27 November 2009

Substance name: Diisobutyl phthalate
EC number: 201-553-2
CAS number: 84-69-5

MEMBER STATE COMMITTEE SUPPORT DOCUMENT FOR IDENTIFICATION OF DIISOBUTYL PHTHALATE AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF ITS CMR PROPERTIES

Adopted on 27 November 2009

CONTENTS

TABLES

Substance Name: **Diisobutyl phthalate**

EC Number: **201-553-2**

CAS number: **84-69-5**

- *The substance is identified as a CMR according to Article 57(c) of Regulation (EC) No 1907/2006 (REACH).*

Summary of how the substance meets the CMR (Cat 1 or 2), PBT or vPvB criteria, or is considered to be a substance of an equivalent level of concern

Pursuant to Annex V of Commission Regulation (EC) No 790/2009[1] diisobutyl phthalate (DIBP) will as of 1 December 2010 be listed in Table 3.2 (the list of harmonised classification and labelling of hazardous substances from Annex I to Directive 67/548/EEC) of Annex VI, part 3, of Regulation (EC) No 1272/2008[2] as toxic to reproduction category 2; R61 (May cause harm to the unborn child).[3]

Therefore, this classification of the substance in Commission Regulation (EC) No 790/2009 shows that the substance meets the criteria for classification as toxic to reproduction in accordance with Article 57 (c) of REACH.

Registration number(s) of the substance or of substances containing the substance:

Not available.

[1] Commission Regulation (EC) No 790/2009 of 10 August 2009 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (1st ATP)

[2] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

[3] Pursuant to the 1st ATP, the classification according to Table 3.1 (list of harmonised classification and labelling of hazardous substances) of Annex VI, part 3, of Regulation (EC) No 1272/2008 will as of 1 December 2010 be toxic to reproduction category 1B, H360Df (May damage the unborn child. Suspected of damaging fertility).

JUSTIFICATION

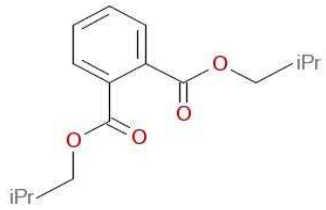
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name: Diisobutyl phthalate (DIBP)
EC Name: Diisobutyl phthalate
CAS Number: 84-69-5
IUPAC Name: Bis(2-methylpropyl)benzene-1,2-dicarboxylate

1.2 Composition of the substance

Chemical Name: 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
EC Number: 201-553-2
CAS Number: 84-69-5
IUPAC Name: Bis(2-methylpropyl)benzene-1,2-dicarboxylate
Molecular Formula: $C_{16}H_{22}O_4$
Structural Formula:



Molecular Weight: 278.35 g/mol
Typical concentration (% w/w): 99
Concentration range (% w/w): 98 – 100

1.3 Physico-chemical properties

Table 1: Summary of physico- chemical properties

REACH ref Annex, §	**Property**	**IUCLID section**	**Value**	**[enter comment/reference or delete column]**
VII, 7.1	Physical state at 20°C and 101.3 kPa	4.1	Colourless, clear , mostly odourless viscous liquid	
VII, 7.2	Melting/freezing point	4.2	-37 °C	Woodward (1988)
VII, 7.3	Boiling point	4.3	320 °C	Härtel (1985)
VII, 7.5	Vapour pressure	4.6	0.01 Pa at 20 °C	Potin-Gautier et al. (1982)
VII, 7.7	Water solubility	4.8	20 mg/l at 20 °C	Leyder and Boulanger (1983)
VII, 7.8	Partition coefficient n-octanol/water (log value)	4.7	logPow: 4.11	Leyder and Boulanger (1983)

2 CLASSIFICATION AND LABELLING

2.1 Classification according Directive 67/548/EEC and in Annex VI of Regulation (EC) No 1272/2008

According to Article 57 (c) of the REACH Regulation, substances meeting the criteria for classification as toxic for reproduction category 1 or 2 in accordance with Directive 67/548/EEC may be included in Annex XIV.

Pursuant to the first ATP to the Regulation (EC) No 1272/2008 (Commission Regulation (EC) No 790/2009) as of 1 December 2010, the classification of diisobutyl phthalate in Annex VI, part 3, Table 3.2 of Regulation (EC) No 1272/2008 (the list of harmonised classification and labelling of hazardous substances from Annex I to Directive 67/548/EEC) will be as follows:

Index Number: 607-623-00-2

Repr. Cat. 2; R61 (May cause harm to the unborn child)
Repr. Cat. 3; R62 (Possible risk of impaired fertility)

T; R61-62; S53-45

Specific concentration limits:
Repr. Cat. 2; R61: C ≥ 25 %
Repr. Cat. 3; R62: C ≥ 5 %

According to the first ATP to Regulation (EC) No 1272/2008, the corresponding classification in Annex VI, part 3, Table 3.1 of this Regulation (EC) No 1272/2008 (list of harmonised classification and labelling of hazardous substances) will be as follows:

Index Number: 607-623-00-2

Hazard Class and Category Code(s):

Repr. 1B

Hazard Statement Code(s):

H360Df (May damage the unborn child. Suspected of damaging fertility.)

