirect sign, but not evidence, of disruption of decidualization	Ema et al. 1998
rat, Wistar, 10–14 ♀	**only on individual days from GD 6–16**, GD 13–15 also 1000 mg/kg body weight and day, 0, 1500 mg/kg body weight and day, gavage, vehicle: olive oil, examination: GD 20	**1000 mg/kg body weight and above**: GD 13, 14, 15: dams: body weight gains transiently ↓; **1500 mg/kg body weight**: GD 6, 7, 8, 9, 10, 11, 12, 16: dams: body weight gains transiently ↓; GD 6, 9, 10, 11, 13, 14, 15, 16: dams: post-implantation losses ↑; GD 9, 10, 13, 14, 15, 16: dams: number of living foetuses per litter ↓; GD 6, 7, 9, 15: foetuses: malformations ↑; GD 7: foetuses: malformations of the cervical vertebrae, dilation of renal pelvis (variation); GD 15: foetuses: cleft palates and fused sternebrae; no unusual findings with respect to: number of corpora lutea, number of implantations	Ema et al. 1999

Table 5 (continued)

Species	Exposure	Findings	References
rat, Wistar, 16 ♀	**GD 15–17**, 0, 250, 500, 1000 mg/kg body weight and day, gavage, vehicle: olive oil, examination: GD 21	**500 mg/kg body weight and above**: dams: body weight gains transiently ↓; foetuses: ♂: absolute and relative (in relation to the cube root of body weight) AGD ↓ (not in ♀, sign of antiandrogenic effect), incidence of undescended testes ↑; **1000 mg/kg body weight**: dams: number of living foetuses per litter ↓, foetuses: body weights ↓; no unusual findings with respect to: number of corpora lutea, number of implantations, number of resorptions	Ema and Miyawaki 2002
rat, Sprague Dawley, 7–13 ♀	**GD 10**, 0, 562, 1125, 1687 mg/kg body weight and day, gavage, vehicle: olive oil, examination: GD 21	**1125 mg/kg body weight and above**: foetuses: incidence of malformations ↑ (exencephaly); **1687 mg/kg body weight**: dams: 1 death; no unusual findings with respect to: body weights, body weight gains, percentage of post-implantation losses/litter, number of living foetuses/litter ↓, foetal body weights	Saillenfait et al. 2003
mouse, CD1-Swiss, 28–30 ♀, 14 in high dose group	**GD 6–15**, 0%, 0.1%, 0.5%, 1.25%, 2% in the diet, 0, 182, 910, 2330, 4121 mg/kg body weight and day, examination: GD 17	**182 mg/kg body weight**: **NOAEL for developmental and maternal toxicity**; **910 mg/kg body weight and above**: dams: body weight gains ↓, resorptions ↑, number of late foetal deaths ↑, number of living foetuses/litter ↓; foetuses: malformations ↑ (exencephaly, short tail, cardiovascular defects, fused ribs, abnormal or fused sternebrae and vertebrae); **2330 mg/kg body weight and above**: dams: relative drinking water consumption ↑, relative liver and kidney weights ↑ (no histological changes in liver and kidneys); foetuses: body weights ↓, variations ↑	NTP 1990
mouse, OF1, 15–23 ♀	**GD 8**, 0, 281, 562, 1125, 1687 mg/kg body weight and day, gavage, vehicle: olive oil, examination: GD 18	**562 mg/kg body weight and above**: dams: percentage of post-implantation losses/litter ↑, foetuses: incidence of malformations ↑ (exencephaly ↑); **1125 mg/kg body weight and above**: dams: body weight gains ↓, number of living foetuses/litter ↓; foetuses: anal atresia ↑, tail missing or atrophied ↑; **1687 mg/kg body weight**: dams: mortality ↑; foetuses: body weights ↓	Saillenfait et al. 2003

Table 5 (continued)

Species	Exposure	Findings	References
rabbit, New Zealand White, 17 ♀	**GD 6–18**, 0, 3, 10 mg/kg body weight and day, gelatine capsules, examination: GD 29	**10 mg/kg body weight: NOAEL for developmental and maternal toxicity**, no unusual findings with respect to: foetal body weights, 24-hour survival, external, skeletal and visceral malformations, no maternal toxicity, not included in the assessment as the original study is not available	NTP-CERHR 2003
prenatal treatment with the metabolites of benzyl butyl phthalate			
rat, Wistar, 11–15 ♀	**MBuP**, **GD 7–15**, 0, 250, 500, 625 mg/kg body weight and day, gavage, examination: GD 20	**250 mg/kg body weight: NOAEL for developmental and maternal toxicity**; **500 mg/kg body weight and above**: dams: body weight gains ↓, feed consumption ↓, post-implantation losses/litter ↑, number of living foetuses/litter ↓; foetuses: body weights ↓, malformations ↑ (cleft palates, fused or missing cervical vertebral arches), variations ↑ (dilated renal pelvis)	Ema et al. 1995 b
rat, Wistar, 10–15 ♀	**MBuP**, **GD 7–9, GD 10–12, GD 13–15**, 0, 500, 625, 750 mg/kg body weight and day, gavage, examination: GD 20	**GD 7–9**: **500 mg/kg body weight and above**: dams: feed consumption ↓; foetuses: body weights ↓, skeletal malformations ↑ (primarily fused or missing cervical vertebral arches); **625 mg/kg body weight and above**: dams: body weight gains ↓, post-implantation losses/litter ↑; foetuses: external malformations ↑; **GD 10–12**: **625 mg/kg body weight and above**: dams: body weight gains ↓, feed consumption ↓, post-implantation losses/litter ↑; no teratogenicity up to 750 mg/kg body weight; foetuses: body weights ↓; **GD 13–15**: **500 mg/kg body weight and above**: dams: body weight gains ↓, feed consumption ↓, post-implantation losses/litter ↑; **625 mg/kg body weight and above**: foetuses: external and skeletal malformations ↑ (primarily cleft palates and fused sternebrae); findings similar to those for BBP and DBP; MBuP or its metabolites thus contribute to embryonic mortality and teratogenicity	Ema et al. 1996 a

Table 5 (continued)

Species	Exposure	Findings	References
rat, Wistar King A, 15 ♀	**MBuP,** **GD 7–10, GD 11–14, GD 15–18,** 0 (GD 7–18), 300 mg/kg body weight and day, gavage, examination: GD 20	**GD 15–18**: maximum inhibition of testicular descent, elongated gubernaculum and hypertrophic cranial suspensory ligament; all animals treated with **MBuP**: epididymis with few small ducti deferentia, testicular testosterone content ↓	Shono et al. 2000
rat, Sprague Dawley, 7–13 ♀	**MBuP or MBeP**, **GD 10**, **MBuP**: 0, 400, 800, 1200 mg/kg body weight and day, **MBeP**: 0, 231, 461, 923, 1684 mg/kg body weight and day, gavage, vehicle: olive oil, examination: GD 21	**MBuP**: no unusual findings in dams; **MBeP**: **923 mg/kg body weight and above**: dams: 1 death; **1684 mg/kg body weight**: mortality ↑; overall, no unusual findings with respect to: body weights, body weight gains, percentage of post-implantation losses/litter, number of living foetuses/litter, foetal body weights, malformations	Saillenfait et al. 2003
mouse, OF1, 15–23 ♀	**MBuP or MBeP**, **GD 8**, **MBuP**: 0, 400, 800, 1200 mg/kg body weight and day, **MBeP**: 0, 231, 461, 923, 1684 mg/kg body weight and day, gavage, vehicle: olive oil, examination: GD 18	**MBuP**: **400 mg/kg body weight and above**: dams: body weight gains ↓, percentage of post-implantation losses/litter ↑, number of living foetuses/litter ↓; foetuses: incidence of malformations ↑ (exencephaly ↑); **800 mg/kg body weight and above**: foetuses: anal atresia ↑, tail missing or atrophied ↑; **1200 mg/kg body weight**: dams: mortality ↑; foetuses: body weights ↓; **MBeP**: **923 mg/kg body weight and above**: dams: mortality ↑; foetuses: incidence of malformations ↑ (exencephaly ↑); **1684 mg/kg body weight**: dams: body weight gains ↓, percentage of post-implantation losses/litter ↑, number of living foetuses/litter ↓; foetuses: anal atresia ↑, tail missing or atrophied ↑	Saillenfait et al. 2003

