

# Existing Chemical Hazard Assessment Report



Australian Government  
Department of Health and Ageing  
NICNAS

## Diethyl Phthalate

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# Preface

This report was compiled under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). This Scheme was established by the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act), which came into operation on 17 July 1990.

The principal aim of NICNAS is to aid in the protection of people at work, the public and the environment from the harmful effects of industrial chemicals.

NICNAS assessments are carried out in conjunction with the Department of Environment and Heritage, which carry out the environmental assessment for NICNAS. NICNAS has two major programs: the assessment of the health and environmental effects of new industrial chemicals prior to importation or manufacture; and the other focussing on the assessment of chemicals already in use in Australia in response to specific concerns about their health/or environmental effects.

There is an established mechanism within NICNAS for prioritising and assessing the many thousands of existing chemicals in use in Australia.

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# Overview

This review of diethyl phthalate (DEP) is a health hazard assessment only. For this assessment, the International Programme on Chemical Safety: Concise International Chemical Assessment Document and Opinion of the Scientific Committee on Cosmetic and Non-Food Products intended for consumers on DEP were consulted. Information from these documents was supplemented with more recent literature surveys conducted up to September 2006.

According to the European Council for Plasticisers and Intermediates (ECPI), DEP is a plasticiser widely used in tools, automotive parts, toothbrushes, food packaging, cosmetics and insecticide.

In Australia, DEP is mainly imported as finished products or mixtures. DEP is used in epoxy resins, cosmetics, personal care and pharmaceutical products and children's toys. DEP is imported in fragrance bases for use in the formulation of household cleaning and personal care products. It is used as an alcohol denaturant. DEP is also imported for distribution to various institutions and laboratories for biotechnological and pharmaceutical research.

Structurally, phthalate esters are characterized by a diester structure consisting of a benzenedicarboxylic acid head group linked to two ester side chains. DEP possesses 2 linear ester side chains each of two carbons (C2).

Toxicity data for DEP 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 hazard assessment reports for phthalates and the NICNAS Phthalates Hazard Compendium, which contains a comparative analysis of toxicity endpoints across 24 *ortho*-phthalates, including DEP.

DEP is readily absorbed via the oral route and eliminated rapidly, with the urine being the major route of excretion following oral administration in rats and mice. Monoethyl phthalate (MEP) is the major metabolite present in urine. Percutaneous absorption of DEP through animal skin is significant, although absorption through human skin may be significantly less than that of animal skin.

DEP has low acute oral and dermal toxicity. DEP causes minimal skin and eye irritation in animals. DEP is not a skin sensitiser in humans or animals.

Repeated dose toxicity studies indicate that the liver is the primary target organ for DEP. However, hypertrophic effects were also observed in other organs such as kidney, stomach and small intestine. From a 16-week rat oral study, a NOAEL of 1% DEP in the diet (approximately 750 mg/kg bw/d) was determined, with a LOAEL of 5% (approximately 3200-3700 mg/kg bw/d) based on increased relative liver, kidney, stomach and small intestine weights.

In vitro data show equivocal evidence of genotoxicity for DEP. In vivo data are not available. Conclusions on the genotoxicity potential of DEP cannot be drawn.

With respect to carcinogenicity, studies in mice and rats provide equivocal evidence of carcinogenic potential for DEP.

A NOAEL for fertility effects of 40-56 mg/kg bw/d was derived from a well-conducted two-generation study in rats. There was no effect on male reproductive organ weights up to dose 1016-1375 mg/kg bw/d; however, there was an increased frequency of abnormal and tailless sperm in the F1 generation exposed to 197-267 mg/kg bw/d and testosterone serum levels were reduced in F0 males. In in vitro studies, DEP was associated with reductions in human sperm motility and increased DNA damage in sperm. In contrast, there was no association between MEP levels and sperm parameters in men attending an andrology clinic.

Reduced testicular and serum testosterone levels were reported in rats after 7 days diet containing 2% DEP (2000 mg/kg bw/d) but no effect on testicular zinc levels were reported after 4 days dosing with 1600 mg/kg bw/d DEP. Changes in Leydig cell ultrastructure by DEP has also been demonstrated in rats treated for only 2 days. This suggests that the observed reduction in testosterone levels seen after administration of DEP might be due to its direct effects on the Leydig cells of the testes despite a lack of testicular atrophy.

Human data are limited and hampered by measurements of multiple phthalates in small sample sizes. Urinary maternal MEP concentration was inversely associated with anogenital distance (AGD) in offspring in a study of 85 mother-sons pairs. The reliability of the measurement of AGD in humans has not been verified. No association was found between breast milk MEP levels and cryptorchidism but MEP showed positive correlations with sex-hormone binding globulin and LH:free testosterone ratio.