Specific concentration limits:
Repr. 1B; H360Df: C ≥ 25 %
Repr. 2; H361f: C ≥ 5 %

2.2 Self classification(s)

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3 ENVIRONMENTAL FATE PROPERTIES

Since this is a support document targeted to the identification of DIBP as a CMR substance, environmental fate properties have not been considered.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Not relevant for this type of document.

4.2 Acute toxicity

Not relevant for this type of document.

4.3 Irritation

Not relevant for this type of document.

4.4 Corrosivity

Not relevant for this type of document.

4.5 Sensitisation

Not relevant for this type of document.

4.6 Repeated dose toxicity

4.6.1 Repeated dose toxicity: oral

In toxicity studies with repeated oral application the male reproductive system was identified as one and most important target organ of toxicity for DIBP. Available early feeding studies in experimental animals revealed reproductive effects in adult males (e.g., decreased testes weight and reduced sperm production in rats) at relatively high oral doses. Sub-acute studies with rats and mice, and sub-chronic studies with rats and dogs are available. Although these studies were not comparable to guideline studies and not in conformity with GLP they point clearly out the critical organs of toxicity for DIBP – the male sex organs. The distinctions in study design compared to published guidelines include e.g., no precise data on strain, number and sex of the used animals; in some studies only animals of one sex were tested.
Similar studies with the correspondent monoester, mono-iso-butyl phthalate (MIBP) to which DIBP is hydrolysed are also available and summarised below. MIBP is likely to be the major metabolite of DIBP (Mentlein and Butte, 1989).

In albino rats (strain unknown), a feeding study over a period of four months is reported by Hodge (1954). Body weights and haematological parameters were measured. Organ weights were determined at autopsy. Livers and kidneys were examined histologically. Groups of rats (5/sex/group) were fed 0, 0.1, 1.0 and 5.0% DIBP in the diet. These dose levels were equivalent to 0 or to about 70, 700 and 3500 mg/kg bw/d in both sexes (calculated on an assumed daily food intake of 7% of the body weight). Retarded growth was observed at dosages of 1.0% and above DIBP in feed. Significantly decreased body weights were observed in both sexes at 5.0% (decrease up to 43% for males and 13% for females). The intake of 5.0% DIBP caused slight reduction in red blood cell counts in males and in haemoglobin values in both sexes. Both absolute and relative testes weights were considerably reduced in the 5.0% group. No statistical analyses were conducted but

reductions were noted to approximately 30% and 50% of control values respectively. Absolute and relative liver weights were raised in the 5.0% groups of both sexes. For males, absolute weights were increased by 5%; relative weights by 80%. For females, absolute weights were increased by 40%; relative weights by 60%. Pathological examinations of liver and kidney were unremarkable.

Hodge (1954) also reported on a feeding study in dogs. One male and one female dog (species unknown) were fed with DIBP via diet at a daily rate of 0.1 ml/kg feed and 2.0 ml/kg feed respectively (equivalent to about 2.6 mg/kg bw/d and 51.9 mg/kg bw/d, calculated on an assumed daily food intake of 25 g/kg bw) for a period of two months. Weight loss was noted in the female dog at the last three treatment weeks. No abnormality was detected in the haematological and urine analyses as well as in gross pathology in both sexes. Organ weight assessment revealed an increase in relative liver weight compared to historical controls in female dog, no histological abnormalities in the liver were observed. In the male dog given 2.6 mg/kg bw/d DIBP, histological examination revealed abnormally few matured sperm in the testes.