Table 5 (continued)

Species	Exposure	Findings	References
rat, Wistar, 10–14 ♀	**MBeP**, **GD 7–15**, 0, 250, 313, 375, 438, 500 mg/kg body weight and day, gavage, examination: GD 20	**250 mg/kg body weight: NOAEL for developmental and maternal toxicity**; **250 mg/kg body weight and above**: dams: feed consumption ↓; **313 mg/kg body weight and above**: dams: body weight gains ↓; foetuses: skeletal malformations ↑ (most frequently effects on the cervical or thoracic vertebrae, ribs); **375 mg/kg body weight and above**: foetuses: body weights ↓, visceral malformations ↑ (most frequently effects on the kidneys: dilated renal pelvis, hypoplasia); **438 mg/kg body weight and above**: dams: post-implantation losses/litter ↑; foetuses: external malformations ↑; **500 mg/kg body weight**: dams: number of living foetuses/litter ↓	Ema et al. 1996 b
rat, Wistar, 10–15 ♀	**MBeP**, **GD 7–9, GD 10–12 or GD 13–15**, GD 7–9: 0, 375, 500, 625 mg/kg body weight and day, GD 10–12 and GD 13–15: 0, 250, 375, 500, 625 mg/kg body weight and day, gavage, examination: GD 20	**GD 7–9**: **375 mg/kg body weight and above**: dams: body weight gains ↓, feed consumption ↓; foetuses: body weights ↓, skeletal malformations ↑ (primarily fused or missing cervical vertebral arches); **500 mg/kg body weight and above**: dams: number of resorptions and dead foetuses ↑, post-implantation losses ↑; foetuses: visceral variations ↑ (dilated renal pelvis); **625 mg/kg body weight**: dams: number of living foetuses ↓; **GD 10–12**: **250 mg/kg body weight and above**: dams: body weight gains ↓, feed consumption ↓; **500 mg/kg body weight and above**: dams: number of resorptions and dead foetuses ↑, post-implantation losses ↑, number of living foetuses ↓; foetuses: body weights ↓; **GD 13–15**: **250 mg/kg body weight and above**: dams: body weight gains ↓, feed consumption ↓; **500 mg/kg body weight and above**: dams: number of resorptions and dead foetuses ↑, post-implantation losses ↑, number of living foetuses ↓; **625 mg/kg body weight and above**: foetuses: external and skeletal malformations ↑ (primarily cleft palates and fused sternebrae)	Ema et al. 1996 c

Table 5 (continued)

Species	Exposure	Findings	References
rat, Wistar, 16 ♀	**MBeP**, **GD 15–17**, 0, 167, 250, 375 mg/kg body weight and day, gavage, examination: GD 20	**167 mg/kg body weight and above**: dams: feed consumption and body weight gains ↓; **250 mg/kg body weight and above**: foetuses: ♂: absolute and relative (in relation to the cube root of body weight) AGD ↓ (not in ♀); **375 mg/kg body weight**: foetuses: body weights ↓	Ema et al. 2003
rat, Wistar King A, 3 ♀	**MBuP**, **GD 15–18**, 0, 300 mg/animal, 0, 1000 mg/kg body weight and day (NTP-CERHR 2003), gavage, vehicle: sesame oil, examination: GD 20, PND 30–40	**1000 mg/kg body weight**: foetuses or offspring: ♂: cryptorchidism GD 20 and PND 30–40	Imajima et al. 1997
prenatal and postnatal treatment			
rat, RIVM WU, 10 ♀	**14 days before and during mating, up to PND 6**, OECD Test Guideline 421 (BBP test substance for validation of test guideline), gavage, 0, 250, 500, 1000 mg/kg body weight and day	**500 mg/kg body weight: NOAEL foetotoxicity and parental toxicity**; **500 mg/kg body weight and above**: dams: feed consumption ↑ (during pregnancy); offspring: body weights ↓ (PND 1, returned to normal on PND 6); **1000 mg/kg body weight**: dams: body weight gains ↓, post-implantation losses ↑, number of living offspring ↓ (PND 2, PND 6), body weights ↓ (PND 6)	Piersma et al. 1995 see also Section "Fertility"

Table 5 (continued)

Species	Exposure	Findings	References
rat, CD (SD), groups of 30 ♂, 30 ♀	**two-generation study**, F0: treatment from 10 weeks before mating until weaning (PND 21); F1: treatment after weaning (from PND 21) for 10 weeks; diet, 0, 750, 3750, 11 250 mg/kg feed (0, 50, 250, 750 mg/kg body weight and day)	**50 mg/kg body weight: NOAEL ♀ parental toxicity**; **250 mg/kg body weight: NOAEL ♂ parental toxicity and foetotoxicity**; **250 mg/kg body weight and above**: F0 ♂ adults: absolute kidney weights ↑ (250 mg/kg body weight: 7%, 750 mg/kg body weight: 8%, no unusual histological findings, relative weights not ↑); F0 ♀ adults: relative kidney weights ↑ (250 mg/kg body weight: 6%, 750 mg/kg body weight: 9%, no unusual histological findings, absolute kidney weights ↑); **750 mg/kg body weight**: F0 ♂ adults: body weights and body weight gains ↓; absolute and relative liver weights ↑; relative kidney weights ↑; liver: minimal to slight diffuse cytoplasmic granules, number of peroxisomes ↑; F0 ♀ adults: body weights and body weight gains ↓; absolute and relative liver weights ↑; absolute and relative ovarian weights ↓; absolute and relative uterine weights ↓; histological changes in liver (as described above); F1 ♂ pups: body weights/litter ↓ (PND 0); F1 ♀ pups: body weights/litter ↓ (PND 0)	Tyl et al. 2004 see also Section "Fertility"
rat, Crj:CD(SD) IGS, groups of 35 ♂, 35 ♀	**two-generation study**, F0: treatment from 12 weeks before mating up to necropsy (age: 23 weeks); F1: treatment after weaning (from PND 22) up to necropsy (age: 10 weeks); gavage, in corn oil, 7 days/week, 0, 20, 100, 500 mg/kg body weight and day	**100 mg/kg body weight: NOAEL foetotoxicity and parental toxicity**; **100 mg/kg body weight and above**: F0 ♀ adults: salivation; absolute and relative kidney weights ↑ (absolute: 100 mg/kg body weight: 7%, 500 mg/kg body weight: 7%; relative: 100 mg/kg body weight: 8%, 500 mg/kg body weight: 6%; no dose dependency, no unusual histological findings); F1 ♂ pups: body weights ↓ (PND 0, 6%, at 500 mg/kg body weight: 7%, effects reversible after 4 days); TSH ↓ (PND 22); F1 ♀ pups: body weights ↓ (PND 0, 6%, at 500 mg/kg body weight: 6%, effects reversible after 4 days); **500 mg/kg body weight**: F0 ♂ adults: body weights ↓; relative brain and lung weights ↑; absolute and relative liver weights ↑; absolute kidney weights ↑; serum testosterone ↓; serum T3 and T4 ↓; F0 ♀ adults: absolute and relative ovarian weights ↓; serum prolactin ↑; serum T4 ↓; F1 pups: survival (PND 0–4) ↓	Nagao et al. 2000 see also Section "Fertility"

