Substance name: Bis(2-ethylhexyl)phthalate
EC number: 204-211-0
CAS number: 117-81-7

MEMBER STATE COMMITTEE SUPPORT DOCUMENT FOR IDENTIFICATION OF BIS(2-ETHYLHEXYL)PHTHALATE (DEHP) AS A SUBSTANCE OF VERY HIGH CONCERN

Adopted on 1 October 2008

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TABLES

Substance Name: Bis(2-ethylhexyl)phthalate

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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, bis(2-ethylhexyl)phthalate (DEHP) is classified as substance toxic to reproduction Repr. Cat. 2; R60-61 (May impair fertility; 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: 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester

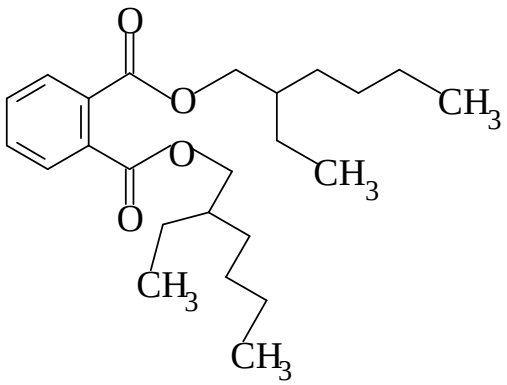
EC Number: 204-211-0

CAS Number: 117-81-7

IUPAC Name: Bis(2-ethylhexyl)phthalate

1.2 Composition of the substance

Data about purity indicate a high purity level (99.7 %). The impurities found are mainly other phthalates.

Chemical Name:	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
EC Number:	204-211-0
CAS Number:	117-81-7
IUPAC Name:	Bis(2-ethylhexyl)phthalate
Molecular Formula:	$C_{24}H_{38}O_4$
Structural Formula:	
Molecular Weight:	390.6 g/mol
Typical concentration (% w/w):	99.7
Concentration range (% w/w):	-

1.3 Physico-chemical properties

Table 1: Summary of physico- chemical properties

REACH ref Annex, §	Property	IUCLID section	Value
VII, 7.1	Physical state at 20°C and 101.3 kPa	3.1	Colourless oily liquid
VII, 7.2	Melting/freezing point	3.2	-55°C or -50°C
VII, 7.3	Boiling point	3.3	230°C at 5 mm Hg 385°C at 1013 hPa
VII, 7.5	Vapour pressure	3.6	0.000034 Pa at 20°C
VII, 7.7	Water solubility	3.8	3 µg/l at 20°C
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	7.5
XI, 7.16	Dissociation constant	3.21	-
	Henry's constant		4.43 Pa m^3/mol

2 CLASSIFICATION AND LABELLING

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

According to Annex I to Directive 67/548/EEC (28th ATP), bis(2-ethylhexyl)phthalate is classified as a substance toxic to reproduction Repr. Cat. 2; R60-61 (May impair fertility; May cause harm to the unborn child).

Index Number (Annex I of Directive 67/548/EEC): 607-317-00-9.

Council Directive 93/42/EEC of 14 June 1993 concerning medical devices, amended by Directive 2007/47/EC, defines the following labelling requirements: “If parts of a device (or a device itself) intended to administer and/or remove medicines, body liquids or other substances to or from the body, or devices intended for transport and storage of such body fluids or substances, contain Phthalates which are classified as carcinogenic, mutagenic or toxic to reproduction, of category 1 or 2, in accordance with Annex I to Directive 67/548/EEC, these devices must be labelled on the device itself and/or on the packaging for each unit or, where appropriate, on the sales packaging as a device containing phthalates.”

2.2 Self classification(s)

Not relevant

3 HUMAN HEALTH HAZARD ASSESSMENT

3.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Generally, DEHP is rapidly absorbed from the gastrointestinal tract following oral administration. The extent of absorption in rats is around 50% for doses up to about 200 mg/kg bw. At higher doses, it appears that absorption in non-human primates is dose-limited in contrast to rodents. For humans, information is not, however, available concerning the dependency of oral uptake on dose. Also, the extent of oral absorption at doses which humans are expected to be exposed is not known. Absorption may be 100% at daily exposure levels. Limited data on toxicokinetics, following inhalation or dermal exposure, indicate that DEHP can be absorbed through the lungs whereas absorption through the skin appears to be limited. Following intra peritoneal injection most of the administered dose remains in the peritoneal cavity.