In a two-generation reprotoxicity study, the main effects included reduced pup weight at weaning and delayed onset of vaginal opening and pinna detachment at the highest dose (maternal effects were also observed at this dose). There was no difference in pup weight at birth suggesting that these effects may be due to lactational exposure to DEP. In developmental studies, effects in offspring (increased skeletal variations, primarily rudimentary extra lumbar ribs) occurred above maternotoxic doses. The NOAEL for development effects was 197-267 mg/kg bw/d and the LOAEL was 1016-1375 mg/kg bw/d based on decreased pup weight and developmental delay. There was little evidence that DEP has oestrogenic activity in vivo or in vitro.

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# Acronyms and Abbreviations

AGD	anogenital distance
AGI	anogenital index
AR	androgen receptor
BBP	butylbenzyl phthalate
bw	body weight
C	Celsius
CAS	Chemical Abstracts Service
CHO	Chinese hamster ovary
d	day
DEHP	diethylhexyl phthalate
DEP	diethyl phthalate
DMBA	7,12-dimethyl-benz[a]anthracene
DNA	deoxyribonucleic acid
ER	oestrogenic receptor
f	female
F0	parental generation
F1	filial 1 (first generation)
F2	filial 2 (second generation)
g	gram
GD	gestation day
GLP	good laboratory practice
h	hour
ip	intraperitoneal
kg	kilogram
kPa	kilopascals
L	litre
LC50	median lethal concentration
LD50	median lethal dose
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
m	male
M	mole
MEP	monoethyl phthalate

mg	milligram
mL	millilitre
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
PND	post-natal day
ppm	parts per million
TPA	12- <i>O</i> -tetradecanoylphorbol-13-acetate
w/v	weight per volume
w/w	weight per weight
Zn	zinc
μ	micro

# 1. Introduction

This review of diethyl phthalate (DEP) is a health hazard assessment only. For this assessment, the International Programme on Chemical Safety: Concise International Chemical Assessment Document 52 (IPCS, 2003) and Opinion of the Scientific Committee on Cosmetic and Non-Food Products intended for consumers (SCCNFP, 2002) on DEP were consulted. Information from these documents was supplemented with relevant studies from more recent literature surveys conducted up to September 2006.

Information on Australian uses was compiled from data supplied by industry in 2004 and 2006.

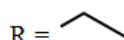
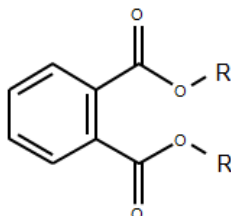
References not marked with an asterisk were examined for the purposes of this assessment. References not examined but quoted from the key documents as secondary citations are also noted in this assessment and marked with an asterisk.

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 24 *ortho*-phthalates (NICNAS, 2008).

## 2. Identity

### 2.1 Identification of the substance

CAS Number:	84-66-2
Chemical Name:	1,2-Benzenedicarboxylic acid, diethyl ester
Common Name:	DEP
Molecular Formula:	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>
Structural Formula:	



Molecular Weight:	222.30
Synonyms:	Diethyl phthalate; Phthalate, diethyl; Ethyl phthalate; Phthalic acid, diethyl ester; o-Benzenedicarboxylic acid diethyl ester, o-Bis(ethoxycarbonyl)benzene
Purity/Impurities/Additives:	Purity: $\geq 99.70 - 99.97$ % w/w Impurities: isophthalic, terephthalic acid and maleic anhydride Additives: none

### 2.2 Physico-chemical properties

**Table 1: Summary of physico-chemical properties**

Property	Value
Physical state	Clear, colourless, odourless liquid
Melting point	No data
Boiling point	298°C (295°C – 302°C)
Density	1120 kg/m <sup>3</sup> at 25°C
Vapour pressure	2.19 × 10 <sup>-4</sup> kPa at 25°C
Water solubility	1 g/L at 25°C
Partition coefficient n-octanol/water (log Kow)	2.47 – 2.51
Henry's law constant	7.9 × 10 <sup>-5</sup> kPa.m <sup>3</sup> /mole
Flash point	No data

Source: SCCNFP (2002); IPCS (2003)

### 3. Uses

According to ECPI (2006), DEP is a plasticiser widely used in tools, automotive parts, toothbrushes, food packaging, cosmetics and insecticide.

In Australia, DEP is mainly imported as finished products or mixtures. DEP is used in epoxy resins, cosmetics, personal care and pharmaceutical products, perfumes and children's toys. DEP is imported in fragrance bases for use in the formulation of household cleaning and personal care products. It is used as an alcohol denaturant. DEP is also imported for distribution to various institutions and laboratories for biotechnological and pharmaceutical research.