Table 5 (continued)

Species	Exposure	Findings	References
rat, Crj:CD (SD) IGS, groups of 24 ♂, ♀	**two-generation study**, validation of a custom protocol for the recognition of non-steroidal chemicals with endocrine activity, gavage, 7 days/weeks, 0, 100, 200, 400 mg/kg body weight and day	**100 mg/kg body weight: NOAEL parental toxicity**; **200 mg/kg body weight: NOAEL effects ♂ reproductive organs of F1 adults**; **200 mg/kg body weight and above**: F0 ♂ adults: absolute weights of left kidney ↑; F0 ♀ adults: absolute and relative liver weights ↑; absolute weights of left and right kidney ↑; **400 mg/kg body weight**: F1 ♂ adults: fraction of animals with preputial separation ↓; absolute weights of right epididymis ↓; small testes (6/24); soft testes (4/24, not statistically significant); epididymis: aplasia, hypoplasia, small epididymis (1/24, 4/24, 3/24; not statistically significant); testes: diffuse atrophy of the seminiferous tubules (9/24), Leydig cell hyperplasia (5/24)	Aso et al. 2005 see also Section "Fertility"

AGD: anogenital distance; ASAT: aspartate aminotransferase; BBP: benzyl butyl phthalate; DBP: di-*n*-butyl phthalate; DES: diethylstilboestrol; FSH: follicle-stimulating hormone; GD: gestation day; Lob1: lobules type 1; MBeP: monobenzyl phthalate; MBuP: monobutyl phthalate; TEB: terminal end bud

An increase in the number of resorptions was observed after the exposure of Harlan-Cpb-WU rats to doses of 450 mg/kg body weight and day and above from gestation days 6 to 15. Extramedullary haematopoiesis was induced in the dams at the low dose of 270 mg/kg body weight and day and above. The NOAEL for developmental toxicity was 350 mg/kg body weight and day and the LOAEL for maternal toxicity was 270 mg/kg body weight and day. A NOAEL could not be derived for maternal toxicity. The incidence of delayed testicular descent was increased only after prolonged exposure from gestation days 6 to 20 (Piersma et al. 2000). However, as described above, the effects induced after prolonged exposure up to the day of birth are not considered relevant for the assessment of workplace exposure.

Treatment of Sprague Dawley rats from gestation days 10 to 21 caused a delay in the onset of puberty in females at doses of 500 mg/kg body weight and day and above. The NOAEL was 120 mg/kg body weight and day (Moral et al. 2011). The changes in gene expression profiles are not relevant to the evaluation as phenotypes were not investigated. As the animals were exposed up to the day of birth, the study is not considered relevant for the assessment of workplace exposure.

In a study with rats given gavage doses from gestation day 14 to birth, changes in sperm parameters and reduced body weights of the offspring 21 days after the end of exposure were observed at the highest dose tested of 100 mg/kg body weight and day (Ahmad et al. 2014). The reduction in body weights is not considered to be substance-induced, because the test substance does not accumulate. The changes in sperm parameters are questionable because no data were provided for the number of animals investigated. The animals were only 11 weeks old (PND 75) instead of 13 to 14 weeks old as specified by OECD Test Guidelines 443 and 416. The study cannot therefore be used for the evaluation of developmental toxicity.

The anogenital distance, corrected for the cube root of body weight, was shortened in male, but not in female, Wistar rats at doses of 500 mg/kg body weight and day and above (Ema and Miyawaki 2002). The in-utero/lactational protocol lists anogenital distance as an assessment parameter (for example EPA OPPTS 870.3800, OECD Test Guideline 416, OECD Test Guideline 443). AGD is a very sensitive parameter. Shortened anogenital distance in males without any changes in females is an indicator for an antiandrogenic effect. The biological significance of anogenital distance and the relevance of the changes are unclear (US EPA 2005).

An increase in the number of resorptions, a reduced number of living foetuses per litter and teratogenic effects were determined in CD1-Swiss mice at doses of 910 mg body weight and day and above. The primary effects were exencephaly, short tails, cardiovascular defects, fused ribs and abnormal or fused sternebrae and vertebrae. A NOAEL of 182 mg/kg body weight and day was derived for developmental and maternal toxicity (NTP 1990).

Single doses of 562 mg/kg body weight and above given to OF1 mice on individual days of gestation caused an increase in post-implantation losses and malformations (Saillenfait et al. 2003).

No developmental or maternal toxicity was observed in New Zealand White rabbits given benzyl butyl phthalate by gelatine capsule up to the highest dose tested of 10 mg/kg body weight and day (NTP-CERHR 2003). The study cannot be included in the assessment because the original study is not available.

Metabolites monobenzyl phthalate (MBeP) and monobutyl phthalate (MBuP)

MBeP and MBuP caused embryo mortality and teratogenicity in rats (Ema et al. 1995 a, b, 1996 b, c). Prenatal developmental toxicity studies determined NOAELs of 250 mg/kg body weight and day for the developmental and maternal toxicity of MBeP and MBuP in rats (Ema et al. 1995 b, 1996 b). MBeP induced malformations in the kidneys after exposure to doses of 375 mg/kg body weight and day and above (Ema et al. 1995 b); these effects were observed also in another rat strain given benzyl butyl phthalate with the diet at a dose level of 1640 mg/kg body weight and day (NTP 1989). The kidneys are the target organ also in adult animals.

Additionally, the exposure of rats to MBeP and MBuP induced malformations in the cervical vertebrae of the offspring (Ema et al. 1995 b, 1996 a, b), which is relevant because of the rarity of these effects and the suggested mechanism, the disruption of gene expression (NTP-CERHR 2003).

MBuP inhibited testicular descent (cryptorchidism) in the offspring of rats exposed to doses of 300 mg/kg body weight and day and above (Imajima et al. 1997; Shono et al. 2000). The antiandrogenic effect of MBeP in male rats was indicated by a shortened anogenital distance (Ema et al. 2003).