Distribution studies in rat indicate that DEHP is widely distributed in the body without evidence of accumulation in the tissues in rats. A comparative study of rats and marmosets showed similar distribution patterns in the two species (oral administration) whereas rats had higher tissue levels than marmosets. Thus, the difference in distribution between species is quantitative rather than qualitative.

The metabolism of DEHP involves several pathways and yields a variety of metabolites. The major step in the metabolism of DEHP is hydrolysis by lipases to MEHP (mono(2-ethylhexyl)phthalate) and 2-ethylhexanol, which is common to all investigated species.

MEHP is a relatively major component in urine of monkeys, guinea pigs and mice but was in most cases not detected in rat urine. However, MEHP is present in plasma in all species tested. The substance is excreted via the urine, mainly as MEHP-metabolites, but some excretion via bile also occurs in rodents. The elimination of DEHP largely depends on its metabolism and it might take 5-7 days to eliminate 80% of the DEHP administrated. The half-life for DEHP and its metabolites was 3-5 days in the adipose tissue and 1-2 days in the liver. The elimination is most rapid in rats.

In the DEHP data base, it has been observed that the oral absorption of DEHP to some extent is age-dependent, and the EU RAR is concluding on oral absorption percentages of 100 % in young animals and 50 % in adult animals.

DEHP can cross the placenta barrier and distribute into foetal tissues. In addition, DEHP can be transferred through the milk from lactating rats to their pups. Since the immature liver may have a lower metabolising capacity than that of older children and adults, infants and foetuses might be especially vulnerable to exposure to DEHP and MEHP.

3.2 Acute toxicity

3.2.1 Acute toxicity: oral

The acute oral toxicity of DEHP has been studied in several experiments of good quality. The LD_{50}-value in rats is > 20,000 mg/kg bw and in mice > 10,000 mg/kg bw. Only one report on the acute oral toxicity in humans has been located; the ingestion of 5 g caused no adverse effects, while 10 g caused mild gastric disturbances and “moderate catharsis”.

3.2.2 Acute toxicity: inhalation

In a study performed according to GLP principles, the toxicity of a single dose of DEHP via inhalation to rats was in excess of 10.6 mg/litre/4 hours.

3.2.3 Acute toxicity: dermal

The acute dermal toxicity of DEHP has not been investigated in any study of good quality. However, due to poor dermal absorption of DEHP, the acute dermal toxicity is expected to be low.

3.2.4 Acute toxicity: other routes

Studies in rats are available. However, the usefulness of the reported results for the risk assessment is uncertain.

3.2.5 Summary and discussion of acute toxicity

The data available on acute toxicity show a low acute toxicity and do not suggest a classification of DEHP according to EU criteria.

3.3 Repeated dose toxicity

3.3.1 Repeated dose toxicity: oral

Numerous studies have investigated the toxicity of DEHP following repeated oral administration to experimental animals, preferably rats. Many of these studies are comparable to guideline studies and conducted in conformity with GLP. Critical organs for DEHP induced toxicity in laboratory animals are the testis, kidney, and liver.

In repeated dose studies, the lowest reported NOAEL for testicular effects, identified in a guideline study, is 50 ppm DEHP in the diet (3.7 mg/kg bw/day for 13 weeks), based on a high incidence of dose-related Sertoli cell vacuolation from the next higher dose level. However, because of some uncertainties caused by a rather high incidence of vacuolisation also in control rats, this NOAEL is not used. Instead, data from a three-generation study in rats, which showed an increased incidence of small testis suggest a NOAEL of 4.8 mg/kg/day, which is used for repeated dose effects on the testis.

The effects on the kidneys include: reduced creatinine clearance, increased absolute and relative kidney weights, increased incidence and severity of mineralization of the renal papilla, increased incidence and/or severity of tubule cell pigment, and increased incidence and/or severity of chronic progressive nephropathy. The majority of these changes were observed in both sexes, in different species following different exposure time. In long-term studies in rats and mice, there was no indication that DEHP-related changes in the kidney were reversible upon cessation of DEHP-exposure. On account of the DEHP-induced kidney toxicity observed in a well-performed 104-week-study in rats, a NOAEL of 28.9 mg/kg/day is suggested for kidney toxicity. The NOAEL is based on increased absolute and relative kidney weight in both sexes at the next higher dose level (LOAEL: 146.6 mg/kg bw/day in the males and 181.7 mg/kg bw/day in the females). A subsequent three-generation study in rats has indicated a LOAEL of 46 mg/kg/day for kidney weight increases, and a NOAEL of 14 mg/kg/day.

