Human Health Hazard Assessment

Diisobutyl phthalate (DIBP)
(CAS No 84-69-5)

TABLE OF CONTENTS

INTRODUCTION

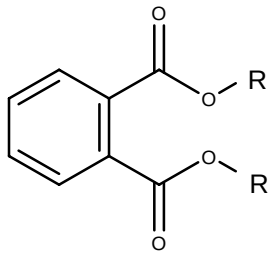
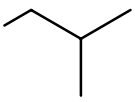
This review of diisobutyl phthalate (DIBP) is a health hazard assessment only. For this assessment, primary references were the main source of information. Information was collected up to September 2006.

References not marked with an asterisk were examined for the purposes of this assessment. References not examined but quoted from the reference sources as secondary citations are also noted in this assessment and marked with an asterisk.

Hazard information from this assessment is published also in the form of a phthalate hazard compendium providing a comparative analysis of key toxicity endpoints for 25 phthalates (NICNAS, 2007).

1. IDENTITY

1.1 Identification of the Substance

CAS Numbers:	84-69-5
Chemical Name:	1,2-Benzenedicarboxylic acid, bis-(2-methoxypropyl) ester
Common Name:	Diisobutyl phthalate (DIBP)
Molecular Formula:	$C_{16}H_{22}O_4$
Structural Formula:	
	R = 
Molecular Weight:	278.35
Synonyms:	Phthalic acid, diisobutyl ester; Di(isobutyl)-1,2-benzenedicarboxylate
Purity/Impurities/Additives:	Purity > 99%

1.2 Physico-Chemical Properties

Table 1: Summary of physico-chemical properties

Property	***Value***
Physical state	Colourless, viscous liquid
Melting point	-37°C
Boiling point	320°C
Density	1038 kg/m^3
Vapour pressure	1 x 10^{-5} kPa at 20°C
Water solubility	1 x 10^{-3} g/L

Partition coefficient n-octanol/water (log Kow)	4.11
Henry's law constant	6.43×10^{-7} atm.m^3/mole
Flash point	185°C (closed cup)

Based on IPCS (2001), HSDB (2006)

2. USES

According to the European Council of Plasticisers and Intermediates (ECPI, 2006), DIBP is a specialist plasticiser often used in combination with other high molecular weight phthalates as a gelling aid. It has very similar application properties to DBP and may therefore be used to substitute for DBP in most, if not all, of its applications. These range from the plasticisation of PVC to the production of paints, printing inks and adhesives.

In Australia, DIBP is imported for use as a plasticiser for the manufacture of PVC and rubber. It is also imported as a component of industrial adhesives and catalyst systems for polypropylene and fibreglass manufacture. Imported DIBP is also sold to various institutions and laboratories for research and product development.

3. HUMAN HEALTH HAZARD

3.1 Toxicokinetics

Previous Evaluations

No data

Data not Reported in Previous Evaluations

The dermal absorption of DIBP has been assessed, along with other phthalates (Elsisi et al., 1989). Fur from an area on the back of male Fischer 344 rats was clipped and ^{14}C phthalate diester was applied at a dose of 157 µmol/kg. Urine and faeces were collected over a seven day period and the amount of ^{14}C excreted was taken as an index of the percutaneous absorption. The cumulative percentage dose excreted in seven days for DIBP was about 51% of the applied ^{14}C. Urine was the major route of excretion for DIBP, with some excretion in the faeces, presumably due to biliary excretion. After seven days, the total recovery for DIBP was 93%.

In humans, DIBP is metabolised to monoisobutyl phthalate (MIBP) which can be detected in the urine (Swan et al., 2005). Apart from this observation in humans, no information on metabolism is available.

Conclusion

DIBP appears to be readily absorbed via the dermal route. Urine was the major route of excretion with minor biliary excretion being observed. There was little accumulation in the rat tissues. In humans, DIBP undergoes primary metabolism into MIBP, which was detected in urine.

3.2 Acute Toxicity

Previous Evaluations

Table 2: Summary of acute toxicity studies

Study	*Species*	*Results (LD50)*	*References*
Intraperitoneal	Mouse	3990 mg/kg bw	Lawrence et al. (1975)
Intraperitoneal	Mouse	6400-12,800 mg/kg bw	Eastman Kodak (1978)
Intraperitoneal	Rat	>1600 mg/kg bw	Eastman Kodak (1978)
Oral	Rat	60,320 mg/kg bw	Hodge (1954)
Oral	Mouse	39,520 mg/kg bw	Hodge (1954)
Oral	Rat	16,000-28,000 mg/kg bw	Eastman Kodak (1978)
Oral	Mouse	12,800 mg/kg bw	Eastman Kodak (1978)

Data not Reported in Previous Evaluations

No data

Conclusion

DIBP has a low order of acute toxicity by the oral route ($LD50_{mouse}$ = 12,800-39,520 mg/kg bw; $LD50_{rat}$ =16,000-60,320 mg/kg bw), and intraperitoneal (i.p.) route ($LD50_{mice}$= 3990-12,800 mg/kg bw; $LD50_{rat}$ >16,00 mg/kg bw).

