Substance name: Dibutyl phthalate
EC number: 201-557-4
CAS number: 84-74-2

MEMBER STATE COMMITTEE SUPPORT DOCUMENT FOR IDENTIFICATION OF DIBUTYL PHTHALATE (DBP) AS A SUBSTANCE OF VERY HIGH CONCERN

Adopted on 1 October 2008

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Substance Name: Dibutyl phthalate

EC Number: 201-557-4

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The substance is identified as a CMR according to Article 57 (c) of Regulation (EC) 1907/2006 (REACH).

Summary of the evaluation:

According to Annex I to Directive 67/548/EEC, Dibutyl phthalate (DBP) is classified as a substance toxic to reproduction Repr. Cat. 2; R61 (May cause harm to the unborn child).

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

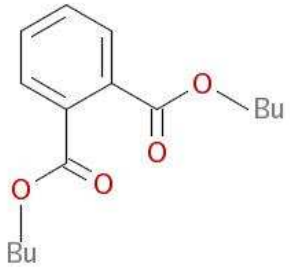
1.1 Name and other identifiers of the substance

Chemical Name: Dibutyl phthalate
EC Number: 201-557-4
CAS Number: 84-74-2
IUPAC Name: Dibutyl phthalate

1.2 Composition of the substance

From EU RAR (2004)

Chemical Name: Dibutyl phthalate
EC Number: 201-557-4
CAS Number: 84-74-2
IUPAC Name: Dibutyl phthalate
Molecular Formula: $C_{16}H_{22}O_4$
Structural Formula:



Molecular Weight: 278.34 g/mol
Typical concentration (% w/w): Degree of purity ≥ 99% (w/w)
Concentration range (% w/w): -
Impurity: ca. 0.01% (w/w) butal-1-ol (CAS 71-36-3)
ca. 0.01% (w/w) butyl benzoate (CAS 136-60-7)
Additives: none

1.3 Physico-chemical properties

Table 1: Summary of physico- chemical properties, from EU RAR 2004

REACH ref Annex, §	Property	IUCLID section	Value	Reference
VII, 7.1	Physical state at 20°C and 101.3 kPa	3.1	oily liquid	
VII, 7.2	Melting/freezing point	3.2	-69°C	DIN-ISO 3016 BASF AG Ludwigshafen; Huels AG Marl Sicherheitsdatenblatt Palatinol C 25.4.1994
VII, 7.3	Boiling point	3.3	340°C at 10,013 hPa	BASF AG Ludwigshafen/Kirk-Othmer 1982; Huels AG Marl/*i.a.* Kemppinen & Gogcen 1956
VII, 7.5	Vapour pressure	3.6	$9.7 \pm 3.3 \times 10^{-5}$ hPa at 25°C	BASF AG Ludwigshafen; Huels AG Marl Banerjee & Howard, 1984
VII, 7.7	Water solubility	3.8	10 mg/L at 20°C	
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	log K_{ow} 4.57 at 20°C	measured (Huels AG Marl/Leyder & Boulanger, 1983) and calculated (BASF AG Ludwigshafen/BASF AG, 1987)
IX, 7.16	Dissociation constant	3.21		

2 CLASSIFICATION AND LABELLING

2.1 Classification in Annex I of Directive 67/548/EEC

Dibutyl phthalate (DBP) was inserted into Annex I of Directive 67/548/EEC with the 28th ATP (Commission Directive 2001/59/EC of 6 August 2001) and is classified as follows:

Index: Number: 607-318-00-4

Repr. Cat. 2; R61 (May cause harm to the unborn child)
Repr. Cat. 3; R62 (Possible risk of impaired fertility)
N; R50 (Dangerous for the Environment: Very toxic to aquatic organisms)

Specific concentration limits: none
Labelling:

Symbols: T; N
R-Phrases: 61-62-50
S-Phrases: 53-45-61

2.2 Self classification(s)

Not relevant.

3 HUMAN HEALTH HAZARD ASSESSMENT

3.1 Toxicity for reproduction

3.1.1 Effects on fertility

3.1.2 Developmental toxicity

3.1.3 Human data

3.1.4 Other relevant information

Estrogenic activity of DBP has been shown in some special *in vitro* assays, however the effects were weak and not confirmed in *in vivo* studies. Therefore the relevance of the estrogenic effects observed in vitro for the in vivo estrogenic toxicity of DBP is questionable. Moreover the results of the developmental studies were indicative of an **antiandrogenic effect** of DBP rather than an estrogenic effect (Mychlreest et al., 1998).

Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Material in Contact with Food (AFC) on a request from the Commission related to Di-Butylphthalate (DBP) for use in food contact materials, Question N° EFSA-Q-2003-192Adopted on 23 June 2005 by written procedure

According to the recent opinion of the Scientific Panel on food additives, flavourings, processing aids and material in contact with food (AFC) on a request from the Commission related to DBP for use in food contact materials (2005) a TDI of 0.01 mg/kg bw based on a LOAEL of 2 mg/kg bw/day (allocating an uncertainty factor of 200) was established. The LOAEL was derived from a recent developmental toxicity study in rats (dietary exposure during late gestation to end of lactation) showing developmental effects at 20 mg/kg bw (Lee et al., 2004 cited in EU RAR).

In vitro assays

The potential estrogenic activity of DBP was demonstrated in an *in vitro* study with human breast cancer MCF-7 cells by induction or repression of endogenous estrogen- regulated marker genes (*pS2, MAO-A, TGFß3, α1ACT).* Estrogenic activity was demonstrated for DBP, but was much lower than those of Estradiol (E_2), Diethylstilbestrol and Zearalenon. For example the expression level of pS2 was induced almost 25-fold after exposure to 10^{-10}M E2 whereas the induction after exposure to 10-4 M DBP was 10- fold (Jorgensen et al., 2000).

Animal studies

Fenell et al. (2004) investigated pharmacokinetics of DBP in pregnant rats and found Mono-butyl-phthalate to be the major metabolite of DBP. With increasing dose, there was a nonlinear increase in area under curve (AUC) for MBP, with a ten-fold increase in fetal plasma between 50 mg/kg and 250 mg/kg. In amniotic fluid, the major metabolite was MBP glucuronide. This study indicated that MBP, the active metabolite of DBP can cross the placenta in late gestation, and that the metabolism of MBP is saturable.

The apparently increasing incidence of the testicular dysgenesis syndrome (TDS) in men is cause of concern, which leads to research for possible causes and reasons by researchers all over the world. In the study of Fisher et al. (2003) a model was established to investigate in-utero exposure of the rat to DBP. According to the authors, it was the abnormal development of Sertoli cells, leading to abnormalities in other cell types the reason for the abnormal changes in DBP-exposed animals. As the testicular and other changes in DBP-exposed rats have all been reported in human TDS, DBP exposure in utero seems to provide a useful model for defining the cellular pathways in TDS (Fisher et al., 2003).

In the study of Mahood et al. (2007) infertility and cryptorchidism and indicators within the fetal testis of dysgenesis were investigated in the offspring of DBP treated rats (4 – 500 mg/kg DBP). The fetal end points analyzed (testicular testosterone levels, abnormal LC aggregation, occurrence of multinucleated gonocytes MNGs) were most sensitive to disruption by DBP, as all were significantly affected at a dose of 100 mg/kg/day DBP, with a trend toward effects occurring at 20 mg/kg/day DBP; adult end points were affected consistently only by 500 mg/kg/day. The fetal end points can be objectively quantified and may prove helpful in evaluating the health risk of exposure to DBP and other phthalates, as well as identifying DBP-sensitive fetal events that have adult consequences/end points that are identifiable in human TDS.

Findings in humans

Studies in humans, with limited value, due to the small number of subjects (n=85) indicate that prenatal exposure with phthalates (i.e. DBP) at environmental levels may affect male reproductive development in humans. Prenatal exposure to DBP, measured as MBP concentrations in the urine of mothers, was inversely related to the anogenital distance (AGD) in the boys (mean age 12,5 month) (Swan et al, 2005 cited in EU RAR 2004; EU RAR, 2007). According to the RAR of BBP, studies with larger sample size are warranted to draw further conclusions (EU RAR, 2007).