## 4. Human Health Hazard

### 4.1 Toxicokinetics

#### Previous evaluations

##### *Oral*

Following oral administration of  $^{14}\text{C}$ -DEP to rats and mice (doses not stated), much of the administered dose was excreted in the urine (90%) within 48 hours post-dosing, with the majority (82%) being eliminated during the first 24 hours (Api, 2001; Ioku et al., 1976\*). There was approximately 3% found in the faeces over the same period of time. Tissue residue levels of radioactivity were low with the highest concentrations of radioactivity observed in the liver and kidney, followed by blood, spleen and adipose tissue. Highest levels were noted within 20 minutes, followed by a rapid decrease to only trace amounts after 24 hours. The major metabolite detected in urine was the ester hydrolysis product, monoethyl phthalate (MEP). Phthalic acid was also detected in urine as a minor metabolite.

Following administration of DEP by stomach intubation at 10 or 100 mg/kg bw in rats, 85 to 93% of the administered dose was excreted in the urine 7 days post-dosing (Kawano 1980\*). For both doses, approximately 77-78% of the administered dose was excreted in urine within 24 hours as MEP ( $\approx 70\%$  of the dose), phthalic acid (9% of the dose) and parent compound (0.1-0.4%).

Following intraperitoneal (ip) injection of  $^{14}\text{C}$ -DEP, radioactivity was detected in amniotic fluid, maternal, placental, and foetal tissues of rats, indicating that the compound can pass through the placenta to the developing foetus (Singh et al., 1975\*). The half-life of the compound in foetal tissue was approximately 2.2 days.

No studies were located on the distribution or excretion of DEP in humans following inhalation, oral, dermal, or other routes of exposure. However, a recent study in humans showed that almost three quarters (71%) of the total amount of MEP, the main metabolite of DEP, excreted in the urine was in the form of free monoester, the rest being MEP glucuronide (Silva et al., 2003).

##### *Dermal*

When  $^{14}\text{C}$ -DEP was applied to male rat skin at 5 to 8 mg/cm<sup>2</sup> under occlusion, around 24% and 1% of the administered dose was excreted in the urine and faeces, respectively within 24 hours (Elsisi et al., 1989\*). Approximately  $74 \pm 21\%$  of the dose was absorbed and very little radioactivity was found in tissues 7 days after exposure. The amounts of radioactivity found in the brain, lung, liver, spleen, small intestine, kidney, testis, spinal cord and blood were each less than 0.5% of the dose. Metabolites were not characterised.

A similar experiment was conducted in female rabbits. When <sup>14</sup>C-DEP was applied to the rabbit skin (dose not stated), around 49% and 1% of the administered dose was excreted in the urine and faeces, respectively after 4 days (RIFM, 1973\*). Very little radioactivity was found in tissues 4 days after exposure. The amounts of radioactivity found in the tissues were: liver (0.003% of dose), kidney (0.004% of dose) and blood (less than 1% of dose). Metabolites were not characterised.

In an in vitro study, comparative percutaneous absorption of DEP between human and rat skin was evaluated in flow-through diffusion cells. Results showed that dermal absorption of <sup>14</sup>C-DEP through male rat dorsal skin was approximately 35.9%, while average absorption in human breast skin in vitro was approximately 3.9% after 72 hours for covered conditions. Hydrolysis to the monoester by skin was demonstrated in vitro for both rats and humans (Mint et al., 1994\*; Hotchkiss, 1994\*). Scott et al. (1987\*), using the same system as Hotchkiss and Mint, reported that the in vitro absorption of DEP through rat skin was significantly higher (37.5%) than through human skin. The steady state absorption rate was 1.27 µg/cm<sup>2</sup>/hour for human and 41.37 µg/cm<sup>2</sup>/hour for rat skin.

### **Data not reported in previous evaluations**

No data.

### **Conclusion**

DEP is primarily metabolized to MEP. It appears to be widely distributed in the body, but does not accumulate in tissue. After oral administration to rats and mice, DEP is readily absorbed and eliminated rapidly, with the urine being the major route of excretion. The hydrolytic mechanism of DEP was found to be qualitatively similar in rodents and humans. Percutaneous absorption of DEP through animal skin is significant, although in vitro studies suggest absorption through human skin is likely to be significantly less than that of animal skin.

## **4.2 Acute toxicity**

### **Previous evaluations**

#### ***Oral***

LD<sub>50</sub> were reported in the range of 1 to 31 g/kg bw by oral route in mice, rats, rabbits, dogs and guinea pigs. Clinical signs following oral administration included CNS depression, convulsion and respiratory paralysis prior to death.

#### ***Dermal***

Rats (3 males and 3 females) received a single application of the test substance (1, 2, 5 and 10 mL/kg, corresponding to up to 11 g/kg) dermally under an occluded patch. No deaths were reported at any dose. Slight redness of the skin at the site of application was observed at 24 h for all concentrations (RIFM 1978c\*).