To evaluate the effects of DIBP on the testes one week feeding toxicity studies in male rats and mice were performed, especially testosterone and zinc concentrations in the testes as an important role in the maintenance of testicular function were examined.
Feeding a diet containing 2.0% (approximately 1500 mg/kg bw/d, calculated on an assumed daily food intake of 7% of the mean body weight of 108 g) of DIBP to 10 male rats (JCL: Wistar, 5 weeks old) resulted in significantly decreased zinc concentrations in the testes and liver. Testosterone concentrations in the testes were increased but appeared normal in the serum. The testes of DIBP-treated rats were reduced in size when compared to controls, and organ weight assessment revealed significantly ($p<0.05$) decreased absolute and relative testicular weights in these rats. Microscopy indicated marked inhibition of spermatogenesis and desquamation of spermatocytes (Oishi and Hiraga, 1980c).
In a comparable study in male mice zinc and testosterone concentrations in tissues were determined, and body and organ weights of testes, liver and kidneys were evaluated, however microscopy was not performed. Administration of 2.0% (approximately 2000 mg/kg bw/d, calculated on an assumed daily food intake of 10% of the body weight) of DIBP in the diet to 10 young male mice (JCL: ICR) revealed significantly decreased zinc concentrations in the testes. The concentration of testosterone in the testes of DIBP-treated mice was not different from control values. The relative weights of the testes and liver of DIBP-treated mice were significantly higher, but the absolute testis weight was not different from control values (Oishi and Hiraga, 1980b).

The purpose of studies with MIBP was to discover whether phthalic acid monoesters including their metabolites have similar effects to their diesters regarding effects on the testes and alterations in zinc and testosterone concentrations.
Administration of 2.0% MIBP (corresponding to total intake of 2300 mg/kg bw/d) in the diet to 10 male rats (JCL: Wistar, 5 weeks old) for 7 days resulted in significantly suppressed food consumption throughout the experimental period, depressed body weight gains (69% of controls), and significantly decreased absolute and relative testes weights (60% of controls). Examination on the concentration of zinc in the testes, liver, kidneys and serum showed significantly decreased values in the testes and liver (60% and 90% of control values). Testosterone concentrations in the testes and serum were significantly increased by 260% and 160% of control values. Microscopy was not performed in this study (Oishi & Hiraga, 1980d).

In a further rat study 800 mg/kg bw/d MIBP was administered by gavage to young male Sprague-Dawley rats (80-100 g) daily for six days. MIBP was given in aqueous solutions as the ammonium salt (pH 6.0). Control animals received an equivalent amount of ammonium chloride (pH 6.0).

Liver, kidneys, testes and accessory sex organs were weighed, and testes and accessory sex organs were examined by light microscopy. Additionally zinc metabolism was examined in 9 rats received [^{65}Zn]Cl_2 (50 µCi/kg body wt.) i.p. 48h prior to treatment with MIBP for 4 days. The ^{65}Zn content was determined in liver, kidney and testes. Urinary ^{65}Zn excretion was examined over a 24-h period following 4 days of treatment. Treated rats developed markedly reduced absolute and relative testes weights (73%, P<0.001), and lowered seminal vesicle weights (not significant) compared to control values. No differences were evident from prostate weights. Microscopy revealed in all six examined animals marked testicular atrophy of the majority of the seminiferous tubules with a diminution of both spermatocytes and spermatogonia. In all instances the lesions were bilateral in origin. No abnormalities were detected in sections of prostate or seminal vesicles. The zinc metabolism was adversely altered by significantly increasing urinary zinc excretion concomitant with decreased ^{65}Zn testicular content and elevated renal ^{65}Zn content (Foster et al., 1981).

In a mice study body weights and organ weights of testes, liver and kidneys were evaluated; and zinc concentration in testes, liver and kidneys was determined, and testosterone concentration in the testes. Feeding of 2.0% (approximately 2000 mg/kg bw/d, calculated on an assumed daily food intake of 10% of the body weight) MIBP in the diet to 10 male mice (JCL: ICR, 5 weeks old) for 7 days resulted in significantly increased relative liver and testes weights associated with decreased body weight, whereas the absolute weights did not differ from control values. The average zinc level in the testes of MIBP-treated mice was significantly lower than the control value and did not differ in liver and kidneys. Testosterone concentration in the testes was significantly decreased (Oishi & Hiraga, 1980a).

4.6.2 Repeated dose toxicity: inhalation

4.6.3 Repeated dose toxicity: dermal

4.6.4 Other relevant information

4.6.5 Summary and discussion of repeated dose toxicity:

DIBP induces microscopic testicular atrophy associated with markedly reduced testes weights in rats and alterations in zinc and testosterone concentrations in rats and mice. Dietary administration of MIBP (the correspondent monoester, mono-iso-butyl phthalate to which DIBP is hydrolysed) induced also severe atrophy of the testes, high testosterone concentration in the testes, and low zinc concentration. Testicular effects have been demonstrated in adult male rats after repeated oral exposures to high doses of DIBP or MIBP. Decreased absolute and relative testes weights were seen in rats fed a diet containing 3500 mg/kg bw/d DIBP for a period of four months, but also in 5-week-old rats fed 2.0% (approximately 1500 mg/kg bw/d) DIBP in their diet for seven days. Markedly reduced absolute and relative testes weights were also observed in rats treated with ≥ 800 mg/kg bw/d MIBP for 7 days. In mice, treatment with approximately 2000 mg/kg bw/d DIBP or MIBP in the diet for the same duration of treatment did not show any effect on absolute testes weights but resulted in significantly increased relative testes weight.