In the liver, the most striking effects observed are hepatomegaly due to hepatocyte proliferation (characterised by increased replicative DNA synthesis/cell division and hypertrophy), peroxisome proliferation, and hepatocellular tumours. A Working Group of the "International Agency for Research on Cancer" (IARC) have concluded that the mechanism by which DEHP increases the incidence of liver tumours in rodents (activation of PPAR-α and peroxisome proliferation) is not relevant to humans.

3.3.2 Repeated dose toxicity: inhalation

In experimental animals three inhalation studies are available. However, these studies are considered inadequate for risk assessment. In humans, there is a study suggesting that toxic damage of the lungs in preterm infants artificially ventilated with PVC respiratory tubes may be causally related to inhalation of DEHP. The estimated inhalative exposure ranged between 1µg/h – 4,200µg/hDEHP.

3.3.3 Repeated dose toxicity: dermal

The only study available following dermal exposure to DEHP is inadequate for risk assessment.

3.3.4 Summary and discussion of repeated dose toxicity:

The data available on repeated dose toxicity (not including reproductive effects) do not suggest a classification of DEHP according to EU criteria. In the risk characterisation for repeated dose toxicity, NOAELs for both testis and kidney toxicity are used. The NOAELs are 4.8 and 28.9 mg/kg/day, respectively.

3.4 Mutagenicity

The possible genotoxic effect of DEHP has been thoroughly investigated in several different short-term tests. The major metabolites of DEHP, MEHP and 2-EH, have also been examined. Most of the studies are performed according to GLP principles and are comparable to guideline studies.

The results have been negative in the majority of the *in vitro* and in *vivo* studies on DEHP, MEHP and 2-EH for detection of gene mutation, DNA damage, and chromosomal effects. The more conclusive positive results were obtained on cell transformation, induction of aneuploidy and cell proliferation. These test systems are, however, also sensitive to several non-genotoxic substances such as tumour promoters and/or peroxisome proliferators. Taking all negative and positive results together, DEHP and its major metabolites are considered to be non-mutagenic substances in humans.

3.4.1 Summary and discussion of mutagenicity

The data available on genotoxicity do not suggest a classification of DEHP according to the criteria for classification and labelling of dangerous substances (Annex IV to Commission Directive 93/21/EEC of 27 April 1993 adapting to technical progress for the 18th time Council Directive 67/548/EEC).

3.5 Carcinogenicity

3.5.1 Carcinogenicity: oral

The carcinogenicity of DEHP has been investigated in numerous animal studies. Four long-term studies performed in rats and mice are of good quality and are considered adequate for evaluation of carcinogenicity of DEHP in experimental animals. DEHP shows clear evidence of hepatocarcinogenicity in both sexes of rats and mice in the four different studies. The increase in tumour incidence in the liver was statistically significant and a dose-response relationship exists. In rats, an increase in the incidence of mononuclear cell leukaemia (MCL) was also observed, significant in males of one study only. In rats, the LOAEL and the NOAEL for tumour induction (both liver tumours and MCL) were established as 2,500 ppm (147 mg/kg bw/day for males) and 500 ppm (29 mg/kg bw/day for males) DEHP in the diet, respectively. In mice the LOAEL and the NOAEL for induction of liver tumour is 1,500 ppm (292 mg/kg bw/day for males) and 500 ppm (98 mg/kg bw/day for males) DEHP in the diet, respectively. Additionally, an increase in the incidence of Leydig cell tumours in male rats exposed for DEHP has been reported.

A feasible mechanistic basis for hepatocarcinogenicity through activation of peroxisome proliferator activated receptor alpha (PPARα) has been accepted by most of the experts in this field. However, there is still no clear evidence showing that the carcinogenicity of DEHP in rodents is mediated through activation of PPARα. It has been suggested that the hepatocarcinogenic effects of peroxisome proliferators, such as DEHP, in experimental animals are rodent-specific and irrelevant for humans. This position is held by a number of experts and is a defensible conclusion based on the available mechanistic data. Notwithstanding, there are also arguments still indicating that a certain human cancer risk cannot, with certainty, be excluded. However, a Working Group of the “International Agency for Research on Cancer” (IARC) has concluded that the mechanism by which DEHP increases the incidence of liver tumours in rodents (activation of PPAR-α) is not relevant to humans.