A comparative study of Sprague Dawley rats and OF1 mice given a single gavage dose of one of the two metabolites or benzyl butyl phthalate itself reported the following findings: MBuP given to OF1 mice on gestation day 8 induced malformations, in particular exencephaly, at doses as low as 400 mg/kg body weight and above. This effect was observed only at doses of MBeP of 923 mg/kg body weight and day and above. MBuP was found to be the more potent of the two metabolites also with respect to the increased incidences of post-implantation losses, which were observed after exposure to MBuP at doses of 400 mg/kg body weight and day and above and after exposure to MBeP at a dose of 1384 mg/kg body weight and day. In contrast, neither foetotoxic nor teratogenic effects were induced in Sprague Dawley rats given single doses of the metabolites on gestation day 10: MBuP up to a dose of 1200 mg/kg body weight and day, MBeP up to a dose of 1684 mg/kg body weight and day. Benzyl butyl phthalate induced teratogenic effects at doses of 1125 mg/kg body weight and day and above (Saillenfait et al. 2003).

Prenatal and postnatal exposure

A NOAEL of 500 mg/kg body weight and day was derived for foetotoxicity from a study in rats carried out according to OECD Test Guideline 421 with 14-day exposure before and during mating and up to postnatal day 6. This was based on an increase in the number of post-implantation losses and a decrease in the number of living offspring at the dose of 1000 mg/kg body weight and day. The NOAEL for parental toxicity was likewise 500 mg/kg body weight and day (Piersma et al. 1995; see also Section "Fertility").

A NOAEL of 250 mg/kg body weight and day was derived for foetotoxicity from a 2-generation study in rats. Reduced body weights in the offspring, the delayed onset of puberty in male and female offspring, shortened AGD and the retention of the areolae in male offspring, and a higher percentage of malformations in the male reproductive tract were observed at the LOAEL of 750 mg/kg body weight and day.

Increased absolute and relative kidney weights were found in female F0 animals at 250 mg/kg body weight and day (Tyl et al. 2004; see also Section "Fertility").

In a 2-generation study in rats, the percentage of surviving F1 pups was decreased at the dose of 500 mg/kg body weight and day. The NOAEL for foetotoxicity and parental toxicity was 100 mg/kg body weight and day. The body weights of the pups, which on the day of birth were reduced after exposure to a dose of 100 mg/kg body weight and day, had returned to the normal range on postnatal day 4 (Nagao et al. 2000; see also Section "Fertility").

In a 2-generation study in which rats were given gavage doses of benzyl butyl phthalate, effects were found in the reproductive organs of adult male F1 offspring at doses of 400 mg/kg body weight and day and above; the NOAEL was 200 mg/kg body weight and day. The NOAEL for parental toxicity was 100 mg/kg body weight and day (Aso et al. 2005; see also Section "Fertility").

Summary

In a prenatal developmental toxicity study in Sprague Dawley rats given benzyl butyl phthalate with the diet from gestation days 6 to 15, the incidence of variations was increased with concurrent maternal toxicity, such as delayed body weight gains, at doses of 1100 mg/kg body weight and day and above. The NOAEL for developmental and maternal toxicity was 420 mg/kg body weight and day (NTP 1989). In two prenatal developmental toxicity studies in Wistar rats given gavage doses of benzyl butyl phthalate on gestation days 7 to 15 and 6 to 15, the incidences of mortality and malformations in the foetuses were increased with concurrent maternal toxicity, such as delayed body weight gains, at doses of 750 mg/kg body weight and day and above (Ema et al. 1992 a). At doses of 450 mg/kg body weight and day and above, the number of resorptions was increased with concurrent maternal toxicity, such as extramedullary haematopoiesis (Piersma et al. 2000). The NOAEL for developmental toxicity and teratogenicity was 500 mg/kg body weight and day (Ema et al. 1992 a) and the NOAEL for developmental toxicity was 350 mg/kg body weight and day (Piersma et al. 2000).

The lowest NOAEL for foetotoxicity, a dose of 100 mg/kg body weight and day, was derived from three 2-generation studies in rats (Aso et al. 2005; Nagao et al. 2000; Tyl et al. 2004) and a study in rats with continuous exposure from 14 days before mating to postnatal day 6 (Piersma et al. 1995). The NOAEL was derived from the decrease in the percentage of surviving F1 pups observed up to postnatal day 4 at a dose of 500 mg/kg body weight and day. Concurrently, maternal and paternal toxicity, such as reduced body weights and changed organ weights, was observed (Nagao et al. 2000).

A prenatal developmental toxicity study in CD1 Swiss mice reported an increase in the number of resorptions and late foetal deaths, a reduced number of living foetuses per litter and an increase in malformations at benzyl butyl phthalate doses of 910 mg/kg body weight and day and above. Concurrently, body weight gains were delayed in the dams. A NOAEL of 182 mg/kg body weight and day was derived for the developmental and maternal toxicity of benzyl butyl phthalate (NTP 1990). The NOAEL for developmental and maternal toxicity may be much higher because of the large margin between the two doses.

5.6 Genotoxicity

In vitro studies of genotoxicity are shown in Table 6, in vivo findings in Table 7.

5.6.1 In vitro

Bacteria

Benzyl butyl phthalate was not found to be mutagenic in the Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 or in the Saccharomyces cerevisiae strain D4 up to a concentration of 10 µl/plate either with or without the addition of metabolic activation. Cytotoxicity was not observed (EU 2007).

Another mutagenicity test carried out by the NTP with the Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 likewise yielded negative results up to a concentration of 10 000 µg/plate both with and without the addition of metabolic activation. Cytotoxicity was not observed (EU 2007; NTP 1997 a).

The negative results obtained in other bacterial test systems (Escherichia coli and Bacillus subtilis) are available only in the form of a review (EU 2007).

Mammalian cells

A test for sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells was carried out with benzyl butyl phthalate at concentrations up to 1.25 µg/ml both with and without the addition of metabolic activation. The results of the first test are questionable because a significantly positive trend was found even though the frequency of SCE was not significantly increased at any of the individual concentrations. The results of the second test were negative. The overall results were assessed as negative (EU 2007; NTP 1997 a).

A chromosomal aberration test in CHO cells did not reveal any clastogenic effects at concentrations up to the highest non-cytotoxic concentration of 1.25 µg/ml either with or without the addition of metabolic activation (EU 02007; NICNAS 2015; NTP 1997 a).

In the L5178Y $TK^{+/-}$ mouse lymphoma assay carried out with benzyl butyl phthalate concentrations of 0, 0.06, 0.16, 0.32, 0.65, 1.25, 2.5 and 5.0 µl/ml both with and without the addition of metabolic activation (S9-mix of mice), the incidence of mutations was not increased up to a concentration of 1.25 µl/ml. Benzyl butyl phthalate was not completely soluble at the two higher concentrations (EU 2007; NICNAS 2015).

Another L5178Y $TK^{+/-}$ mouse lymphoma assay was carried out with benzyl butyl phthalate concentrations of 5, 10, 20, 30, 40 and 60 nl/ml both with and without the addition of metabolic activation. Precipitates formed at the high concentration and the test substance induced cytotoxic effects. The formation of mutant colonies was increased in the absence of metabolic activation only at this concentration. The results of the assay, which was carried out by the NTP, were considered to be negative (EU 2007; NTP 1997 a).