Marked inhibition of spermatogenesis and desquamation of spermatocytes were observed in 5-week-old rats fed 2.0% (approximately 1500 mg/kg bw/d) DIBP in their diet for seven days. Diminished sperm was noted in a study used one adult dog given 2.6 mg/kg bw/d DIBP in the diet for two months. Severe testicular injury as seen as marked testicular atrophy of the majority of the

seminiferous tubules with a diminution of both spermatocytes and spermatogonia was caused by treatment with 800 mg/kg bw/d MIBP for one week.

The role of zinc and testosterone as elements essential for the maintenance of normal testicular function has been analysed in short term studies with male rats and mice. The examination of DIBP effects on the zinc content in the testes showed related results in both species. In rats and mice significantly decreased zinc concentrations were measured in the testes after feeding of 2.0% DIBP in their diet for seven days. The average zinc level in the testes of rats and mice treated with ≥ 800 mg/kg bw/d MIBP for one week was also significantly decreased. Testicular testosterone concentration was significantly increased in rats fed 2.0% (approximately 1500 mg/kg bw/d) DIBP, and also in rats fed 2300 mg/kg bw/d MIBP in their diet for seven days. No effect on the testosterone content was observed in mice receiving approximately 2000 mg/kg bw/d DIBP for 7 days, but was significantly decreased in mice treated with MIBP for the same study period.

4.7 Mutagenicity

Not relevant for this type of document.

4.8 Carcinogenicity

Not relevant for this type of document.

4.9 Toxicity for reproduction

4.9.1 Effects on fertility

Whereas no fertility studies (one-, two- or multigeneration studies) could be identified in the current toxicological data base for DIBP, adverse effects on male reproductive organs (testicular toxicity) and on spermatogenesis had been observed during repeat dose toxicity studies with DIBP and with MIBP at relatively high dosages (c.f. 5.6.1 Repeated dose toxicity: oral), indicating that the monoester MIBP to which DIBP is hydrolysed by human and rat hepatic esterases (Mentlein and Butte, 1989) should be considered an active metabolite.

4.9.2 Developmental toxicity

Prenatal developmental toxicity studies

In a comparative study on eight different phthalate esters Singh et al. (1972) treated pregnant Sprague Dawley rats (n = 5/group) with DIBP at single doses of 0.375, 0.75 and 1.25 ml/kg bw (approximately 390, 780 and 1300 mg/kg bw) by intraperitoneal injection on three different days during gestation. Animals of the control groups were either untreated or received a similar volume of distilled water, normal saline or cottonseed oil. The pregnant females were treated on 3 days during gestation (GD 5, 10 and 15) and were sacrificed on GD 20, one day prior to parturition. Ovaries were taken for recording of the numbers of corpora lutea; uterine horns were taken for recording the numbers of resorption sites, and of dead and viable fetuses. Fetuses were weighed and examined for gross malformations. A randomly selected number of fetuses (30-50% of the total) was taken for evaluation of skeletal malformations. Investigation on any maternal parameters is not reported from the study. As a result, there was no difference observed in the number of corpora lutea at any dose level in comparison to the controls. An increase in resorptions (25.8%) was revealed at the high dose level, indicating an embryotoxic potential and leading to a decrease of the

number of live fetuses. At the dose level of 0.75 mL/kg bw 2 out of a total of 52 fetuses were found dead, however, at the low and high dose level only life fetuses were recorded. The average weight of fetuses was reduced in comparison to controls at all dose levels. Gross abnormalities (not further specified) were observed in two fetuses, however at the dose level of 0.75 ml/kg bw only and an increased incidence in skeletal abnormalities was reported for the high dose level (not further specified).