3.3 Irritation

Skin Irritation

Previous Evaluations

No data

Data not Reported in Previous Evaluations

Lawrence et al. (1975) reported negative results of irritation tests on undiluted DIBP using intradermal injections. However, the type of animals used was not stated and limited information is provided.

Eastman Kodak Co. (1978) reported that DIBP was a slight skin irritant in guinea pigs. No further information is available.

Conclusion

DIBP has been reported to cause minimal skin irritation in guinea pigs, although no data are presented.

Eye Irritation

Previous Evaluations

No data

Data not Reported in Previous Evaluations

Lawrence et al. (1975) reported negative results of tests on undiluted DIBP in the eyes of rabbits. No further information is available.

Conclusion

DIBP did not cause eye irritation in rabbits.

3.4 Sensitisation

Previous Evaluations

No data

Data not Reported in Previous Evaluations

Eastman Kodak Co. (1978) reported that DIBP was not a skin sensitiser in guinea pigs. No further information is available.

Conclusion

DIBP has been reported to not cause skin sensitisation in guinea pigs, although no data are presented.

3.5 Repeated Dose Toxicity

Previous Evaluations

No data

Data not Reported in Previous Evaluations

Hodge (1954) reported on a four month repeated dose dietary study involving albino rats (species not provided) (5/sex/group) fed 0, 0.1, 1.0 and 5% DIBP (doses in mg/kg bw not provided). Body weights and haematological parameters were measured. Organ weights were determined at autopsy. Livers and kidneys were examined histologically. Significant decreased body weights were observed in both sexes at 5.0% (decrease up to 43% for males and 13% for females at 4 months). Red blood cell counts in the 5% male group and haemoglobin levels in both sexes receiving this dose were slightly reduced. These effects were not dose-related. Both absolute and relative testes weights in the 5% group were considerably reduced. 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% groups in 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 limited short term 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 for 2 months. Weight loss was noted in the female dog but

haematological and urine analyses were all normal. At study termination, there was an increase in relative liver weight in the female dog compared to historical controls. No histological changes in liver were observed. In the male dog, histological examination of testes revealed abnormally few sperm. The study was poorly reported. No conclusions can be drawn from this study.

Conclusion

The main target organ for DIBP following a 4-month repeat dose toxicity study in rat was the liver. The NOAEL was determined to be 1% (dietary level), with a LOAEL of 5% based on decreased body and testes weights and increased liver weights.

3.6 Genetic Toxicity

Previous Evaluations

No data

Data not Reported in Previous Evaluations

DIBP was found not to be mutagenic with and without S9 activation in an 8-azaguanine resistance assay in *Salmonella typhimurium* TA 100 (concentration not provided) (Seed, 1982). Results of a *Salmonella typhimurium* assay (NTP Technical Bulletin, 1982) with S9 activation were negative. Zeiger et al. (1985) tested 34 phthalates and related chemicals, including DIBP, for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 with and without S9 activation, up to 10 mg/plate unless limited by solubility and/or toxicity. DIBP was not mutagenic.

Kleinsasser et al. (2000a) reported, using an *in vitro* comet assay, that DNA damage (single-strand breaks) was significantly induced by DIBP (354μmol/ml) in human oropharyngeal (n=40) and nasal (n=30) mucosa samples, as compared to the negative control (DMSO).

In further work, Kleinsasser et al. (2000b) found that DIBP induced strand breaks in DNA, in both blood lymphocytes and normal mucosal cells from the oropharynx or larynx of 60 human patients with head and neck cancer.

Conclusion

DIBP induced DNA damage (single-strand breaks) in an *in vitro* Comet Assay. It was not mutagenic in bacterial mutation assays.

No *in vitro* chromosomal aberrations, mammalian mutation and *in vivo* genotoxicity studies are available. Overall, the genotoxic potential of DIBP cannot be determined.

3.7 Carcinogenicity

Previous Evaluations

No data

Data not Reported in Previous Evaluations

No data

Conclusion

No data

3.8 Reproductive Toxicity

Traditional hazard assessments consider reproductive toxicity separate from developmental toxicity. Reproductive toxicity is tested by exposing sexually mature adults to a chemical and examining the effects on the animals capacity to reproduce. Developmental toxicity is studied by exposing pregnant dams and looking for effects in the foetuses. However, these tests generally do not detect chemicals that induce effects that only appear postnatally. Thus, chemicals that affect the developing reproductive system following prenatal exposure may also affect sexual maturation or functional reproductive disorders that are only apparent at maturity. Developmental toxicity can therefore lead to reproductive toxicity and the two endpoints cannot be clearly distinguished.