Table 6 Studies of the genotoxic effects of benzyl butyl phthalate in vitro

End point	Test system	Concentration [µl/plate][a]	Effective concentration[a]	Cytotoxicity[a]	Results		References
					–m.A.	+m.A.	
gene mutations	Escherichia coli, Bacillus subtilis	30 mg/plate	–	no data	–	no data	EU 2007
gene mutations	Saccharomyces cerevisiae D4	0.1–10	–	> 10	–	–	EU 2007
gene mutations	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	0.1–10	–	> 10	–	–	EU 2007
gene mutations	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	0.001–10	–	> 10	–	–	EU 2007
gene mutations	Salmonella typhimurium TA98, TA100, TA1535, TA1537	100–10 000 µg/plate	–	> 10 000 µg/plate	–	–	EU 2007; NTP 1997 a
SCE	CHO cells	up to 1.25 µg/ml	–	> 1.25 µg/ml	–	–	EU 2007; NTP 1997 a
CA	CHO cells	up to 1.25 µg/ml	–	> 1.25 µg/ml	–	–	EU 2007; NTP 1997 a
gene mutations ($TK^{+/-}$)	mouse lymphoma cell line L5178Y	5–60 nl/ml	–	60 nl/ml	–	–	EU 2007; NTP 1997 a
gene mutations ($TK^{+/-}$)	mouse lymphoma cell line L5178Y	0.06–5 µl/ml (insoluble at 1.25 µl/ml and above)	–	no data	–	–	EU 2007; NICNAS 2015

[a] if not otherwise specified: [µl/plate]
CA: chromosomal aberration test; SCE: sister chromatid exchange; $TK^{+/-}$: thymidine kinase

Table 7 Studies of the genotoxic effects of benzyl butyl phthalate in vivo (EU 2007)

End point	Species	Dose	Results	References
SLRL test	male Drosophila melanogaster	0, 250, 10 000, 50 000 mg/kg feed or 0, 500 mg/l (injection)	–	EU 2007; NICNAS 2015; NTP 1997 a
SCE test in the bone marrow	groups of 5 male B6C3F1 mice	0, 1250, 2500, 5000 mg/kg body weight, intraperitoneal	–	EU 2007; NTP 1997 a
chromosomal aberration test in the bone marrow	groups of 5 male B6C3F1 mice	0, 1250, 2500, 5000 mg/kg body weight, intraperitoneal	–	EU 2007; NTP 1997 a
micronucleus test in the bone marrow	19 female Alpk:APf-SD rats	0, 182.6 µg/kg body weight and day in the drinking water during gestation and lactation	–	Ashby et al. 1997; EU 2007; NICNAS 2015
dominant lethal test	24 DC-1 mice or 36 B6C3F1 mice	0, 400–600, 1280–1840 or 3200–4560 mg/kg body weight and day subcutaneous, day 1, 5 and 10	–	Bishop et al. 1987; EU 2007; NICNAS 2015

SCE: sister chromatid exchange; SLRL: sex-linked recessive lethal

5.6.2 In vivo

In the SLRL (sex-linked recessive lethal) test in Drosophila melanogaster, mutations were not induced in the germ cells of male animals after exposure to benzyl butyl phthalate concentrations of up to 500 µl/l (injection) or up to 50 000 mg/kg feed (EU 2007; NICNAS 2015; NTP 1997 a).

In a SCE test carried out in the bone marrow cells of B6C3F1 mice, 5 males were given doses of 0, 1250, 2500 or 5000 mg/kg body weight by intraperitoneal injection. Samples were taken after 23 or 42 hours. A positive trend was determined after 23 hours except at the high dose, at which a decline in the reaction was observed. A weak positive trend was found also after 42 hours. None of the trials were replicated by the NTP (EU 2007; NTP 1997 a).

In a chromosomal aberration test in bone marrow cells, groups of 10 male mice were given intraperitoneal injections of 0, 1250, 2500 or 5000 mg/kg body weight. The trend analyses were positive for two tests with sampling after 17 hours. In the first and second test, the percentage incidence of cells with chromosomal aberrations was 0.75% and 1.0%, respectively, in the control group, 1.5% and 2.25% at 1250 mg/kg, 0.75% and 2.0% at 2500 mg/kg and 3.25% and 4.15% at 5000 mg/kg. Induction was statistically significant in the high dose group. The number of chromosomal aberrations per cell was not significantly different from the number found in the control group. When samples were taken after 36 hours, the percentage fraction of cells with chromosomal aberrations was 0.25%, 1.5%, 0.25% and 0.5% at 0, 1250, 2500 and 5000 mg/kg body weight, respectively, and thus not significantly increased at any dose (EU 2007; NICNAS 2015; NTP 1997 a). There are no data available for toxicity.

In a study with 19 female Alpk:APf-SD rats given benzyl butyl phthalate with the drinking water during gestation and lactation at a dose of 182.6 µg/kg body weight and day, micronuclei were not induced in the polychromatic erythrocytes of the bone marrow. The ratio of polychromatic to normochromatic erythrocytes remained unchanged (Ashby et al. 1997; EU 2007; NICNAS 2015). It is assumed that the substance was administered at a dose that was too low for effects to be observed.

In a dominant lethal test in mice, which was published only in a review, benzyl butyl phthalate was given by subcutaneous injection to groups of 24 male CD-1 mice and 36 male B6C3F1 mice on days 1, 5 and 10 at doses of 0, 400–600, 1280–1840 or 3200–4560 mg/kg body weight and day. Each male mouse was mated for four days with 3 untreated female animals on day 2, 6, 11, 15, 22, 29, 42 and 49. The females were sacrificed and examined 17 days after the beginning of mating. No effects were induced by benzyl butyl phthalate in this test (Bishop et al. 1987; EU 2007; NICNAS 2015). No further data are available for the dose ranges administered.

Summary/Evaluation of the genotoxic effects

Benzyl butyl phthalate did not cause genotoxic effects in most of the tests (see Table 6 and 7).

The marginally positive findings in vitro were not confirmed in replication tests.

Benzyl butyl phthalate did not induce mutations in germ cells in vivo in the SLRL test with Drosophila melanogaster. In the SCE test in bone marrow cells of B6C3F1 mice, a positive trend was observed after intraperitoneal injection, which, however, was not significant when compared with the effects at the individual doses. The test was not replicated to validate the findings. In the chromosomal aberration test in the bone marrow cells of mice, the findings were positive at the high dose at 1 of 2 validated sampling times. No clastogenic effects were induced in the dominant lethal test in mice and in the micronucleus test in polychromatic erythrocytes of the bone marrow of rats.

Like for other phthalates that were previously evaluated by the Commission (see documentation "Di(2-ethylhexyl)phthalate" 2009; documentation "Di-*n*-butyl phthalate" 2013), the majority of the genotoxicity data available for benzyl butyl phthalate provides no evidence of genotoxic effects in vitro or in vivo. Cytotoxic effects may also have contributed to the isolated positive findings obtained after exposure to high doses or in the indicator test (SCE).

5.7 Carcinogenicity

5.7.1 Short-term studies

Benzyl butyl phthalate (purity 98%) was tested in a cell transformation test with Syrian hamster embryo cells at a pH of 6.7. The study was carried out according to GLP guidelines. The 24-hour test concentrations were 25, 50, 150 and 250 µg/ml, the 7-day test concentrations were 1, 2, 5, 10 and 20 µg/ml. The data do not indicate whether an exogenous metabolic activation system was added. Precipitates formed at concentrations above 25 µg/ml. The 7-day study with 2, 5 and 10 µg/ml yielded positive results. The fact that positive results were observed only after a longer pe-

riod of exposure was attributed to non-genotoxic mechanisms (changes in gene expression) (EU 2007).