In a study by Main et al. (2005) monoester metabolite contamination of human breast milk was investigated on the possible influence on the postnatal surge of reproductive hormones in newborn boys as a sign of testicular dysgenesis (Danish - Finnish cohort study in cryptorchism 1997-2001). The median concentration of MBP in breast milk was 9,6 µg/l. Higher MBP concentrations were positively correlated with SHBG (sex-hormone binding globuline), LH (luteinising hormone)/free testosterone ratio and negatively correlated with free testosterone. No association was found between phthalate monoester levels in breast milk and cryptorchidism.

Duty et al. (2005) investigated the relationship of urinary phthalate levels and serum hormone levels in men. An increase in hormone levels of Inhibin B was found in men with higher urinary metbolites of MBP, whereas according to findings in previous studies a decrease was expected. No increase of FSH levels could be detected. The authors conclude that further analyses with a larger study population are warranted.

Hauser et al., (2006) reported altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites in humans. Semen from 463 male partners of subfertile couples was investigated and dichotomized according WHO reference values for sperm concentration and motility as well as the Tygberg Kruger Strict criteria for morphology. Results were adjusted for age, abstinence time and smoking status. MBuP (mono-n-butyl-phthalate) was

found in 97% of the samples. A dose -response relationship of MBuP with low sperm concentration and motility was found. This result is consistent with studies in laboratory rodents showing testicular toxicity. The study confirms previous results on the relationship of altered semen quality with exposure to MBuP at general population levels. Another study of Jonsson et al. (2005), performed in Swedish young men recruited to medical examination prior to military service found no relationships of MBuP with any of the semen parameters; however there were important differences in study population, study design, analytical methods and statistical analyses.

In studies commissioned by DG Environment of the European Commission a list of 146 substances with endocrine disruption properties has been established (http://ec.europa.eu/environment/docum/pdf/bkh_annex_13.pdf). DBP has been classified as Cat. 3 for wildlife, Cat. 1 for Humans and Combined as Cat. 1 (Cat.1: Evidence for endocrine disruption in living organisms; Cat. 2: Evidence of potential to cause endocrine disruption; Cat.3: No evident scientific basis). DBP is also listed in the list of 66 potentially endocrine substances with classification of high exposure concern (http://ec.europa.eu/environment/docum/pdf/bkh_annex_15.pdf) (EUROPEAN COMMISSION DG ENV, 2000).

3.1.5 Summary and discussion of reproductive toxicity

Based on the available reproduction, fertility and developmental studies and according to EC criteria, DBP is classified in Category 3 for effects on fertility and in Category 2 for effects on developmental toxicity and is labelled with R-phrase 62 (Possible risk of impaired fertility) and R-phrase 61 (May cause harm to the unborn child). A LOAEL of 52 mg/kg bw was established based on embryotoxic effects in rats in the absence of maternal toxicity in a two-generation reproduction study with a continuous breeding protocol including improved sensitive endpoints (such as sperm parameters, estrous cycle characterisation and detailed testicular histopathology) and with exposure of male and female animals. The protocol of this study was supposed to adequately identify compounds with endocrine activity (NTP, 1995; Wine et al., 1997 both cited in EU RAR). Based on the available developmental studies in mice an oral NOAEL of 100 mg/kg bw was derived for teratogenicity, embryotoxicity and maternal toxicity. At the next higher dose-level of 400 mg/kg bw embryotoxic and teratogenic effects were seen in the presence of maternal toxicity (Hamano et al., 1977 cited in EU RAR). In rats developmental studies with exposure during gestation or during gestation and lactation, revealed delayed preputial separation and reproductive tract malformations in male offspring at oral doses ≥ 250 mg/kg bw. Maternal toxicity was seen at doses ≥ 500 mg/kg bw. At the lowest oral dose-level of 100 mg DBP/kg bw, studied in developmental studies in rats, still delayed preputial separation in male progeny was seen. A NOAEL could not be derived from the developmental studies in rats (Mychlreest et al., 1999 cited in EU RAR). No reproduction, fertility or developmental studies with dermal exposure or exposure by inhalation to DBP are available. An epidemiological study on possible reproductive effects in occupationally exposed women is inadequate for assessment of possible reproductive effects caused by DBP in humans in the working environment due to several limitations such as lack of an adequate control group, small study population size, adequate documentation of protocol and results and mixed exposure (Aldyreva et al., 1975 cited in EU RAR).

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