In this hazard assessment, data will be presented on the basis of test procedure. Studies include repeat dose toxicity studies that dose adult animals for varying duration, prenatal developmental toxicity studies (only the dam is dosed, study ends before parturition) and developmental/postnatal studies (dam is dosed during gestation and allowed to litter, study ends during weaning). The effects on fertility (as adults) and development (as foetuses) will then be discussed separately.

Previous Evaluations

No data

Data not Reported in Previous Evaluations

3.8.1 Human Studies

Association between 11 maternal urinary phthalate monoester concentrations and genital parameters such as anogenital distance (AGD) and testicular descent in children was determined in 85 mother-son pairs (Swan et al., 2005). Urinary MIBP concentration was inversely related to anogenital index (AGI) (i.e. anogenital distance normalized for body weight). This study has been criticised by McEwen et al. (2006) from the Cosmetic and Fragrance Associations of America and Europe. They suggested that AGD is more likely to be proportional to height rather than weight and that maternal phthalate urinary concentrations were not normalized for urine volume. The reliability of the measurement of AGD in humans has not been verified. One study of 87 neonates that has assessed the correlation of AGD with body weight found it was 0.48 in males and that body length may be a slightly better predictor for AGD than weight (Salazar-Martinez et al., 2004).

<u>Laboratory animals</u>

3.8.2 Repeat Dose Toxicity Studies

Oishi & Hiraga (1980b, c) found that feeding a diet containing 2% of DIBP for a week resulted in significantly ($p<0.05$) decreased absolute and relative weight of the testes in rats but significantly increased relative testes weight in mice (there was no difference in absolute testes weight). Zinc concentrations in the testes and liver were significantly decreased in both species. On gross examination, the testes of DIBP-treated rats were reduced in size and microscopic examination indicated marked inhibition of spermatogenesis and desquamation of spermatocytes.

The effects of MIBP on rat and mouse testes have been studied (Oishi & Hiraga 1980a, d; Foster et al., 1981). Mice fed diets containing 2% of MIBP had significantly increased relative testes weight associated with decreased body weight. The average zinc and testosterone levels in the testes of treated mice were significantly lower than the controls. In rats fed diets containing 2% MIBP, body weight was decreased as was absolute and relative testes weights. Testicular zinc and testosterone concentrations as well as serum testosterone concentration were significantly reduced. Foster et al. (1981) reported that when MIBP was orally administered to young male rats at 800 mg/kg bw/d for six days, the animals developed marked testicular atrophy and zinc metabolism was altered, with increasing urinary excretion of zinc and depletion of its concentration in testicular tissues.

3.8.3 Prenatal Developmental Toxicity Studies

Singh et al. (1972) administered doses of 0.375, 0.750 and 1.250 ml/kg bw (approximately 390, 780 and 1300 mg/kg bw) of DIBP by intraperitoneal (ip) injection to pregnant Sprague Dawley rats on GD 5, 10 and 15. Effects observed included decreased average weight of foetuses at all dose levels and increased numbers of resorptions and skeletal abnormalities ("partially elongated and fused ribs") at the highest dose (1300 mg/kg bw). Dead foetuses were found. The possible effect on maternal health was not described.

Four groups of pregnant Wistar rats were gavaged from GD7 to GD 19 or 20/21 with either vehicle (corn oil) or 600 mg/kg bw/day of DIBP (Borch et al., 2006). Administration of DIBP resulted in significant reduction in AGD in male pups (and increased AGD in female pups) at GD 20/21 together with reduction in bodyweights of male and female fetuses and reductions in testicular testosterone production and testicular testosterone content in the male offspring. Testicular pathological changes were also noted: clustering of small Leydig cells on GD19 or GD20/21 and vacuolisation of Sertoli cells on GD 20/21. A NOAEL was not established.