Male rats exposed to DEHP showed an increase in the incidence in Leydig cell (LC) tumours. The relevance for humans of rodent LC tumours has been evaluated in an international workshop as well as in a published review. It was concluded that the pathways for regulation of the Hypothalamo-Pituitary-Testis (HPT)-axis in rats and humans are similar and hence, compounds that induce LCTs in rats by disruption of the HPT-axis pose a risk to human health with exception of two classes of compounds GnRH and dopamine agonists. Since it has been demonstrated that DEHP and other phthalates has a direct effect on the foetal testes the two latter mechanisms are not relevant for phthalates, and the induction of LC tumours in rats exposed for pthalates should be regarded as relevant to humans taking into consideration the species differences in sensitivity. In conclusion, the presented evidence for the phthalates-induced LC tumours in rats, and the possible endocrine effects of phthalates, together with the fact that developing rats are more sensitive to the phthalates-induced testicular toxicity than sexually mature animals, should be considered seriously. Especially, when related to the limited human data suggesting an increased risk for testicular cancer in workers in PVC-industry. However, a careful evaluation of the available data is necessary before concluding on the possible carcinogenic risk of DEHP.

An increase in the incidence in mononuclear cell leukemia (MCL) was observed in male rats exposed to DEHP. Whereas some experts consider MCL in F344 rats as having similar pathology to an uncommon human tumour (large granular lymphocytic leukemia) and representing a unique model for study of natural tumour immunity, other experts regard MCL as F344 rats-specific, with little relevance for humans. Based on the available data the relevance for humans of the DEHP-induced MCL in F344 rats is not clear.

3.5.2 Carcinogenicity: inhalation

In experimental animals, the only inhalation study available is on hamsters, and is considered inadequate for risk assessment as only one dose of DEHP was used in the study. Also, the dose of DEHP used was very low and the maximal tolerated dose (MTD) was not reached as no signs of any toxicological effects were reported.

3.5.3 Carcinogenicity: dermal

No data available.

3.5.4 Carcinogenicity: human data

No relevant study in humans on the carcinogenicity of DEHP is available.

3.5.5 Summary and discussion of carcinogenicity

Based on the overall evaluation of the available data, no classification for carcinogenicity is proposed.

3.6 Toxicity for reproduction

The most important studies are briefly described below in Table 2 and Table 3 (section 3.6.1 and 3.6.2). An overall discussion of the data follows in section 3.6.5.

3.6.1 Effects on fertility

Table 2: Summary of important reproductive studies with DEHP in laboratory animals

Species	Protocol	Results	References
Repeated dose (testicular) toxicity studies			
Rat, F344 10 rats/sex/group	13 weeks, via the *diet* 0, 1,600, 3,100, 6,300, 12,500 or 25,000 ppm (0, 80, 160, 320, 630, or 1,250 mg/kg/day)	↓bw gain at 25,000 ppm testis atrophy from 12,500 ppm NOAEL 6,300 ppm (320 mg/kg/day)	NTP (1982); see RAR
Rat, F344 50 rats/sex/group	103 weeks, via the *diet* 0, 6,000, or 12,000 ppm (0, 322, or 674 mg/kg/day [males])	↓bw at 12,000 ppm anterior pituitary: hypertrophy at 12,000 ppm (22/49 males, 45%) testis: seminiferous tubular degeneration at 6,000 ppm (2/44, 5%)	NTP (1982); see RAR

		and 12,000 ppm (43/48 males, 90%), histologically devoid of germinal epithelium and spermatocytes	
Rat, Wistar 6 males (25-day-old) per dose group	0, 50, 100, 250, or 500 mg/kg bw for 30 days	Dose-dependent and significant ↑ LDH and GGT and ↓ SDH from 50 mg/kg bw; ↑ β-glucuronidase and ↓ acid phosphatase testis: marked destructive changes in the advanced germ cell layers and vacuolar degeneration at 250 and 500 mg/kg	Parmar *et al.* (1995); see RAR
Rat, F344 70-85/sex/group recovery group: 55/sex	104 weeks, *diet* 0, 100, 500, 2500, or 12,500 ppm (0, 5.8, 28.9, 146.6, or 789.0 mg/kg bw/day [males]; 0, 7.3, 36.1, 181.7, or 938.5 mg/kg bw/day [females] or 12,500 ppm for 78 weeks, followed by a recovery period of 26 weeks	Pituitary: ↑ castration cells (30/60 males) at 12,500 ppm; Testis: ↓ weight, ↑ incidence and severity of bilateral hypospermia at 12500 ppm; Epididymis: ↑ immature or abnormal sperm forms and hypospermia from 12,500 ppm; Changes in the testis and pituitary were not reversible upon cessation of exposure NOAEL for testicular effects 500 ppm (28.9 mg/kg bw/day)	Moore (1996); see RAR
Rat, Sprague-Dawley 10 rats/sex/group	13 weeks, *diet* 0, 5, 50, 500, or 5,000 ppm (0, 0.4, 3.7, 37.6, or 375.2 mg/kg bw/day [males])	Testis: mild Sertoli cell vacuolation at 500 ppm (7/10); decreased absolute and relative testicular weight, mild to moderate Sertoli cell vacuolation, testicular atrophy and complete loss of spermatogenesis at 5,000 ppm (9/10), in-	Poon *et al.* (1997); see RAR