No effects were induced by benzyl butyl phthalate in a cell transformation test with BALB/3T3 cells at concentrations of 0.49 to 8000 nl/ml. The test concentrations were determined via a cytotoxicity study. The poor solubility of benzyl butyl phthalate in the medium was described as problematic (EU 2007). No data for the exposure period are provided; the original study is not available.

Groups of 27 female Sprague Dawley rats (43 days old) were given benzyl butyl phthalate (purity not specified) in doses of 250 or 500 mg/kg body weight and day for 7 days by intragastric administration. The animals were subsequently given dimethylbenz(a)anthracene (DMBA) at a dose of 31 mg/kg body weight. The incidence of mammary tumours was determined in the animals after 15 weeks. The body weight gains were found to be similar in the treated and untreated animals. The incidence of palpable mammary tumours was significantly reduced via pretreatment with benzyl butyl phthalate, 58% and 71% at 250 and 500 mg/kg body weight and day, respectively. Additionally, the average number of adenocarcinomas was significantly reduced at 4.0 after exposure to DMBA alone, 1.6 after exposure to a benzyl butyl phthalate dose of 250 mg/kg and 1.2 after exposure to a benzyl butyl phthalate dose of 500 mg/kg (EU 2007).

In summary, benzyl butyl phthalate induced morphological cell transformations in Syrian hamster embryo cells, but no cell transformations in BALB/3T3 cells. The incidence of mammary tumours induced by DMBA was reduced by pretreatment with benzyl butyl phthalate.

5.7.2 Long-term studies

Oral carcinogenicity studies in rats are available from the 1980s (NTP 1982) and 1990s (NTP 1997 a, b) and one study in mice from the 1980s (NTP 1982).

Mouse

In groups of 50 male and 50 female B6C3F1 mice given benzyl butyl phthalate doses of 0, 6000 or 12 000 mg/kg feed for 24 months, which is equivalent to doses of about 900 and 1800 mg/kg body weight and day (conversion factor 0.15 for chronic studies according to EFSA (2012)), neither an increased incidence of tumours nor other histopathological findings were observed (NTP 1982).

Rat

Groups of 50 male and 50 female F344 rats were fed a diet containing benzyl butyl phthalate concentrations of 6000 or 12000 mg/kg feed for 2 years, which is equivalent to doses of about 300 and 600 mg/kg body weight and day, respectively (conversion factor 0.05 for chronic studies according to EFSA (2012)). The body weights of the females were reduced in comparison with those of the controls over the entire study period, the feed consumption of the treated animals was 70% to 80% of that of the control animals. Increased mortality was observed in the males in week 14; the males had to be removed completely from the study by week 30 (NTP 1982). The non-neoplastic findings are reported in detail in Section 5.2.2, Table 3; tumours and preneoplasms are shown in Table 8.

Table 8 Studies of the carcinogenicity of benzyl butyl phthalate in rats

Author:	NTP 1982		
Substance:	benzyl butyl phthalate (purity: 97.2%)		
Species:	rat, F344/N, groups of 50 ♂/50 ♀ (♂ animals not evaluated)		
Administration route:	diet		
Dose:	♂/♀: 0, 6000, 12 000 mg/kg feed (doses of about 0, 300, 600 mg/kg body weight and day[1)])		
Duration:	105–106 weeks		
Toxicity:	♂: increased mortality in week 14, study aborted in week 30 ♀: body weight gains ↓ at 300 mg/kg body weight and day and above		
	Exposure (mg/kg body weight and day)		
	0	300	600
surviving animals	♀ 31/50	29/50	32/50
tumours and preneoplasms			
haematopoietic system:			
mononuclear cell leukaemia	♀ 7/49 (14%)[a)]	7/49 (14%)	18/50 (36%)[*]
pituitary gland:			
adenomas	♀ 20/45 (44%)[T)]	21/40 (53%)	26/41 (63%)
liver:			
neoplastic nodules	♀ 1/49 (2%)	1/48 (2%)	3/50 (6%)
hepatocellular carcinomas	♀ 0/49 (0%)	0/48 (0%)	1/50 (2%)

[a)] historical control since 1977: 12%–24%, mean 19% (NTP 1982); historical control 16%–42%, mean 29.3% (EU 2007)
[*] p = 0.01; [T)] positive trend test, p = 0.05
[1)] conversion factor 0.05 (long-term) according to EFSA (2012)

Author:	NTP 1997 a, b
Substance:	benzyl butyl phthalate (purity: 97%–99%)
Species:	**rat**, F344/N, groups of 50 ♂, 50 ♀
Administration route:	diet
Dose:	♂: 0, 3000, 6000, 12 000 mg/kg feed (doses of 0, 120, 240, 500 mg/kg body weight and day) ♀: 0, 6000, 12 000, 24 000 mg/kg feed (doses of 0, 300, 600, 1200 mg/kg body weight and day)
Duration:	24 months
Toxicity:	**300 mg/kg body weight and above**: ♀: renal toxicity (see Section 5.2.2) **500 mg/kg body weight and above**: ♂: body weights ↓, renal toxicity (see Section 5.2.2) The mean body weights of both sexes were 10% lower in the high dose group and the weight-matched control group than in the ad libitum control group

Table 8 (continued)

		Exposure (mg/kg body weight and day)					
		0 (ad libitum)	0 (weight-matched)[h]	120	240/300	500/600	1200
surviving animals	♂	28/50	**34/50**	20/50	22/50	22/50	n. t.
	♀	25/50	**41/50**	n. t.	29/50	29/50	29/50
tumours and preneoplasms							
pancreas:							
focal acinar cell hyperplasia	♂	4/50 (8%)	2/50 (4%)	0/49 (0%)	9/50 (18%)	**12/50 (24%)***	n. t.
	♀	1/50 (2%)		n. t.	4/50 (8%)	2/50 (4%)	0/50 (0%)
acinar cell adenomas	♂	3/50 (6%)[a]	0/50 (0%)	2/49 (4%)	3/50 (6%)	**10/50 (20%)*** (***)**	n. t.
	♀	0/50 (0%)[b]		n. t.	0/50 (0%)	0/50 (0%)	2/50 (4%)
acinar cell carcinomas	♂	0/50 (0%)[c]	**1/50 (2%)**	0/49 (0%)	0/50 (0%)	**1/50 (2%)**	n. t.
	♀	0/50 (0%)		n. t.	0/50 (0%)	0/50 (0%)	0/50 (0%)
bladder:							
urothelial hyperplasia	♀	4/50 (8%)	0/50 (0%)	n. t.	0/50 (0%)	1/50 (2%)	**10/50 (20%)*** (***)**
urothelial papillomas	♀	1/50 (2%)[d]	0/50 (0%)	n. t.	0/50 (0%)	0/50 (0%)	2/50 (4%)
adrenal medulla:							
hyperplasia	♂	3/50 (6%)	1/50 (2%)			**11/50 (22%)*** (***)**	n. t.
benign phaeochromocytomas	♂	9/50 (18%)[e]	3/50 (7%)			**8/50 (20%)(*)**	n. t.
malignant phaeochromocytomas	♂	2/50 (4%)	1/50 (2%)			2/50 (4%)	n. t.
haematopoietic system:							
mononuclear cell leukaemia	♂	31/50 (62%)[f]	15/50 (30%)			**30/50 (60%)(**)**	n. t.
	♀	21/50 (42%)[g]	13/50 (26%)				**19/50 (38%)(**)**