A dermal LD<sub>50</sub> of 3000 mg/kg bw in guinea pig was also reported.

The results for acute toxicity studies in laboratory animals are summarised in Table 2.

## Conclusion

Based on the LD50/LC50 reported from the studies, DEP is considered to have a low order of acute toxicity in laboratory animals.

## Data not reported in previous evaluations

No data.

**Table 2: Acute toxicity of DEP in animals**

Route of administration	Species	Results (LD50/LC50)
Oral	Mouse	6200 mg/kg bw
	Rat	>5600 to 31000 mg/kg b w
	Guinea pig	>4000 to 8600 mg/kg bw
	Rabbit	1000 mg/kg bw
	Dog	5000 mg/kg bw
Dermal	Rat	>11000 mg/kg bw
	Guinea pig	3000 mg/kg bw
Inhalation	Mouse	4.9 mg/L
	Rat	7.5 mg/L

Source: SCCNFP (2002)

## 4.3 Irritation

### 4.3.1 Skin irritation

#### Previous evaluations

Application of undiluted DEP (concentration and duration not stated) on intact and braded rabbit skin (6 animals) in a closed patch test caused slight to moderate irritation at both sites after 24 hours. A slight (40%) reduction in irritation was noticed at the 72 hours evaluation (Api, 2001; RIFM 1974\*). In contrast, in two other 4-h semi-occlusive patch tests in rabbits, 0.5 mL of undiluted DEP did not cause skin irritation (Api, 2001; RIFM, 1984\*; 1985\*).

Studies in rats showed application of undiluted DEP (2 mL/kg/d) on rat skin for 2 weeks (6 h/d) in a semi-occlusive patch test caused slight erythema and/or desquamation. Histological examination revealed very mild epidermal thickening and slight hyperkeratosis (Api, 2001; RIFM 1994\*). In addition, the NTP (1995\*) reported that long-term dermal DEP (99% pure, 100 or 300 µL) administration is associated with mild, dermal acanthosis in rats.

The Research Institute for Fragrance Materials Inc (RIFM) database contains reports of 576 human volunteers exposed to undiluted DEP in occluded/closed patch test and no dermal reactions were reported (Api, 1997\*).



### **Data not reported in previous evaluations**

No data.

### **Conclusion**

DEP caused minimal skin irritation.

## **4.3.2 Eye irritation**

### **Previous evaluations**

Several eye irritation studies were conducted in rabbits. Application of 0.1 mL of undiluted DEP caused minimal eye irritation after one hour. No reactions were noted at 24, 48 or 96 hours (Api, 2001; Draize, 1944\*). Similarly, undiluted DEP (0.1 mL) resulted in transient slight redness of the conjunctivae (Api, 2001; RIFM, 1978a\*) and minimal eye irritation was noted (ATSDR, 1995\*).

### **Data not reported in previous evaluations**

No data.

### **Conclusion**

DEP caused minimal eye irritation.

## **4.4 Sensitisation**

### **Previous evaluations**

Several skin sensitisation studies were conducted in guinea pigs. One Buehler study, purity not given (Api, 2001; RIFM, 1978b\*), and two Magnusson and Kligman maximization studies, using undiluted and 50% aqueous solution of DEP respectively, (Api, 2001; Buehler, 1996\*; Klecak et al., 1977\*) did not show any skin sensitisation effect. Negative dermal sensitisation responses were also observed using undiluted DEP in an open epicutaneous test, the Draize intradermal test and the Freund's complete adjuvant test (Api, 2001; Klecak et al., 1977\*, Klecak, 1979\*).

In a local lymph node assay, DEP (25µL of 25-100% DEP in acetone-olive oil) did not induce significant stimulation of thymidine incorporation into lymph node cells (Ryan et al., 2000\*)

DEP has not been reported to be a dermal sensitiser in healthy volunteers in several human trials, although sensitisation was reported in some studies, those incidences were mostly involving persons with skin diseases.

### **Data not reported in previous evaluations**

DEP (2% v/v) was applied to the skin of 203 volunteers under semi-occlusive patch for 3 consecutive weeks and reactions to a challenge application were recorded following a 2-week rest period (David et al., 2003). Results showed slight erythema (score less than 1) in only one subject at 96 hours after challenge.

### **Conclusion**

DEP did not cause skin sensitiser in animals and humans.

## 4.5 Repeated dose toxicity

### Previous evaluations

#### *Oral*

Ten male JCL: Wistar rats were given 2% DEP in the diet for 1 week (Api, 2001; Oishi and Hiraga, 1980\*). A significant increase (12%) in relative liver weight was observed, with no changes in kidney and testis weights.