In a further study (Borch et al., 2006; cited in: Boberg et al., 2008) mated female Wistar rats (n=8/group) were gavaged from GD 7 until GD 19 or until GD 20/21 with either vehicle (corn oil) or 600 mg/kg bw/d of DIBP (purity 99%), when they were sacrificed and their male offspring evaluated. At sacrifice on GD 19 five dams from the control and six dams from the treated group provided litters and at sacrifice on GD 20/21 six dams from the treated group provided litters. Anogenital distance (AGD) was measured in all fetuses, fetuses were decapitated and their trunk blood collected, and from males testes removed for histopathology and for immunohistochemistry, for measurement of testosterone production ex vivo, respectively measurement of testosterone content. Administration of DIBP resulted in statistically significant reduction in AGD in male pups (and increased AGD in female pups) at GD 20/21 together with 10 % reduction in bodyweights of male and female fetuses and in a significant reduction in testicular testosterone production and testicular testosterone content in the male offspring at GD 20/21. Histopathological investigations revealed testes pathology as seen with other phthalates, in particular clustering of small Leydig cells on GD19 or GD20/21 and vacuolisation of Sertoli cells on GD 20/21. Immunohistochemistry revealed reduced staining for StAR and P450scc, indicative for reduced expression of these two proteins and thereby reduced capacity of the testicular steroid synthesis. Further results from this study were reported by Boberg et al. (2008), who quantified levels of insulin, leptin, MCP1, IL-1B, PAI-active, IL6, and TNFα in pooled samples of plasma. In addition, livers, adrenals and testes tissue from the male fetuses and ovaries from the females had been used for gene expression (mRNA expression) analysis and for steroid hormone measurements (estradiol, testosterone). Treatment with DIBP had resulted at GD 21 in statistically significant reduction of protein levels of insulin and of leptin, whereas no alterations were seen in plasma levels of MCP1, IL-1B, PAI-1 active, IL6 or TNFα. Gene expression analysis on genes involved in steroid synthesis revealed reduced testicular mRNA levels of SR-B1, StAR, P450c17, P450scc and Insl-3 at GD 19 and GD 21. In addition testicular SF-1 mRNA levels were reduced on GD 19, whereas no alterations were seen for testicular mRNA levels of aromatase or PBR. In the ovaries of DIBP treated animals an increase in mRNA levels of aromatase was revealed at GD 21. Gene expression analysis on PPARα and on PPARγ revealed significantly reduced mRNA levels of PPARα in livers and testes of DIBP exposed males at GD 19 but not at GD 21. PPARγ mRNA levels were very low in both testes as well as livers and appeared unaltered by DIBP treatment. In the ovaries of DIBP treated animals no alterations were seen in the expression of ERα, ERβ, PPARα, or PPARγ. Besides reductions in mRNA levels there were also indications for reduced protein levels of P450c17 and of PPARγ in the Leydig cells of DIBP treated animals at GD 19 and GD21 (evidenced from reduced immunostaining intensity).

In a dose-range finding study on Sprague-Dawley rats, DIBP was administered to pregnant animals (10-14 animals per treatment group) by gavage at doses of 0 (olive oil), 250, 500, 750 and 1000 mg/kg bw/d (Saillenfait et al., 2005) on GD 6-20. Maternal body weights and clinical signs were recorded. Dams were euthanised on GD 21, and the uterine contents were evaluated for number of implantations, resorptions, fetal deaths, and live fetuses. All live fetuses were submitted to external examination and to internal gross examination of the reproductive tract. Maternal body weight gain was transiently depressed on GD 6-9 at the two higher dose levels. However, the weight gains during GD 6-21 corrected for uterine weight were comparable across groups. A marked increase in

the number of resorptions of 38% and of 61% was observed at the 750 and 1000 mg/kg bw/d dose level. A dose-related reduction in fetal body weight was observed amounting to 21% at 1000 mg/kg. Gross internal examination of the reproductive tract revealed undescended testes in 56% and 70% of the male fetuses at 750 and 1000 mg/kg. No further visceral or skeletal examinations were conducted.

In a further guideline according prenatal toxicity study on Sprague-Dawley rats, DIBP was administered to pregnant animals (23-24 animals per treatment group) by gavage at doses of 0 (olive oil), 250, 500, 750 and 1000 mg/kg bw/d on GD 6-20 (Saillenfait et al., 2006). Endpoints included in addition were determination of the degree of transabdominal testicular migration (TTM). There were no maternal deaths. Signs of transient maternal toxicity were observed, as evidenced by reduction in body weight gain, at the beginning of treatment (GD 6-9) at 500 mg/kg bw/d and higher doses, however, overall weight gain corrected for gravid uterus was not different from controls at the end of gestation. No changes could be observed for maternal food consumption, pregnancy rate or number of implantations. The incidences of resorptions were statistically significantly increased to 28% at 750 mg/kg bw/d and to 59% at 1000 mg/kg bw/d. Mean fetal body weight was statistically significantly reduced at 500 mg/kg/d and higher doses amounting to a decrease of 24% -26% at 1000 mg/kg/d in comparison to controls. The incidence of total external malformations (neural tube closure defects, anophthalmia) and of total visceral malformations (urinary tract and vascular defects) was statistically increased at 750 and 1000 mg/kg bw/d. Skeletal evaluations revealed malformations primarily of the axial column with the incidences of fused sternebrae statistically significantly increased at 750 and 1000 mg/kg bw/d and variations (delayed ossification and supernumerary ribs) at 750 and 1000 mg/kg bw/d with supernumerary ribs in 95% of the fetuses of the 1000 mg/kg group. Visceral variations involved mainly the urinary tract with statistically significantly increased incidences of ureter variations in the 1000 mg/kg group and the male reproductive system. Unilateral or bilateral undescended testes occurred at 500 mg/kg/d and was significantly increased at 750 mg/kg/d (in 30/55 male fetuses and in 16/20 litters) and at 1000 mg/kg bw/d (in 30/34 male fetuses and in 16/17 litters). In addition the degree of transabdominal descent was significantly impaired at 500 mg/kg/d with about two third of the testes located in the upper half of the abdominal cavity at the 1000 mg/kg dose group. Thus, it appeared that alterations of the male reproductive system occurred at lower doses than those producing structural malformations/variations and embryotoxicity. No evidence of embryo or fetal effects was found at the 250 mg/kg dose level. Therefore, a NOAEL/developmental toxicity of 250 mg/kg/d can be derived from the study.