In a study on Sprague-Dawley rats, DIBP was administered to pregnant rat by gavage at doses of 0 (olive oil), 250, 500, 750 and 1000 mg/kg bw/day on GD 6 to 20 (Saillenfait et al, 2006). Signs of 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/day and higher doses although overall weight gain corrected for gravid uterus was no different than controls at the end of gestation. The incidences of resorptions was significantly increased at 750 mg/kg bw/d, and reached 60% at 1000 mg/kg bw/day. For the offspring, there was a dose-related decrease in foetal weight, which was significantly lower than control from 500 mg/kg/day. There were significant increases in the incidence of foetuses with skeletal malformations (supernumerary lumbar and cervical ribs). At the two highest doses (750 and 1000 mg/kg bw/d), the incidence of male foetuses with undescended testes was significantly increased and the degree of trans-abdominal testicular migration was increased in a dose-related fashion, in treated pups (significant from 500 mg/kg bw/d). The NOAEL for maternal and developmental toxicity

was 250 mg/kg/d. The LOAEL was 500 mg/kg bw/d based on the increased incidence of undescended testes and decreased weight in pups and decreased body weight gain in adults.

3.8.4 Developmental/Postnatal Toxicity Studies

DIBP was 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 exposed dams died.

3.8.5 Mode of Action

DIBP was negative for estrogenic activity in a yeast two-hybrid assay (Nishihara et al., 2000) and showed extremely weak estrogenic activity in recombinant yeast assay (Harris et al., 1997). DIBP (up to 10^{-5}M) had no binding affinity for the oestrogen receptor α or β *in vitro* (Toda et al., 2004) but was also found to induce estrogen receptor (ER)α-mediated estrogenic activity and possess antiandrogenic activity *in vitro* but showed no activity towards ERβ in CHO-K1 cells (Takeuchi et al., 2005).

Conclusion

Effects on fertility

Administration of DIBP at high dose (approximately 2000 mg/kg bw/d) resulted in decreased testes weight in rats but increased relative testes weight in mice and inhibition of spermatogenesis in the rat. The development of these effects within a week suggests a specific effect rather than a secondary effect of generalised toxicity (Oishi & Hiraga, 1980c). A NOAEL was not established.

Effects on development

A recent human study (Swan et al., 2005) showed urinary MIBP concentration was inversely related to anogenital index (AGI) (i.e. anogenital distance normalized for body weight). However, multiple exposures to different phthalates may have contributed to this effect. In addition, the endpoint measured, anogenital index, has not been verified in humans.

Limited developmental toxicity studies are available. Oral exposure to DIBP during gestation was associated with complete loss of litters at materno-toxic doses. A NOAEL of 250 mg/kg bw/day and a LOAEL of 500 mg/kg bw/day were established, based on decreased pup weight and increased transabdominal migration of testes (Saillenfait et al., 2006). In another experiment, male foetuses at term demonstrated decreased testicular testosterone production *ex vivo*, decreased testosterone levels in testes and plasma, decreased AGD, and pathological changes in the testes including clustering of small Leydig cells and vacuolisation of Sertoli cells at 600 mg/kg bw/day (Borch et al., 2005, 2006).

Table 4: Summary of reproductive toxicity studies

Study type	***Route***	***Doses (mg/kg***	***NOAEL (mg/kg***	***LOAEL (mg/kg bw/d) & endpoint***	***Reference***

		bw/d)	***bw/d)***		
Rats GD 5-15	ip	0.375, 0.750, 1.250 ml/kg bw (0, 390, 780, 1300)	NE	375; Fetotoxicity	Singh et al., 1972
Rats,Wistar, male 10/gp 7 days	diet	0, 2% (0, 2000)	NE	2000; ↓ testes wt, ↓ testicular testosterone, ↓ liver & testes zinc, spermatogenesis and desquamation of spermatocytes	Oishi & Hiraga, 1980c
Mice 50/gp GD 6-13	gavage	0, 4000	NE	4000; no viable litters	Hardin et al., (1987)
Mice, JCL: ICR, male 10/gp 7 days	diet	0, 2% (0, 4000)	NE	4000; ↓ body wt gain, ↑ testes wt, no effect on testosterone	Oishi & Hiraga, (1980b*)
Rats, Sprague Dawley 24/gp GD 6-20	gavage	0, 250, 500, 750, 1000	250	500; ↓ fetal wt, ↑ incidence of undescended testes	Saillenfait et al., 2006
Wistar rats 10-12/gp GD 7-19/21	gavage	0, 600	NE	600; ↓ testicular testosterone content; ↓ testicular testosterone production; ↓ AGD (absolute & relative, male), ↓ body weight	Borch et al., 2006

NE = not established
↓ = decreased
↑ = increased
wt = weight

4. HAZARD CHARACTERISATION

Toxicity data for DIBP were not available for all health endpoints. For endpoints with missing or incomplete data, information from structurally similar phthalates, where available, was used to extrapolate potential toxicity. Relevant read-across information was obtained from other NICNAS assessment reports for relevant phthalates and the NICNAS Phthalates Hazard Compendium (NICNAS, 2007), which contains a comparative analysis of toxicity endpoints across 25 phthalates, including DIBP. DIBP has a linear portion of carbon sidechain of 3 carbon atoms in length (branched at C2).