Rats (15/sex) were treated with DEP in the diet at levels of 0, 0.2, 1 and 5% for 16 weeks (approximately 150, 770/750 and 3200/3700 (m/f) mg/kg bw/d) (Api, 2001; Brown et al., 1978\*). Body weight gain was significantly reduced (15-32%) in both sexes at 5% and in females at 1% (8%). Concurrent paired-feeding experiments indicated that the decrease in body weight was primarily attributable to lower food consumption and/or poorer food utilisation. Relative kidney and liver weights were increased significantly in both sexes at 5%. The increases in relative liver weights of females at all doses were significant and dose-dependent. There were also significant dose dependent increases in the absolute and relative weights of stomach and small intestine in the female rats at all dietary levels at week 16. In male rats, small intestine weights were increased at 5% only, whereas stomach weights were increased at 1 and 5%. There was no effect on the gross or microscopic pathology of the lungs or trachea and no abnormal histopathology findings in the liver, kidney, or digestive organs. Unusually low control values were observed in stomach and small intestine weights at doses below 5%.

A NOAEL of 1% DEP in the diet (approximately 750 mg/kg bw/d) was determined based on increased relative liver, kidney, stomach and small intestine weights at 5% (approximately 3200-3700 mg/kg bw/d).

#### *Dermal*

In a 4-week study, mice (10/sex) were treated dermally 5 days per week with DEP at 0, 12.5, 25, 50, and 100  $\mu$ L/animal, approximately equal to 0, 560, 1090, 2100, or 4300 mg/kg bw/d for males and 0, 630, 1250, 2500, or 5000 mg/kg bw/d for females (Api, 2001; NTP, 1995\*). There was no histological evidence of damage to any organ. Absolute and relative liver weights were greater than those of the controls in females treated with 25 and 100  $\mu$ L DEP. No effects were observed at 12.5  $\mu$ L /d.

Rats (10/sex) treated dermally with DEP at 0, 37.5, 75, 150, and 300  $\mu$ L/animal/d (approximately equal to 0, 200, 400, 800, or 1600 mg/kg bw/d in males and 0, 300, 600, 1200, or 2500 mg/kg bw/d in females) for 4 weeks exhibited no clinical signs of toxicity (Api, 2001; NTP, 1995\*). Gross observations and histopathology were also not affected. Increased relative liver weights were observed in 300  $\mu$ L/animal/d males (9%) and females (7%) and 150  $\mu$ L/animal/d females (10%) as compared to controls. Relative kidney weights of 150  $\mu$ L and 300 $\mu$ L/animal/d males and 150  $\mu$ L/animal/d females were greater than those of controls. No other adverse effects were observed in this study.

### Data not reported in previous evaluations

A 5 month dietary study was undertaken to evaluate the toxicity of DEP in young male Wistar rats (6-7 weeks old) (Pereira et al., 2006). DEP was administered in diet at 0, 0.57, 1.4 and 2.85 mg/kg bw/d. A significant increase in relative liver weights

was reported only for the lowest dose. Liver glycogen, cholesterol, triglycerides and lipid peroxidation were statistically significantly increased in all treatment groups, however only increases for glycogen and cholesterol showed dose dependence. Liver and serum levels of acid phosphatase, lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase were also increased. Electron micrographs of liver from low dose animals showed severe intra- and intercellular vacuolation, loss of hepatocyte architecture, fatty degeneration of centrilobular and periportal hepatocytes and increased numbers of peroxisomes. Higher doses showed granular deposits in hepatocytes and mild vacuolations in centrilobular and periportal hepatocytes. A dose-dependent liver mitochondrial proliferation across all dose groups was also reported. No other organs were examined. Several inconsistencies in data presentation are noted which hamper the reliability of this publication.

## **Conclusion**

Repeated dose toxicity studies indicate that the liver is the primary target organ for DEP. However, hypertrophic effects were also observed in other organs such as kidney, stomach and small intestine. A NOAEL of 1% DEP in the diet (approximately 750 mg/kg bw/d) was determined from a 16-week rat oral study based on increased relative liver, kidney, stomach and small intestine weights at 5% (approximately 3200-3700 mg/kg bw/d, males, females).

A NOAEL of 12.5  $\mu$ L/d (560-630 mg/kg bw/d) on dermal application was established based on a 4-week study in mice.

## **4.6 Genetic toxicity**

### **Previous evaluations**

The majority of in vitro mutagenic assays for DEP yielded negative results except for the following conflicting results: Kozumbo et al. (1982\*) reported that DEP was weakly mutagenic for *S. typhimurium* strains TA100 and TA1535 in the absence of metabolic activation. However, results obtained by the National Toxicology Program (Api, 2001; NTP, 1995\*) demonstrated absence of mutagenic responses with DEP (up to 10 mg/plate) in *S. typhimurium* strains TA100, TA1535, TA98 or and TA1537 with or without activation. MEP, the hydrolysed monoester of DEP also showed no mutagenic effect when tested with *S. typhimurium* strains TA100 and TA98 and *E. coli* WP2 strain *uvr A*<sup>+</sup> and *uvr A*<sup>+</sup>, with or without rat liver S9 (Api, 2001; Yoshikawa et al., 1983\*).