In a further study on Sprague-Dawley rats, which was designed to provide dose-response information on the effects of a series of individual phthalates on fetal testosterone production and on the use of the data obtained for the prediction of effects of phthalate mixtures on fetal testosterone production, DIBP was administered to pregnant animals (5-8 animals per treatment group) by gavage at doses of 0 (corn oil), 100, 300, 600, and 900 mg/kg bw/d on GD 8-18 (Howdeshell et al., 2008). Maternal body weights were taken on GD 8 and on GD 18 at sacrifice, when the uterus was removed and the number of fetuses (live and dead) and resorptions were counted and recorded. The total number of implantations was calculated by adding together the number of live and dead fetuses with the total number of resorptions. Fetal mortality was calculated by adding together the number of resorptions and dead fetuses then divided by the total number of implantations. Testes from three males/dose group (2 replicate determinations in individual testes) in were used for investigation of ex vivo testis testosterone production. Maternal body weight gain was reduced from 73 g in controls to 48 and 43 g in the 600 and 900 mg/kg/d dose group.
DIBP-induced complete litter loss in 1/5 dams at 900 mg/kg/d, and induced greater than 50% resorptions in 2/5 dams at 900 mg/kg/d and in 1/5 dams at 600 mg/kg/d resulting in increased

percentages of fetal mortality of 17% at 600 mg/kg/d and of 59% at 900 mg/kg/d as compared to 1.3% in the controls. It is reported, that many of the testes collected from DiBP fetuses at dosages of 600 and 900 mg/kg/d were smaller, mucinilagous, and/or located higher in the abdominal cavity. The functional assay on testes ability for hormone production revealed that fetal testicular testosterone production was statistically significantly ($p<0.001$) reduced at dosages of 300 mg/kg/d or higher. The overall results indicated that DIBP (as well as DBP and BBP) was of equivalent potency to DEHP at reducing fetal testosterone production. Dosage levels reducing fetal testosterone production were about one-half to one-third of that required to increase fetal mortality, indicating changes in fetal testicular testosterone production to be a sensitive parameter. Based on statistically significantly lower fetal testosterone production at 300 mg/kg/d a NOAEL of 100 mg/kg bw/d can be derived from this study.

A further guideline according prenatal toxicity study on Wistar rats (BASF, 2003; cited in Saillenfait and Laudet-Hesbert, 2005) with dietary administration is indicated in the data base, for which the study report is not available to the rapporteur. It is reported that a decrease in fetal weights and an increase in skeletal variations was observed in rats that had ingested 942 mg/kg DIBP with their diet during pregnancy.