DIBP appears to be readily absorbed via the dermal route. It undergoes primary metabolism into the hydrolytic monoester, MIBP, before excretion. Urine was the major route of excretion with minor biliary excretion being observed. There was little accumulation in the rat tissues.

DIBP has a low order of acute toxicity by the oral, intraperitoneal and dermal route. DIBP is reported to cause minimal skin irritation in guinea pigs. No eye irritation or skin sensitisation was observed in animals, however, no further information was available.

A 4-month repeated dose toxicity study reported low body and testes weights and increased liver weights in rats with a 5% diet. The NOAEL was 1% in diet. DIBP was not mutagenic in bacterial mutation assays but there is evidence that it induced DNA damage in human cells *in vitro*. No *in vitro* chromosomal aberrations, mammalian mutation and *in vivo* genotoxicity studies are available. Overall, the genotoxic potential of DIBP cannot be determined.

No carcinogenicity data are available for DIBP. Due to insufficient testing on other phthalates, it is not possible to extrapolate carcinogenic potential for DIBP.

With respect to reproductive toxicity, DIBP induced decreased body weight after 1 week oral dosing in rats and mice as well as effects on testis weight and testosterone content (Oishi & Hiraga, 1980 b; c). Relative testes weight was increased in mice and decreased in rats while testicular testosterone content was decreased in both species. Similar results were obtained when rats and mice were fed diets containing MIBP (Oishi & Hiraga, 1980 a; d). A NOAEL was not established in any of the animal studies.

Limited developmental toxicity studies are available. Oral exposure to DIBP during gestation was associated with complete loss of litters at materno-toxic doses. At lower doses, DIBP induced decreased foetal weight and increased incidence of undescended testes (Saillenfait et al., 2006) and in male foetuses at term decreased testicular testosterone production *ex vivo* and testosterone levels in testes and plasma, decreased AGD, and induced pathological changes in the testes including clustering of small Leydig cells and vacuolisation of Sertoli cells (Borch et al., 2005). The NOAEL was 250 mg/kg bw/day based on decreased pup weight and increased incidence of undescended testes (Saillenfait et al., 2006). A recent human study (Swan et al., 2005) showed urinary MIBP concentration was inversely related to anogenital index (AGI) (i.e. anogenital distance normalized for body weight) in male children. However, multiple exposures to different phthalates may have contributed to this effect. In addition, the reliability of endpoint measured, anogenital index, has not been verified in humans.

Although data for DIBP are limited, the fertility and developmental effects observed are similar to those phthalates with sidechain backbone of carbon sidechains of 4-6 carbon atoms in length (C4-6) (NICNAS, 2007). These C4-6 phthalates previously referred to as 'transitional' phthalates (Phthalate Esters Panel HPV Testing Group, 2001) have also been associated with male reproductive (decreased in testicular testosterone production) and developmental (decreased anogenital distance and pathological changes in the testes) effects. Therefore, it could be argued that DIBP has a similar reproductive toxicity profile to 'transitional' (C4-6) phthalates for which reproductive and developmental effects are recognised.

5. HUMAN HEALTH HAZARD SUMMARY TABLE

Phthalate	***Acute***	***Irritation & Sensitisation***	***Repeated Dose Toxicity***	***Genetic Toxicity***	***Carcino-genicity***	***Fertility***	***Developmental Toxicity***
Diisobutyl phthalate (DIBP)	Oral Mouse: LD50 =12,800-39,520 mg/kg bw Rat: LD50 =16,000-60,320 mg/kg bw Intraperitoneal Mouse: LD50= 3990-12,800 mg/kg bw Rat: LD50 >16,00mg /kg bw	Skin irritation: minimal effects Eye irritation: negative Skin Sensitisation: negative	Rat: NOAEL = 1% in diet LOAEL = 5% ↓ body and testes weights (m) and increased liver weights (m+f)	<u>*In vitro*</u> Negative in bacterial mutation assays Positive in comet (DNA damage) assay using human mucosa (sample size = 70) <u>*In vivo*</u> No data	No data	*Male reproduction study* Rat: NOAEL= not established LOAEL = 2000 mg/kg bw/day ↓ testes weight and histology	*Developmental study* Rat: NOAEL <u>Devp</u> = 250 mg/kg bw/d LOAEL <u>Maternal</u> = 500 mg/kg <u>Devp</u> = 500 mg/kg ↓ pup weight and ↑ trans-abdominal testes migration

↓: decrease.

6. REFERENCES

Borch J, Axelstad M, Vinggaard AM, Dalgaard M (2006) Diisobutyl phthalate has comparable anti-androgenic effects to di-n-butyl phthalate in fetal rat testis, Toxicology Letters, 163, 183-190.