[a] historical control: 0%–10%; [b] historical control: 0%–4%; [c] historical control: 0%; [d] historical control: 0%–2%; [e] historical control: 33.7% (12%–63%); [f] historical control: 46.7% (18%–62%); [g] historical control: 26.8% (14%–52%); [h] NTP 1997 b

* $p < 0.1$; ** $p < 0.05$; *** $p < 0.001$ in relation to ad libitum (weight-matched) control group

n. t. not tested

In the second NTP study with F344 rats, benzyl butyl phthalate was given to groups of 50 males in concentrations of 0, 3000, 6000 or 12 000 mg/kg feed (doses of 0, 120, 240, 500 mg/kg body weight and day) and to groups of 50 females in concentrations of 0, 6000, 12 000 or 24 000 mg/kg feed (doses of 0, 300, 600, 1200 mg/kg body weight and day) for 2 years. The non-neoplastic findings are reported in detail in Section 5.2.2, Table 3; tumours and preneoplasms are shown in Table 8. Survival was similar in both the treated and untreated groups (NTP 1997 a). A follow-up study of the NTP included a weight-matched control group to determine the tumour incidence in animals with body weights that matched those of the group given the highest dose (by means of restricted diets). In each of the high dose groups survival was reduced in comparison with that in the control group (NTP 1997 b).

In the additional 2-year restricted feed study, which included a control group and the two high dose groups (males 12 000 mg/kg feed, females 24 000 mg/kg feed) (NTP 1997 b), a marginally increased incidence of pancreatic adenomas was found only at interim necropsy, but not at the end of the study. According to the NTP, restricted feed intake influences the formation of pancreatic neoplasms and in this case probably prevented the development of substance-induced tumours. In this study, neoplasms were found in the bladder after lifelong exposure, but not after 2 years. The authors concluded from this that the length of the study, and not the body weight, was the critical determinant for the induction of this type of tumour.

Summary and discussion of the studies

Three carcinogenicity studies are available from the NTP:

(1) one carcinogenicity study in female F344 rats (the males were removed prematurely from the study because of increased mortality) (NTP 1982),
(2) one study in male and female F344 rats (NTP 1997 a) with an additional weight-matched control group and a trial with a low-calorie diet (NTP 1997 b),
(3) and one study in male and female B6C3F1 mice (NTP 1982).

The tumour incidence was not increased in mice.

In male rats (no data available from the first study), the incidence of benign **pancreatic** tumours was increased. This effect was only marginal in female rats (NTP 1997 a). The effect was not induced when the animals were fed a low-calorie diet (NTP 1997 b). The single pancreatic carcinoma that was found in the high dose group was evaluated as a rare occurrence by the NTP (NTP 1997 a), but was not considered to be substance-induced because it was also found in the weight-matched control group (NTP 1997 b).

Pancreatic tumours induced by peroxisome proliferation in rats are considered to be of little relevance for humans, as the expression of the CCK_A receptor in the human pancreas is much lower than in rats (Klaunig et al. 2003; see also Section "Mechanism of Action").

In male rats (no data available from the first study), the incidence of hyperplasia and benign **phaeochromocytomas** was increased in the adrenal medulla; however, this was within the range of the historical controls (NTP 1997 a, b).

In female rats in the first study (no data available for the males; NTP 1982) and in both sexes in the second study (NTP 1997 a), the incidence of **mononuclear cell leukaemia** was increased. This was apparent in the second study only in comparison

with the weight-matched controls. The incidences were all within the range of the historical controls (NTP 1997 b).

Also not in the first (NTP 1982), but in the second study (NTP 1997 a), a significant increase in urothelial hyperplasia of the **bladder** of female rats and a marginal increase in papillomas were observed. These were within the range of the historical controls. There was also a slight, but not significant, increase in the incidence of bladder carcinomas in the animals fed a restricted diet after lifelong exposure, but not after exposure for 2 years. The authors concluded that the length of the study, and not the body weight, was the critical determinant for the induction of this type of tumour (NTP 1997 b).

The marginally increased incidence of bladder carcinomas after lifelong exposure is difficult to interpret due to a lack of historical controls (EU 2007).

Overall, carcinogenic effects for humans cannot be concluded from the three studies.

5.8 Effects on the immune system

In vivo studies in different animal models are available that investigated the effects of benzyl butyl phthalate or its metabolites on the (respiratory) immune system.

In BALB/cJ mice given subcutaneous injections of MBuP (monobutyl phthalate) or MBeP in doses of about 5 to 5000 µg/kg body weight after first being sensitized to ovalbumin (OVA), no significant increase or decrease in the levels of OVA-specific IgE (immunoglobulin E) or IgG1 (immunoglobulin G1) was observed (Larsen et al. 2003).

Multiple topical applications of 50 µl undiluted benzyl butyl phthalate (2 weeks, 5 times per week) did not increase interleukin 4 or interleukin 13 levels in the auricular lymph nodes or increase IgE production in female B6C3F1 mice (Butala et al. 2004).

The studies thus did not provide any evidence that benzyl butyl phthalate has an adjuvant effect.

In an in vitro study, benzyl butyl phthalate suppressed CpG-induced (Cytosine-phosphate-Guanine) interferon-alpha, interferon-beta and interferon-gamma expression of human plasmacytoid dendritic cells, but promoted interleukin-13 secretion (Kuo et al. 2013).

6 Manifesto (MAK value/classification)

The critical effects are the effects on the liver and kidneys. In addition, at higher doses, benzyl butyl phthalate, like DEHP and di-*n*-butyl phthalate, induces effects on the male reproductive organs, fertility and development.

MAK value. There are no data available for humans that can be used to derive a MAK value.

In a 13-week inhalation study in rats (Monsanto Co 1982), an increase in the absolute and relative kidney and liver weights was observed in males and females at a concentration of 789 mg/m^3. The increase in liver weights was caused by peroxi-

some proliferation, which is not relevant to humans (see Section 2). The NOAEC was 218 mg/m³. The study, which was described in great detail and had only minor methodological uncertainties (Section 5.2.1), did not report any effects on the respiratory tract up to high concentrations. No organ weight changes were observed in the 28-day inhalation study up to a concentration of 526 mg/m³.

In the feeding studies in rats carried out by the NTP (1997 a), an increase in the relative kidney weights was observed at a dose of 550 mg/kg body weight and above (NOAEL 180 mg/kg body weight and day) after 26 weeks and at a dose of 120 mg/kg body weight and day and above (no NOAEL) after 15 months. It is therefore possible that the effects on the kidneys may intensify over time with long-term exposure by inhalation. A LOAEL of 120 mg/kg body weight and day was determined from the chronic feeding studies in rats because of the increase in kidney weights observed after 15 months (no determination after 2 years) (NTP 1997 a). A LOAEL of 900 mg/kg body weight and day was determined on the basis of the reduced body weights observed after B6C3F1 mice were given benzyl butyl phthalate in the diet for 2 years (NTP 1982). The following toxicokinetic data are taken into consideration for the extrapolation of the estimated NAEL (no adverse effect level = LOAEL/3) of 40 mg/kg body weight and day in rats and 300 mg/kg body weight and day in mice to a concentration in workplace air: the daily exposure of the animals in comparison with the 5 days a week exposure at the workplace (7:5), the corresponding species-specific correction values for the rat or mouse (1:4; 1:7), the measured oral absorption (100%), the body weight (70 kg) and the respiratory volume (10 m³) of the person and the assumed 100% absorption by inhalation. The concentrations calculated from this are 98 mg/m³ (rat) and 420 mg/m³ (mouse). On the basis of the study in rats (98 mg/m³) (NTP 1997 a) and after extrapolating the data from animal studies to humans (1:2) and applying the preferred value approach, a MAK value of 50 mg/m³ I would result. The MAK value would be even higher (200 mg/m³) if it were derived from the data from the study in mice.