Postnatal developmental toxicity studies

In a study on Sprague-Dawley rats, which was performed to determine whether in utero exposure to DIBP would induce permanent and dose-responsive alterations of male reproductive development, DIBP was administered to pregnant animals (11-13 animals per treatment group) by gavage at doses of 0 (olive oil), 125, 250, 500, and 650 mg/kg bw/d on GD 12-21 (Saillenfait et al., 2008). Doses were based on an unpublished preliminary study in which 625 mg DIBP/(kg day) on GD 12-21 caused reproductive tract malformations in male offspring and had no effects on litter size or pup survival. Litters of the definite study were examined as soon as possible after birth to determine the number of viable and stillborn pups. Pup body weights were recorded on PND 1, 4, 7, 14 and 21. AGD was measured on PND1 and litters culled to 10 pups on PND 4. All pups were examined for the presence of areola and/or nipples on the ventral surface of the thorax on PND 12-14. At weaning on PND 21 three to four male pups from each litter were randomly selected and retained and unselected pups sacrificed and submitted to internal examination. After weaning the dams were sacrificed and the number of implantations recorded from their uteri. All retained males were examined for preputial separation (PPS) and individual body weights recorded at acquisition. Adult males were necropsied on PND 76-86 (two males in each litter) or on PND 111-122 (the remaining males in each litter). They were examined for the presence of areolas and/or nipples on the ventral surface of the thorax, for gross abnormalities of external and internal genitalia, and for position of testes. Testes, epididymides, seminal vesicles (with the coagulating glands and seminal fluid), and prostate were weighed. Histopathology was conducted on testes and epididymides of all DIBP animals necropsied on PND 76-88. No differences in maternal body weight gain were observed between the controls and the treatment groups. All dams delivered live pups. Post-DIBP implantation loss, litter size, sex ratio, and pup survival to PND 4 and PND 21 were unaffected by treatment. AGD measured on PND 1 was dose-dependently significantly reduced in male pups from 250 mg DIBP/(kg day) to the higher doses with or without adjustment for body weight. The decrease amounted to 11% at 250 mg DIBP/(kg day) and 22% at 625 mg DIBP/(kg day), compared to controls. AGD of females was not affected at any dose. Pup body weight at PND 1 of both sexes was statistically significantly decreased at 625 mg DIBP/(kg day), and remained lower in comparison to controls in the male pups at weaning. During the post weaning period mean body weights of the offspring were lower than controls at 500 and 625 mg DIBP/(kg day) (6-8% and 10-12%, respectively). On PND 12-14 or at adult necropsy retained areolas and/or nipples were

apparent in males at 250 mg DIBP/(kg day) and their incidence increased with dose. No such effects were observed in animals from vehicle controls or the 125 mg DIBP/(kg day) treated group. Acquisition of PPS was delayed by approximately 4 days at 500 mg DIBP/(kg day). Evaluation of PPS was precluded in half of the males at the high dose by presence of hypospadias. Mature males displayed severe malformations (hypospadias with exposed os penis in the more severely affected animals, and non-scrotal testis) at the two high doses. Non-descended testes were always located in the inguinal or supra-inguinal area; none were in the intra-abdominal position. Markedly underdeveloped (less than 10% of control weight) or absent testes and/or epididymes were seen in 2%, 16% (7 males from 5 litters), and 13% (5 males from 4 litters) of the animals in the 250, 500 and 625 mg /(kg day) dose groups. At sacrifice (PND 76-86, resp. PND 111-122) organ weights of the testes, epididymes, seminal vesicles and prostate were significantly reduced (with or without body weight as covariate) at 500 and 625 mg DIBP/(kg day). These reductions amounted to 39-59% for the testes and the epididymes, and 28-33% for the seminal vesicles and the prostate. Histological examinations revealed testicular damage in all DIBP treated groups with moderate or severe degeneration of seminiferous tubules (including Sertoli cell only tubules). The lesions were uni- or bilateral and associated with oligospermia or total azoospermia in the corresponding epididymides. Based on these observations a NOAEL/developmental toxicity could not be determined. Therefore, a LOAEL/developmental toxicity of 125 mg DIBP/kg bw/day can be derived from this study.

DIBP was further evaluated in a Chernoff-Kavlock screening assay in which CD-1 mice (50 dams/group) were gavaged on GD 6-13 with a single dose level of 4000 mg/kg bw/d or corn oil (Hardin et al., 1987). Dams were allowed to litter and a postnatal evaluation was conducted. At that dose, no pregnant dams gave birth to a live litter and 27/50 exposed dams died.

4.9.3 Human data

In the attempt to explore whether prenatal exposure to phthalates would be reflected in postnatal performance of genital parameters concentrations of 11 maternal urinary phthalate monoesters were determined in spot urine samples taken prenatally during pregnancy and associated to parameters such as anogenital index (AGI) – a biomarker suspected to be indicative of androgen action also in humans - and testicular descent in the male infants in a cohort of 85 mother-son pairs (Swan et al., 2005). In this investigation maternal urinary MIBP concentration was found to be inversely related to AGI, and that in general the boys classified as having a short AGI (AGI below 25th percentile for age) also had a higher prevalence of concomitant cryptorchidism. Although of limited value, due to the small number of subjects (n=85) and to other shortcomings (e.g., concentrations of phthalate metabolites in spot urine samples may not be representative for and adequately reflect maternal exposure during pregnancy), data of this study may support the hypothesis that prenatal phthalate exposure at environmental levels may affect male reproductive development also in humans. It should be noted, in addition, that little is known on the normal variation of AGD in human infants to adequately interpret the findings on AGI values lower than expected and that any long-term clinical implications of a shorter than expected AGD in humans has not yet been revealed.