Borch J, Dalgaard M, & Ladefoged O (2005) Early testicular effects in rats perinatally exposed to DEHP in combination with DEHA-apoptosis assessment and immunohistochemical studies. Reproductive Toxicology, 19: 517-525.

Eastman Kodak Company (1978). Toxicity and health hazard summary: diisobutyl phthalate. OTS 0206525. DOC #878214402.

ECPI (2006) DIBP Information Centre-CAS Number 84-69-5 [on line]. Available from URL: http://www.dibp-facts.com/ [Assesses 2006 Oct 30).

Elsisi AE, Carter DE & Sipes, IG (1989). Dermal absorption of phthalate diesters in rats. Fundamental and Applied Toxicology 12, 70-77.

Foster PMD, Lake BG, Thomas, LV, Cook MW, & Gangolli, SD (1981). Studies on the testicular effects and zinc excretion produced by various isomers of monobutyl-*o*-phthalate in the rat. Chem.-Biol. Interactions, 34, 233-238.

Hardin BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, MackKenzie KM, Piccirillo VJ, & Smith KN (1987) Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratogen Carcinogen Mutagen, 7: 29-48

Harris CA, Henttu P, Parker MG, & Sumpter JP (1997) The estrogenic activity of phthalate esters *in vitro*. Environmental Health Perspectives, 105 (8): 802-811.

Hazardous Substances Data Bank (HSDB) (1998) US Department of Commerce National Technical Information Service, data bank on diisobutyl phthalate

Hodge HC (1954). Preliminary acute toxicity tests and short term feeding tests of rats and dogs given di-isobutyl phthalate and di-butyl phthalate. US EPA/OPTS public files.

IPCS Inchem (2001) International Programme on Chemical Safety on diisobutyl phthalate (http://www.inchem.org/documents/icsc/icsc/eics0829.htm)

Kleinsasser NH, Kastenbauer ER, Weissacher H, Muenzenrieder RK & Harreus UA (2000a). Phthalates demonstrate genotoxicity on human mucosa of the upper aerodigestive tract. Environmental and Molecular Mutagenesis 35, 9-12.

Kleinsasser NH, Wallner BC, Kastenbauer ER, Muenzenrieder & Harreus UA (2000b). Comparing the genotoxic sensitivities of human peripheral blood lymphocytes and mucosa cells of the upper aerodigestive tract using Comet assay. Mutation Res., 467, 21-30.

Lawrence WH, Malik M, Turner JE, Singh AR & Autian J (1975). A toxicological investigation of some acute, short-term, and chronic effects of administering di-2-ethylhexyl phthalate (DEHP) and other phthalate esters. Environmental Research 9, 1-11.

McEwen GJ, & Renner G (2006) Validity of Anogenital Distance as a Marker of in Utero Phthalate Exposure. Environmental Health Perspectives, 114(1): A19-20.

NICNAS (2007) Phthalate Hazard Compendium: A summary of physicochemical and human health hazard data for 25 phthalate chemicals. Sydney, National Industrial Chemicals Notification and Assessment Scheme.

Nishihara T, Nishikawa J, Kanayama T, Dakeyama F, Saito K, Imagawa M, Takatori S, Kitagawa Y, Hori S, & Utsumi H (2000). Estrogenic activities of 517 chemicals by yeast two-hybrid assay. Journal of Health Science 46 (4): 282-298.

NTP (1982) Mutagenesis testing results. Technical Bulletin 7, 5-9.

Oishi S & Hiraga K (1980a) Effects of phthalic acid monoesters on mouse testes. Toxicology Letters 6, 239-242.

Oishi S & Hiraga K (1980b) Effect of phthalic acid esters on mouse testes. Toxicology Letters 5, 413-416.

Oishi S & Hiraga K (1980c) Testicular atrophy induced by phthalate acid esters: Effect on testosterone and zinc concentrations. Toxicology and Applied Pharmacology 53, 35-41.

Oishi S & Hiraga K (1980d) Testicular atrophy induced by phthalic acid monoesters: Effects of zinc and testosterone concentrations. Toxicology 15, 197-202.

Phthalate Esters Panel HPV Testing Group (2001) High production volume (HPV) chemical challenge programme test plan for the phthalate esters category. December 10, 2001.

Saillenfait AM, Sabaté JP, & Gallissot F (2006) Developmental toxic effects of diisobutylphthalate, the methyl-branched analogue of di-n-butyl phthalate. Toxicology Letters, 165: 39-46.

Salazar-Martinez E, Romano-Riquer P, Yanez-Marquez E, Longnecker M, & Hernandez-Avila M (2004) Anogenital distance in human male and female newborns: a descriptive, cross-sectional study. Environmental Health, 3: 3-18.