		creased liver and kidney weights (all rats of both sexes), and mild histological changes of the thyroid at 5,000 ppm NOAEL 50 ppm (3.7 mg/kg bw/day)	
Mouse, B6C3F1 70-85/sex/group; recovery group: 55/sex	104 weeks, *diet* 0, 100, 500, 1,500 or 6,000 ppm (0, 19.2, 98.5, 292.2 or 1,266.1 mg/kg bw/day [males] or 6,000 ppm followed by a recovery period of 26 weeks	Testis: from 1,500 ppm ↓ weight, ↑ incidence and severity of bilateral hypospermia; Epididymis: from 1,500 ppm ↑ immature or abnormal sperm forms and hypospermia; changes in testes partially reversible; NOAEL 500 ppm (98.5 mg/kg bw/day)	Moore (1997); see RAR
Continuous breeding studies			
Mouse, ICR 20 animals/sex/dose group, 40 control animals of each sex	*Diet*, 98 days 0, 0.01, 0.1, or 0.3% (0, 20, 200 or 600 mg/kg bw/day)	Dose-dependent ↓ in the number of litters and proportion of pups born alive from 0.1% (0.1%: 14/19 fertile, 0.3%: 0/18); ↑ absolute and relative liver weight (both sexes) and ↓ reproductive organ weights and atrophy of seminiferous tubules at 0.3%; no effect on bw NOAEL for maternal and developmental toxicity 20 and 600 mg/kg bw/day, respectively crossover mating trial: treated males and control females: 4/20 fertile; control males and treated females: 0/16 fertile	Lamb *et al.* (1987); see RAR
See also the **multi-generation studies** described below in section 3.6.2			

3.6.2 Developmental toxicity

Table 3: Summary of important developmental toxicity studies in laboratory animals

Developmental toxicity Studies			
Rat, F344/CrlBr 34-25 females/group	*Diet* 0, 0.5, 1.0, 1.5, or 2% gestation days 0-20	↓ maternal food intake and mean foetal bw from 0.5%; ↓ maternal bw gain, ↑ absolute and relative liver weights, ↓ foetal bw/litter from 1.0% ↑ number and percentage of resorptions, non-live and affected implants/litter at 2%; NOAEL for maternal and developmental toxicity 0.5% (~357 mg/kg bw/day)	NTIS (1984); Tyl *et al*. (1988); see RAR
Rat, Wistar 9-10 females/group	*Gavage*, oil 0, 40, 200 or 1,000 mg/kg bw/day on gestation days 6-15	↓ maternal bw and ↑ maternal relative kidney and liver weights at 1,000 mg/kg bw ↓ number of live foetuses/dam ↓ foetal body weights, ↑ number of malformed foetuses/dam (tail, brain, urinary tract, gonads, vertebral column, and sternum) at 1,000 mg/kg bw; NOAEL for maternal and developmental toxicity 200 mg/kg/day	BASF (1995); Hellwig *et al*. (1997); see RAR
Mouse, 1-CR 30-31 females/group	*Diet*; 0, 0.025, 0.05, 0.10 or 0.15% (0, 44, 91, 190.6 or 292.5 mg/kg bw/day); gestation days 0-17	↓ maternal body weight gain from 0.10% (mainly due to ↓ uterine weight, ↓ foetal body weight and number of live foetuses per litter); ↑ number and percent of resorptions, late foetal deaths, dead and malformed foetuses,	NTIS (1984); Tyl *et al*. (1988); see RAR