No chromosomal aberrations was induced by DEP at 70, 151 and 324  $\mu$ g/mL in Chinese ovary cells, with or without rat liver S9, at concentrations up to 0.324 mg/mL. However, DEP has been reported to induce sister chromatid exchanges at concentrations 167-750  $\mu$ g/mL, in the presence of S9 (Api, 2001; NTP, 1995\*).

### **Data not reported in previous evaluations**

Duty et al. (2003b) reported a significant association between human urinary MEP, at environmental levels, and increased DNA damage in sperm (sample size = 141) as measured by an in vitro Neutral Comet assay.

## **Conclusion**

There were conflicting results in both mutagenic and clastogenic assays. DEP was negative in the majority of in vitro mutagenic assays. It was positive in sister chromatid exchanges but negative in chromosomal aberrations assays. There were no in vivo genotoxicity studies. Overall, the genotoxic potential of DEP cannot be determined.

## **4.7 Carcinogenicity**

### **Previous evaluations**

#### ***Oral***

Rats (15/sex/dose) were fed with DEP in the diet at 0, 0.5, 2.5 or 5% for 2 years (Api, 2001; RIFM, 1955\*). The approximate doses were 0, 250, 1250 or 2500 mg/kg bw/d, respectively. Decreased bodyweight gains without depression in food consumption was observed in both sexes at 5% throughout the study. There were no treatment-related effects on haemocytology, blood sugar, non-protein nitrogen levels, urinalyses or gross or microscopic organ pathology. Due to the small study size, this study is not adequate for the evaluation of carcinogenicity.

#### ***Dermal***

B6C3F1 Mice (60/sex/dose) were treated dermally with DEP at 0, 7.5, 15 and 30  $\mu$ L/animal/d (corresponding to approximately 0, 280, 520 or 1020 mg/kg bw/d for males and 0, 280, 550, or 1140 mg/kg bw/d for females) in 100  $\mu$ L acetone, given 5 days per week for 103 weeks (Api, 2001; NTP, 1995\*). Survival and mean body weights of the dosed animals were similar to controls throughout the study. An increased incidence of non-neoplastic proliferative lesions (basophilic foci) in the liver was reported which was statistically significant at the 15  $\mu$ L dose in males, but not females. This effect was not dose-related. The incidence of combined hepatocellular adenomas or carcinomas was increased in both sexes at all doses but the incidence was dose-related (statistically significantly) only in males, from the lowest dose of 280 mg/kg bw/d. Effects were considered equivocal evidence of carcinogenic activity due to lack of dose relationship in females and similar incidence of combined hepatocellular adenomas or carcinomas in males at the highest dose compared to historical controls.

F344/N rats (60/sex/dose) were treated dermally with DEP at 0, 100 or 300  $\mu$ L per animal/d (approximately 320 or 1010 mg/kg bw/d for males and 510 or 1560 mg/kg bw/d for females), 5 days per week for 2 years (Api, 2001; NTP, 1995\*). No evidence of dermal toxicity was noted. Survival rates of treated animals were similar to control. The mean body weights of 300  $\mu$ L males were slightly less (4-9%) than those of the controls throughout the study. A treatment-related increased incidence of minimal to mild epidermal acanthosis at the site of application was observed in dosed males and females. This was considered an adaptive response to irritation. A decrease in the incidence of fibroadenomas of the mammary glands was observed in female treated rats. The incidence of fatty degeneration of the liver was also notably decreased in treated animals compared to controls, possibly attributable to the hypolipidemic action of DEP. No evidence of skin neoplasia due to DEP was found in male or female rats.

One year initiation/promotion studies in male Swiss (CD-1) mice were conducted to evaluate the potential of dermally applied DEP to initiate tumorigenesis when followed by a strong promoter (TPA: 12-*O*-tetradecanoylphorbol-13-acetate) or to promote tumorigenesis following administration of a known initiator (DMBA; 7,12-dimethyl-benz[*a*]anthracene). No initiator or promoter activity of DEP was demonstrated (Api, 2001; Marsman et al., 1994\*; NTP, 1995\*). The promoting activity of TPA following DMBA initiation was confirmed in these studies.

### **Data not reported in previous evaluations**

No data.