However, on the basis of the NOAEC of 218 mg/m³ determined after subchronic exposure by inhalation and the extrapolation of the data from animal studies to humans (1:2) and taking into consideration the increased respiratory volume at the workplace (1:2) and the increase in the effects over time (1:2) and after applying the preferred value approach, a MAK value of 20 mg/m³ I is derived. It should be noted that even at the LOAEC of 789 mg/m³ only changes in organ weights without histopathological findings were reported. The NOAEC was therefore a very conservative value and may be higher.

As inhalation best reflects the exposure at the workplace, a MAK value of 20 mg/m³ I has been established.

Peak limitation. As the critical effect is a systemic effect, benzyl butyl phthalate has been classified in Peak Limitation Category II. As data for the half-life are not available and the critical metabolite has yet to be determined, the default excursion factor of 2 has been set.

Prenatal toxicity. The concentrations in the air at the workplace and the margins to the MAK value of 20 mg/m³ calculated by toxicokinetic extrapolation from the studies of developmental toxicity are shown in Table 9.

Table 9 Relevant NOAELs in rats and mice, toxicokinetic extrapolation (see text) of the NOAELs to a concentration in the air and the resulting margins to the MAK value of 20 mg/m³

References	Species, exposure	NOAEL: end point	Toxicokinetic extrapolation[a] (mg/m³)	Margin to the MAK value of 20 mg/m³
	rat			
Ema et al. 1992 a	prenatal, gavage	500 mg/kg body weight and day: developmental toxicity and teratogenicity	875	44
NTP 1989	prenatal, diet	420 mg/kg body weight and day: developmental toxicity 1100 mg/kg body weight and day: teratogenicity	735 1925	37 96
Piersma et al. 2000	prenatal, gavage	350 mg/kg body weight and day: developmental toxicity	613	31
Nagao et al. 2000	prenatal and postnatal, gavage	100 mg/kg body weight and day: foetotoxicity	245[b]	12
	mouse			
NTP 1990	prenatal, diet	182 mg/kg body weight and day: developmental toxicity and teratogenicity	182 910 (LOAEL)	9[c] 46

[a] (1:4 or 1:7) × 1.0 (oral absorption in animals)/1.0 (absorption by inhalation in humans) × 70 kg/10 m³
[b] taking into consideration the extrapolation from 7-day to 5-day exposure
[c] may be higher because LOAEL of 910 mg/kg body weight and day is 5 times as high

The margins between the MAK value and the calculated NOAECs for developmental toxicity and foetotoxicity are therefore sufficiently large, also in the study in mice, as effects (increase in the number of resorptions, late foetal deaths, a decrease in the number of living foetuses per litter, malformations) were induced in the offspring only at a LOAEL that was five times as high and with concurrent maternal toxicity (delayed body weight gains). Benzyl butyl phthalate has been classified in Pregnancy Risk Group C.

Carcinogenicity. Most of the data available for benzyl butyl phthalate in vitro and in vivo provide no evidence of genotoxic effects.

Benzyl butyl phthalate induced morphological cell transformations in Syrian hamster embryo cells after exposure for 7 days, but not after 24 hours. A BALB/3T3A cell transformation assay yielded negative results; no data were provided for the length of exposure. The incidence of mammary tumours induced by exposure to DMBA was reduced by pretreatment with benzyl butyl phthalate.

Benzyl butyl phthalate induced peroxisome proliferation in rats, but to a lesser degree than DEHP. Unlike the findings for DEHP, neither the oral carcinogenicity study in rats nor that in mice provided evidence of the induction of liver tumours (documentation "Di(2-ethylhexyl)phthalate" 2009).

Three carcinogenicity studies were carried out by the NTP, one in female rats (NTP 1982), one in male and female F344 rats (NTP 1997 a) and one in male and female B6C3F1 mice (NTP 1982). The tumour incidence was not increased in B6C3F1 mice. An increased incidence of mononuclear cell leukaemia was found in F344 rats at the high doses, which was, however, in the range of the historical controls. There was an increase in the incidence of benign pancreatic tumours; this finding is of little relevance for humans. In addition, the incidence of hyperplasia and benign phaeochromocytomas was increased in the adrenal medulla of male F344 rats; this was likewise in the range of the historical controls. Hyperplasia and papillomas were found in the bladders of the females; these were again within the range of the historical controls. The incidence of bladder carcinomas was slightly, but not significantly, increased in the animals fed a restricted diet after lifelong exposure, but not after 2 years.

In summary, only the incidence of adenomas, but not that of carcinomas, was increased; the incidence of adenomas was usually within the range of the historical controls. Carcinogenic effects for humans cannot be derived from the findings of the three NTP carcinogenicity studies. For this reason, benzyl butyl phthalate has not been classified in a category for carcinogens.

Germ cell mutagenicity. Most of the data available for benzyl butyl phthalate in vitro and in vivo provide no evidence of genotoxic effects. As was concluded for DEHP and di-*n*-butyl phthalate (see documentation "Di(2-ethylhexyl)phthalate" 2009; documentation "Di-*n*-butyl phthalate" 2013), cytotoxic effects may be involved. A dominant lethal test with subcutaneous injection in mice yielded negative results. For this reason, the substance has not been classified in a category for germ cell mutagens.

Absorption through the skin. An in vivo study in rats and an in vitro study in human skin are available for the evaluation of the absorption of benzyl butyl phthalate through the skin. Assuming uniform penetration into and through the skin and

1-hour exposure of 2000 cm^2 of skin to undiluted benzyl butyl phthalate, a maximum amount of 55 mg is estimated to be absorbed through the skin. As the systemic NOAEL for rats was 218 mg/m^3 after sub-chronic exposure by inhalation, a systemically tolerable amount of 272.5 mg is calculated after extrapolation to chronic exposure (1:2), assuming 100% absorption by inhalation, taking into consideration an increased respiratory volume (1:2), at a respiratory volume of 10 m^3 and after extrapolation of the data from animal studies to humans (1:2). Absorption through the skin is thus less than 25% of the systemically tolerable amount and the substance is not designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. None of the studies yielded positive results for the contact sensitizing potential of benzyl butyl phthalate; the substance has therefore not been designated with "Sh" (for substances which cause sensitization of the skin). The data from epidemiological studies or animal studies do not plausibly support an association between exposure to benzyl butyl phthalate and an increased risk for the development of allergic asthma induced by ubiquitous allergens. The substance has therefore not been designated with "Sa" (for substances which cause sensitization of the airways).

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