		and percent malformed foetuses/litter from 0.05% (open eyes, exophtalmia, exencephaly, short, constricted or no tail); visceral malformations and skeletal defects (fused and branched ribs, misalignment, and fused thoracic vertebral centra); NOAEL for maternal toxicity 0.05% (91 mg/kg bw/day) and for develop-mental toxicity 0.025% (44 mg/kg bw/day)	
Mouse, CD-1 15 females/dose group30 controls	*Oral*, gavage 0, 40, 200 or 1,000 mg/kg bw/day gestation days 6-15	Foetotoxic effects at 200 mg/kg bw/day ↓ number of viable foetuses ↑ number of resorptions and post-implantation losses at 1,000 mg/kg bw/day and also cardiovascular abnormalities, tri-lobed left lungs, fused ribs, fused thoracic vertebral centres and arches, immature livers, and kidney abnormalities NOAEL 200 mg/kg bw for maternal toxicity and NOAEL 40 mg/kg bw/day for developmental toxicity	Huntingdon (1997); see RAR
Two-generation studies			
Rat, Sprague-Dawley 17/males/group	3 generations via *diet*; 1.5, 100, 300, 1,000, 7,500 and 10,000 ppm (0.1, 0.5, 1.4, 4.8, 14, 46, 359, and 775 mg/kg/day	Dose-dependent effects on numerous testis-related parameters. NOAEL for testicular toxicity and developmental toxicity and 46 mg/kg/day for fertility	Wolfe *et al. (*2003); see RAR
Rat, Wistar, 25	0, 1,000, 3,000 or	3,000 ppm: reduced	Schilling *et al.*

animals/group	9,000 ppm DEHP via the *diet* (corresponding to approximately 0, 113, 340 or 1,088 mg/kg/day)	testis weight in F2, focal tubular atrophy and a feminisation of 49% of the male offspring. Minimal focal tubular atrophy also occurred at 1,000 ppm (113 mg/kg and day), which thus constitutes a conservatively chosen LOAEL	(2001); see RAR
Muse, CD-1 (number not specified)	*Diet*, 0.01, 0.025, or 0.05% (0, 19, 48 or 95 mg/kg bw/day)	↑ prenatal mortality for F1-litters at 0.05% ↓ number of viable pups neonatally at 0.05% NOAEL for parental toxicity and F2-offspring: 0.05% (95 mg/kg bw/day) NOAEL for F1-offspring: 0.025% (48 mg/kg bw/d)	NTIS (1988); see RAR
Post-natal studies			
Rat, Sprague-Dawley 10 males/group	*Gavage*, corn oil 5 days from the age of 1 week, 2 weeks, 3 weeks, 6 weeks, or 12 weeks 0, 10, 100, 1,000 or 2,000 mg/kg bw/day	Two doses of 2,000 mg/kg bw were fatal for most pups in the three youngest age groups, ↓ bw for 6- and 12-week-old rats but no mortalities; 5 doses of 1,000 mg/kg bw: ↓ bw gain in 1-, 2-, and 3-week-old rats; ↑ absolute and relative liver weights at 100 mg/kg bw/day in all age groups (except for 1-week-old rats) and in all age groups at higher dose levels; ↓ plasma cholesterol levels in weanling and adult rats from 1,000 mg/kg/day	Dostal *et al.* (1987b); see RAR

3.6.3 Human data

No human data on the effect of DEHP on fertility is available.

3.6.4 Other relevant information

Studies performed after the EU RAR on DEHP was agreed have not been thoroughly evaluated. However, there are recent studies in rodents that may seem to support a NOAEL for testicular toxicity of the magnitude agreed in the RAR (e.g., Andrade *et al.*, 2006a, 2006b, 2006c). In contrast, toxicity studies in primates have generally been negative. (Tomonari et al , 2006).

Several phthalates seem to have similar toxicological profiles with respect to testicular effects, and there are good indications that DEHP, dibutylphthalate (DBP) and diisobutylphthalate (DiBP) may have similar mechanisms of action (Borch *et al.*, 2006a, 2006b). The risks from the combined exposure to several different phthalates thus need to be considered.

3.6.5 Summary and discussion of reproductive toxicity

Available data demonstrate that exposure to DEHP affects both fertility and reproduction in rodents of both sexes and also produces developmental effects in offspring. In males, DEHP induces severe testicular effects, including testicular atrophy. Testicular effects have been observed in numerous repeated dose toxicity studies in rats, mice and ferrets. In addition, minor effects were observed in hamster exposed to DEHP and more severe effects induced by MEHP. In the available studies marmosets were not sensitive to DEHP. No studies on testicular effects in rabbits are available. MEHP is believed to be the active metabolite of DEHP affecting testes and reproductive functions both *in vivo* and *in vitro*. The possible role of other metabolites is, however, not fully elucidated.