4.9.4 Other relevant information

Mode of action – in vitro assays

DIBP was negative for oestrogenic activity in a yeast two-hybrid assay (Nishihara et al., 2000) and showed extremely weak oestrogenic activity in a recombinant yeast assay and in cell proliferation assays with MCF-7 and ZR-75 cells (Harris et al., 1997). In a commercial Ligand Screening Assay DIBP (up to 10^{-5}M) had no binding affinity for the oestrogen receptor α or β in vitro (Toda et al.,

2004). In a reporter gene assay DIBP was found to induce oestrogen receptor hERα-mediated oestrogenic activity (at 10^{-5}M) and possess antiandrogenic activity in vitro but showed no activity towards hERβ in CHO-K1 cells (Takeuchi et al., 2005).

4.9.5 Summary and discussion of reproductive toxicity

The available data base has been evaluated for the toxic potential of DIBP adverse to reproduction and development. DIBP was found to adversely affect the reproductive organs in adult males in experimental studies which may affect their fertility. DIBP was also found to be a developmental toxicant. The results of these evaluations are reflected in the classification as Repr. Cat 3; R62 and Repr. Cat 2; R 61 according to directive 67/548/EEC.

Any generation or fertility studies are not available in the toxicological data base for DIBP. However, adverse effects on male reproductive organs (testicular toxicity) and on spermatogenesis had been induced in studies with young adult male rats and mice after repeated oral administration of DIBP at relatively high dosages. Similar effects were also revealed after treatment with MIBP, the major monoester metabolite of DIBP. A NOAEL has not been established from theses studies for testicular toxicity in adult males. Further, the data base lacks information for evaluation of any effects on female fertility or effects on the female adult reproductive system.

Studies related to developmental toxicity revealed embryotoxic, fetotoxic and teratogenic properties of DIBP. In a guideline according study embryolethality in terms of an increase in resorptions, fetal growth retardation in terms of significantly reduced fetal body weight and structural defects were observed in the skeletal system and in various organ systems including the male reproductive system at dosages ($\geq$ 500 DIBP mg/kg bw/d) without signs of maternal toxicity, respectively early transient maternal weight gain effects only. DIBP exposures focused to a sensitive period (GD 12-21) caused preferential and permanent effects on the male reproductive system at lower doses than those inducing embryolethality and malformations not related to the reproductive system. Impact on AGD at birth and areola/nipple retention in male progeny during early postnatal live ($\geq$ 250 mg/kg bw/d) and changes in testicular histopathology of in utero exposed male progeny ($\geq$ 125 mg/kg bw/d) appeared to be sensitive markers for DIBP induced effects on the development of the male reproductive system. Changes in fetal testicular testosterone production, however, revealed to be the most sensitive parameter for the adverse effects of DIBP on development of the male reproductive system, based on which a NOAEL/developmental toxicity of 100 mg/kg bw/d. The pattern and types of malformation of the male reproductive system observed with DIBP did not differ from those seen after treatment with DNBP, principally consisting of cleft prepuce, hypospadias, and inguinal or supra-inguinal testis. Incidences of hypospadias and of undescended testes were lower for DIBP when compared for equal dosages. However, in reducing fetal testosterone production DIBP was of equivalent potency as DEHP, DBP and BBP, phthalates which are also potent reproductive toxicants.

The structures affected by in utero exposure to DIBP are indicative of an antiandrogenic mode of action. In particular, the development of dihydrotestosterone-regulated tissues (e.g. areolas/nipples, external genitalia including AGD and hypospadias) were severely affected. DIBP had also marked effects on the final inguinoscrotal descent, which is known to require androgens. The changes in the androgen-dependent endpoints induced by DIBP treatment are congruent with the findings of lowered fetal levels of testosterone and changes in the expression of several genes in the cholesterol uptake, transport and testicular testosterone biosynthesis in other studies. Although phthalates do not act as classical antiandrogenic chemicals by binding to the androgen receptor, they obviously

have the same effects of blocking androgen-action at the target tissue and therefore may be considered as acting antiandrogenic.

4.10 Other effects

Not relevant for this type of document.

4.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant for this type of document.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Since this is a support document targeted to the identification of DIBP as a CMR substance, no environmental hazard assessment has been carried out.

6 PBT, VPVB AND EQUIVALENT LEVEL OF CONCERN ASSESSMENT

Since this is a support document targeted to the identification of DIBP as a CMR substance, no PBT, vPvB and equivalent level of concern assessment has been carried out.

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