Seed JL (1982). Mutagenic activity of phthalate esters in bacterial liquid suspension assays. Environmental Health Perspectives 45, 111-114.

Singh AR, Lawrence WH & Autian J (1972). Teratogenicity of phthalate esters in rats. Journal of Pharmaceutical Sciences 61 (1), 51-5.

Swan S, Main K, Liu F, Stewart S, Kruse R, Calafat A, Mao C, Redmon J, Ternand C, Sullivan S, Teague J, & The-Study-for-Future-Families-Research-Team (2005) Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect, 113: 1056-1061.

Takeuchi S, Iida M, Kobayashi S, Jin K, Matsuda T, & Kojima H (2005) Differential effects phthalate ester human estrogen receptors. Toxicology, 210: 223-233.

Toda C, Okamoto Y, Ueda K, Hashizume K, Itoh K, & Kojima N (2004) Unequivocal estrogen receptor-binding affinity of phthalate esters feaured with ring hydroxylation and proper alkyl chain size. Arch Biochem Biophys, 431: 16-21.

Zeiger E, Haworth S, Mortelmans K & Speck W (1985). Mutagenicity testing of di(2-ethylhexyl)phthalate and related chemicals in *Salmonella*. Environmental Mutagenesis 7, 213-232.

7. ROBUST STUDY SUMMARIES

Genetic Toxicity/Carcinogenicity

Test substance	**:**	Diisobutyl phthalate (DIBP).
Species	**:**	Specimens from human mucosa of the upper aerodigestive tract, harvested during surgery.
Route of admin.	**:**	Cell aliquots incubated with DIBP.
Exposure period	**:**	Incubation time 60 minutes.
Study Duration	**:**	N/A
Frequency of treatm.	**:**	N/A
Post exposure period	**:**	N/A
Doses	**:**	130, 216, 354, 650 µmol/ml.
Control group	**:**	Positive and negative control chemicals were used.
Guidelines	**:**	None
NOAEL / NOEL	**:**	N/A
LOAEL / LOEL	**:**	N/A
Method	**:**	The microgel electrophoresis technique (comet assay) was applied to detect DNA-strand breaks in single cells. The extent of DNA damage was quantified using the "Olive tail moment" (OTM), which is defined as the percentage of DNA in the tail of the comet multiplied by the migration distance.
Results	**:**	In n=40 samples of oropharyngeal mucosa, DIBP at 354 µmol/ml produced an average OTM of 9.6 ± 5.8 (negative controls, 1.3 ± 0.4; positive controls, 63.5 ± 11.1). In n=30 samples of biopsies from the inferior nasal turbinates, DIBP at 354 µmol/ml produced an average OTM of 13.4 ± 5.7 (negative controls, 1.6 ± 1.1; positive controls, 55.7 ± 10.6).
Conclusion	**:**	DIBP caused DNA strand breaks in isolated human mucosal cells from the upper aerodigestive tract.
Reference	**:**	Kleinsasser NH, Kastenbauer ER, Weissacher H, Muenzenrieder RK & Harreus UA (2000a). Phthalates demonstrate genotoxicity on human mucosa of the upper aerodigestive tract. Environmental and Molecular Mutagenesis **35**, 9-12.

Reproductive Toxicity

Test substance	:	Diisobutyl phthalate (DIBP)
Type	:	Dietary administration of test material to assess effects on reproductive organs.
Species	:	JCL: Wistar young male rats were used, weighing 90-120 g (mean 108 g, 5 weeks old).
Route of admin.	:	Dietary
Exposure period	:	One week
Study Duration	:	N/A
Frequency of treatm.	:	Ad libitum
Premating exposure period		N/A
Male	:	N/A
Female	:	N/A
Duration of test	:	N/A
Doses	:	Two percent DIBP in the diet (approx. 2000 mg/kg bw/d)
Control group	:	A group fed the basal diet.
NOAEL parental	:	N/A
NOAEL F1 offspring	:	N/A
Other: systemic effects	:	N/A
Guidelines	:	None
GLP	:	None
Method	:	Ten rats were given diets containing 2% DIBP. Twenty rats were in the control group. Body weight and food consumption were measured daily. After one week of treatment, the rats were killed by decapitation, samples of blood were collected, and the fresh weights of the testes, liver and kidneys were obtained. Serum and tissue samples were stored at -80°C until analysis.
Result	:	At the end of the experiment, the mean body weights of DIBP-treated rats were slightly lower than that of controls, but not significantly depressed. Food consumption was decreased during the first three days and then recovered to the control level. Absolute and relative liver weights were increased in treated rats. Absolute and relative testicular weights were significantly decreased and the testes were decreased in size, with histological examination revealing a decrease in spermatogonia and spermatocytes. There was a significant increase in testicular testosterone in treated rats but no difference in serum dihydrotestosterone levels. Zinc concentrations in the testes and liver were decreased.
Conclusion	:	The effects of DIBP on the testes (decrease in organ weight, size and histological changes) in this study involved high doses but their development within a week suggests a specific effect rather than a secondary effect of generalised toxicity.
Reference	:	Oishi S & Hiraga K (1980c). Testicular atrophy induced by phthalate acid esters: Effect on testosterone and zinc concentrations. Toxicology and Applied Pharmacology 53, 35-41.