The NOAEL for testicular effects, as identified in a guideline three-generation reproductive toxicity study (Wolfe *et al*., 2003; see RAR), is 4.8 mg/kg/day. A NOAEL of 3.7 mg/kg bw in rats was indicated based on a high incidence (7/9) of Sertoli cell vacuolation at the next higher dose level (500 ppm equivalent to 37.6 mg/kg bw) in a 13-week guideline study (Poon *et al.*, 1997; see RAR). At the highest dose level (5,000 ppm, equivalent to 375.2 mg/kg body weight) also a high incidence of atrophy of the seminiferous tubules with complete loss of spermatogenesis was found in addition to a higher incidence of cytoplasmic Sertoli cell vacuolation (9/10). However, as there remains some doubts as to the toxicological significance of the Sertoli cell vacuolisation observed in the Poon study, a NOAEL of 4.8 mg/kg/day (100 ppm) is chosen from the Wolfe study (2003), based on occurrence of small male reproductive organs (testis/epididymes/seminal vesicles) and minimal testis atrophy (exceeding those of the current controls as well as historical control groups) at 300 ppm and above.

Both *in vivo* and *in vitro* experiments have demonstrated that the Sertoli cell is one of the main targets for DEHP/metabolite-induced testicular toxicity producing subsequent germ cell depletion (Poon *et al.*, 1997; Arcadi *et al*., 1998; Li *et al*., 1998; see RAR). Sertoli cells provide both physical support as well as secreting factors that are required for germ cell differentiation and survival and may also influence the signal transduction mechanism between these cells. Study results have also shown that DEHP and MEHP may exert a direct effect on Leydig cell structure and function as determined by testosterone output and also that DEHP and MEHP produce similar changes *in vivo* and *in vitro* in both Leydig cells and in Sertoli cells (Jones *et al.*, 1993; see RAR). It is plausible

that malfunction of Leydig cells affects the physiology of adjacent Sertoli cells. Findings also indicate that different phthalates may exert changes that are unique to one or common to both cell types.

Developing and pre-pubertal rats have been found to be much more sensitive to exposure to DEHP than adults (Gray and Butterworth, 1980; Sjöberg *et al*., 1985c; 1986b, Arcadi *et al*., 1998; Wolfe *et al*., 2003; see RAR). The younger animals respond to a much lower dose or produce a more serious lesion with a comparable dose on a mg/kg/day basis. In some instances, the onset for the production of the lesion is also more rapid. Exposure of rats prenatally and during lactation has produced irreversible effects at dose levels inducing only minimal effects in adult animals at the same exposure levels (Arcadi *et al*., 1998; Wolfe *et al*., 2003; see RAR).

Based on the available data, which varies in both the study designs and number of animals included**,** testicular effects have been demonstrated in both male rodents and non-rodents: rat (NOAEL = 4.8 mg/kg bw/day), mouse (NOAEL = 98.5 mg/kg bw/day), and ferret (LOAEL = 1,200 mg/kg/day) (Poon *et al*., 1997, Moore, 1997; Lake *et al*., 1976; see RAR). In addition, minor effects were observed in hamster exposed to DEHP and more severe effects were induced by MEHP (Gray *et al*., 1982; see RAR). In the available studies with marmosets, testicular toxicity has not been observed after treatment with DEHP (Kurata *et al*., 1995; 1996; 1998; see RAR). The reasons for the differences in study results have been suggested to be caused by toxicokinetic differences. Moreover, other factors such as animal age, study design, animal model selection have also to be considered. For instance, marmosets which are new-world monkeys vary in their metabolic pathways and capacities and are not as closely related to humans as are cynomolgus and Rhesus monkeys (old-world monkeys) **(**Caldwell, 1979a; 1979b; see RAR). Although Sertoli cell replication seems to be more similar in man and marmosets, and the efficiency of spermatogenesis is poor in marmosets as well as in humans, there is, however, no evidence to support that the results obtained in pre-pubertal rats are not relevant for man or that use of adult marmosets should be preferred. Other mechanism(s) and/or factors that caused the observed differences in the DEHP-induced testicular toxicity have not, however, been fully substantiated. Based on the available animal data it is not possible to definitely conclude the relevance of these differences to humans. However, in the limited toxicokinetic data in humans, MEHP, the testicular toxicant, is formed following exposure to DEHP. Therefore, DEHP-induced testicular effects observed in animal studies are considered relevant for humans.