### **Conclusion**

In a 2-year rat dermal study, minor dermal acanthosis was observed following dermal application of DEP but was likely an adaptative response to local irritation. In a 2-year dermal study in mice, the incidence of combined hepatocellular adenomas or carcinomas was increased in both sexes at all doses but the incidence was dose-related only in males. Effects were considered equivocal evidence of carcinogenic activity due to lack of a dose response relationship in females and similar incidence of combined hepatocellular adenomas or carcinomas compared to historical controls. A one-year tumorigenesis initiation/promotion study in mice showed no initiator or promoter activities for DEP. Taken as a whole, these studies provide equivocal evidence of carcinogenic potential for DEP.

## **4.8 Reproductive toxicity**

Traditional hazard assessments consider effects on fertility separate from developmental toxicity. Fertility is tested by exposing sexually mature adults to a chemical and examining the effects on reproductive capacity. Developmental toxicity is studied by exposing pregnant dams and looking for effects in the foetuses. 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 effects on fertility and the two endpoints cannot be clearly distinguished.

In this hazard assessment, data are presented on the basis of test procedure. Test procedures include repeat dose toxicity studies that dose adult animals for varying durations, 2-generation studies, prenatal developmental toxicity studies (only the dam is dosed, study ends before parturition) and postnatal developmental toxicity studies (dam is dosed during gestation and allowed to litter, study ends during weaning). The effects on fertility (as adults) and development (as foetuses) are then discussed separately.

The effects of DEP on reproductive endpoints have been tested in a variety of species. Reproductive and developmental data are summarised in Table 4.

### **4.8.1 Human studies**

#### **Previous evaluations**

When human sperm suspensions were incubated with DEP (33, 330, 3300  $\mu\text{mol/L}$ ), the mean motility was dose-dependently decreased at doses higher than 330  $\mu\text{mol/L}$  (about 10% inhibition at 3300  $\mu\text{mol/L}$ ) (Fredricsson et al., 1993\*).

## Data not reported in previous evaluations

Jonsson et al. (2005) studied semen parameters and urinary phthalate monoester levels in 234 military recruits. Subjects in the highest quartile for MEP had fewer motile sperm, more immotile sperm and lower luteinizing hormone (LH) values.

Duty et al. (2003a; b) examined the levels of phthalate monoesters in urine in men attending an andrology clinic and semen quality and DNA damage in sperm. Eight urinary phthalate monoesters, including MEP, were measured in a single spot urine sample collected on the same day as the semen sample. While there was no dose-response relationship between MEP and sperm or semen parameters, urinary MEP was associated with increased sperm DNA damage (as measured by the comet assay).

Association between 11 maternal urinary phthalate monoester concentrations and genital parameters such as anogenital index (AGI) [i.e. anogenital distance (AGD) normalized for body weight] and testicular descent in children was investigated in 85 mother-son pairs (Swan et al., 2005). Urinary MEP concentration was inversely related to AGI. 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 in males a correlation of 0.48 and that body length may be a slightly better predictor for AGD than weight (Salazar-Martinez et al., 2004).

A follow-up study to Swan et al. (2005) was conducted by their collaborator (Marsee et al., 2006) who estimated, based on metabolite concentrations and pharmacokinetic modelling, the average individual daily exposures of phthalates in the mothers of male infants exhibiting reduced AGD. Results estimated the median and 95<sup>th</sup> percentile of daily exposure for DEP to be 6.64 and 112.3 µg/kg/d respectively.

Breast milk samples were analysed for six different phthalate monoesters in a Danish-Finnish cohort study (62 cryptorchid and 68 healthy boys) and serum measurements for gonadotropins, sex-hormone binding globulin, testosterone and inhibin B (Main et al., 2006). No association was found between MEP and cryptorchidism, but MEP showed positive correlations with sex-hormone binding globulin and with LH:free testosterone ratio.

## 4.8.2 Repeat dose toxicity studies

### Previous evaluations

Groups of young male Sprague Dawley rats were dosed with 1.6 g/kg bw/d DEP for 4 days (Foster et al., 1980\*). There was no significant effect on food intake or body weight gain nor testes weight or zinc content of testes. Histological examination did not reveal any testicular lesions. In a second study, young male rats (5 weeks old) were treated with 2% DEP in the diet for 7 days (Oishi and Hiraga, 1980\*). Relative liver weights were significantly elevated. The concentration of testosterone in both serum and testes was significantly decreased by approximately 40%. Testes weights and Zn levels in the testes, an element thought to be essential for the maintenance of normal testicular function, were unaffected.