Developmental Toxicity/ Teratogenicity

Test substance	**:**	Diisobutyl phthalate
Species	**:**	Adult, virgin female rats of the Sprague-Dawley strain, weighing between 200 and 250 g. Male rats of the same strain and age were used for mating
Route of admin.	**:**	Intraperitoneal injections.
Exposure period	**:**	Intraperitoneal injections were given on days 5, 10 and 15 of gestation
Study Duration	**:**	2 estrus cycles preior to mating – GD20
Frequency of treatm.	**:**	As above
Doses	**:**	Approx. 390, 780 and 1300 mg/kg bw.
Control group	**:**	Cottonseed oil, normal saline, distilled water and untreated controls employed
NOAEL maternal tox.	**:**	Not determined
NOAEL teratogen.	**:**	Not determined
Guidelines	**:**	Nil
GLP	**:**	Nil
Method	**:**	All treatments were administered by intraperitoneal injections on days 5, 10 and 15 of gestation. On day 20, each rat was killed with ether. The uterine horns and ovaries were surgically exposed to permit counting and recording of the numbers of corpora lutea, resorption sites, and viable and dead foetuses. Foetuses were weighed and examined for gross malformations. A proportion of foetuses were prepared to permit visualisation of the skeletal system.
Results	**:**	Effects observed were statistically significant decreases in the average weight of foetuses at all dose levels and increased numbers of resorptions and skeletal abnormalities ("partially elongated and fused ribs") at the highest dose.
Conclusion	**:**	Decreased average foetal weight is indicative of developmental toxicity.
Reliability	**:**	The study is old and not of a standard design and doses were administered intraperitoneally. The effect observed (decreased foetal weight) were compared to untreated controls rather than vehicle control.
Reference		Singh AR, Lawrence WH & Autian J (1972). Teratogenicity of phthalate esters in rats. Journal of Pharmaceutical Sciences 61 (1), 51-5.

Developmental Toxicity/ Teratogenicity

Test substance	**:**	Diisobutyl phthalate
Species	**:**	Sprague-Dawley rats approximately 180-200 g.
Route of admin.	**:**	gavage
Exposure period	**:**	GD 6 to GD20
Study Duration	**:**	GD 0 to GD21
Frequency of treatm.	**:**	As above
Doses	**:**	250, 500, 750 and 1000 mg/kg bw/day
Control group	**:**	Olive oil
NOAEL maternal tox.	**:**	250 mg/kg bw/day
NOAEL teratogen.	**:**	250 mg/kg bw/day
Guidelines	**:**	Nil
GLP	**:**	Nil
Method	**:**	Pregnant females were given daily doses of DIBP by gastric intubation from GD 6 to GD20. All females were observed daily for clinical signs of toxicity with body weight recorded. On GD 21, the females were sacrificed. Uterine contents were examined to determine the number of implantation sites, resorptions, and dead and live foetuses. All live foetuses were weighted, and examined for external anomalies including those of the oral cavity. Half were examined for internal soft tissue changes and the other half for skeletal examination.
Results	**:**	Signs of 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 and higher doses. The incidences of resorptions was significantly increased at 750 mg/kg bw, and reached 60% at 1000 mg/kg bw. For the offspring, there was a dose-related decrease in foetal weight, which was significantly lower than control from 500 mg/kg/day. There were significant increases in the incidence of foetuses with skeletal malformations (supernumerary lumbar and cervical ribs). At the two highest doses (750 and 1000 mg/kg bw/d), the incidence of male foetuses with undescended testes was significantly increased and the degree of trans-abdominal testicular migration was increased in a dose-related fashion, in treated pups (significant from 500 mg/kg bw/d).
Conclusion	**:**	Decreased average foetal weight and increased incidence of undescended testes are indicative of developmental toxicity.
Reference		Saillenfait Am, Sabaté JP, Gallissot F (2006) Developmental toxic effects of diisobutylphthalate, the methyl-branched analogue of di-n-butyl phthalate. Toxicology Letters, **165**, 39-46.