Effects on male fertility have been observed in mice and rats. In mice, DEHP adversely affects the number of fertile matings. In a continuous breeding study, an oral NOAEL of 0.01% in the diet (20 mg/kg bw/day) was identified for fertility (Lamb *et al*., 1987; see RAR). In rat, the oral NOAEL for body weight, testis, epididymis and prostate weights and for endocrine and gonadal effects in male rats was considered to be 69 mg DEHP/kg bw/day in a 60 day study (Agarwal *et al.*, 1986a; 1986b; see RAR). In a complementary crossover mating trial, females given 0.3% DEHP were more seriously affected than males. None of the females were able to produce pups: the fertility index was 0 (0/16) for females and 20% (4/20) for males compared to 90% for the control group (18/20).

Developmental toxicity has been observed in several studies. The rat has been shown to be the most sensitive species to DEHP-induced malformations. Irreversible testicular damage in the absence of obvious effects on the dams was shown in male pups exposed *in utero* and during lactation at very low dose levels (LOAEL = 3.5 mg/kg bw/day) (Arcadi *et al*., 1998; see RAR). Their mothers were exposed to DEHP in drinking water at doses from about 3 mg/kg/day during pregnancy and lactation. However, there is some uncertainty with regard to the actual concentration of DEHP in the water. Alterations in kidneys tended to ameliorate with time; the testicular lesions did, however, not appear to reduce with growth. Histopathological changes were still observed at termination of the study, 8 weeks after delivery. The same levels of exposure did not produce similar effects in

adult male rats. Effects on the male reproductive system, partly induced during the gestational period, were also observed in a three-generation study with a NOAEL of 4.8 mg/kg/day (Wolfe *et al*., 2003; see RAR). In mice, DEHP is embryotoxic and teratogenic at oral dose levels below those producing observable evidence of toxicity to the dams.

In a continuous breeding study in mice, an oral NOAEL for maternal and developmental toxicity of 600 and 20 mg/kg bw/day were identified, respectively (Lamb *et al*., 1987; see RAR). In a developmental toxicity study an oral NOAEL of 44 mg/kg bw/day was identified. The NOAEL for maternal toxicity was 91 mg/kg bw/day (NTIS, 1984; Tyl *et al*., 1988; see RAR). In a dietary 2-generation study in mice, the maternal NOAEL was 0.05% DEHP (91 mg/kg bw/day) and the NOAEL for F1 offspring 0.025% (48 mg/kg bw/day) (NTIS 1988; see RAR).

A few developmental toxicity studies have been performed in other species. These studies are, however, inconclusive. Only one developmental study is available concerning the effects of exposure to DEHP by inhalation (Merkle *et al*., 1988; see RAR) However, this study is considered inconclusive and not useful for risk assessment. Because of uncertainties regarding the actual dosing in the study by Arcadi *et al.* (1998), which has given the lowest effect level, the NOAEL of 4.8 mg/kg/day (Wolfe *et al*., 2003; see RAR) is selected for developmental toxicity.

Animal data have shown that DEHP and its metabolites can be transferred to pups via mother's milk in concentrations sufficient to cause toxicity (Parmar *et al*., 1985, Dostal *et al*., 1987a, Tandon *et al*., 1990; see RAR).

Both *in vivo* and *in vitro* study results indicate that DEHP can interfere with the endocrine function and also influence the sexual differentiation (e.g. Gray *et al*., 1999 and Jones *et al*., 1993; see RAR). Due to the effects on the Leydig cells as measured by a decreased testosterone output, it cannot be excluded that DEHP may exert an antiandrogen effect. The results of recently performed *in vivo* studies in rats exposed to DEHP or DBP support the hypothesis that exposure to phthalates may be provoked by an antiandrogen mechanism (Gray *et al*., 1999, Mylchrest and Foster, 1998; see RAR). The present data in experimental animals are of concern for humans.

To summarize, based on a 3-generation study in rats, a NOAEL of 4.8 mg/kg/day is chosen for developmental toxicity and for repeated dose toxicity on the testis based on testicular toxicity in developing rats at a dose of 14 mg/kg/day (Wolfe *et al*., 2003; see RAR). For effects on fertility a NOAEL of 20 mg/kg/day was chosen from a continuous breeding study in mice, where fertility was affected at the next higher dose of 200 mg/kg/day (Lamb *et al*., 1987; see RAR).

REFERENCES

This support document mainly builds on the agreed European Union Risk assessment report (RAR) on DEHP (EC, 2008) developed under regulation EEC 793/93. Information from this document is used in this supporting document without giving full references in the supporting document. Thus the reader is referred to the RAR. New information and new studies not used in the RAR are given as full reference.

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