In an investigation of ultrastructural changes of Leydig cells caused by treatment of DEP, young male Wistar rats were treated by oral gavage with 2000 mg/kg bw/d for 2 days (Jones et al., 1993\*). Testosterone production was measured and cells were examined by electron microscopy. DEP produced ultrastructural changes in Leydig cells in vivo, characterised by smooth endoplasmic reticulum focal dilation, swelling of the mitochondria associated and with a loss of matrix granules in all treated animals. Increased interstitial macrophage activity was also seen with the activity located around Leydig cells showing substantial cytoplasmic alterations such as swollen mitochondria. Testes weights and zinc levels in the liver and testes were unaffected. The histological effects were not replicated in vitro when Leydig cells were cultured with 1000 µM of methylethyl phthalate, a major DEP metabolite (Jones et al., 1993\*).

The effect of administration of 5 different phthalates on the levels of laurate hydroxylase (a marker for the CYP 4 family of isozymes, which is responsible for the metabolism of testosterone) was examined in rat liver microsomes (Okita & Okita, 1992). Administration of DEP to rats increased the specific activity of laurate hydroxylase by 1.6 times more than that induced in control rats but 6.75 fold less than that induced by 2-diethylhexyl phthalate (DEHP).

### **4.8.3 Continuous breeding reproductive toxicity studies**

#### **Previous evaluations**

DEP was administered to CD-1 mice in the diet at 0, 0.25, 1.25, 2.5% in a continuous breeding protocol (corresponding to 0, 340, 1770 or 3640 mg/kg bw/d) (Lamb et al., 1987). In this protocol, dosing was initiated 1 week prior to individual males and females being cohabited. This cohabitation continued for the next 14-week dosing period. Litters were removed as the offspring were born. After 14 weeks, the last litter remained with the mother until weaning. A second generation (20 pairs each) was produced by pairing the F1 pups from the litters of control dams and of dams treated with DEP at 2.5% only (within group). Results showed no adverse effect of DEP on the physiology, fertility, or reproductive performance of the F0 generation. In the F1 generation, high-dose female body weights were significantly decreased and liver weights were significantly increased. Prostate weights were significantly increased compared to controls. There was no effect on testes, epididymis or female reproductive organ weights. Epididymal sperm concentrations in the F1 males at the 2.5% dose were significantly decreased (30%) although motility and morphology were unaffected. The number of F2 pups per litter of treated dams was significantly decreased (the F1 litter had 14% fewer pups than controls). As only one dose was used in the second generation study, a NOAEL could not be established. The LOAEL in this study was estimated to be 3640 mg/kg bw/d, based on reproductive effects and changes in body, liver and prostate weights in the F1 generation.

### **4.8.4 Prenatal developmental toxicity studies**

#### **Previous evaluations**

In a preliminary developmental toxicity study, 50 CD-1 mice received DEP at 0 or 4500 mg/kg bw/d by oral gavage during gestation day (GD) 6 to 13 (Hardin et al., 1987). No effect on body weight of dams, numbers of viable litters, neonatal survival, or neonatal body weights were observed. The NOAEL was 4500 mg/kg bw/d.

Thirty pregnant rats per dose were treated in the diet with DEP (31-32/group) during GD 6 to 15 at levels of 0, 0.25, 2.5 and 5.0% for DEP (approximately 0, 200, 1910, 3200 mg/kg bw/d). The rats were sacrificed on GD 20 (Field et al., 1993\*). Maternal body weight gains during treatment were significantly lowered at 2.5 and 5% ( $p < 0.05$ ) but fell within those of the control group on the day of post-mortem examination. Food consumption was decreased in the mid and high dose groups through much of the treatment period. Maternal liver weights were unaffected. There was no effect on number of resorptions, live litter size, mean pup weight or frequency of malformations. The only treatment related effect in the foetuses was a 3 to 4 fold increase in the incidence of variations, primarily rudimentary extra lumbar ribs in the high dose group with no clear dose response (21% versus 8.8%). It is unknown whether the frequency fell within the historical control range. There were no significant effects on the dams at 0.25% and foetal development was unaffected at 2.5%. The developmental NOAEL was 2.5% (1910 mg/kg bw/d). The LOAEL was 5% (3200 mg/kg bw/d) based on significantly increased frequency of skeletal variations.

Twenty pregnant mice per dose were treated with DEP percutaneously during days 0 to 17 of gestation at levels of 0, 500, 1600, 5600 mg/kg bw/d (Tanaka et al., 1987\*). A significant reduction in thymus weight relative to the controls were observed in all dose groups. The body weights of the dams in all dose groups were not affected. Increased maternal adrenal and kidney weights were observed at 5600 mg/kg bw/d. There was no effect on number of implantations, live born foetuses or malformations. Lower foetal weight and increase in the incidence of variations, primarily rudimentary cervical and lumbar ribs were observed 5600 mg/kg bw/d ( $p < 0.05$ ) perhaps related to maternal toxicity. A dose of 1600 mg/kg bw/d was identified as the NOAEL for effects on the offspring.

#### **Data not reported in previous evaluations**

Pregnant rats (5/group) were injected intraperitoneal (ip) with 0, 0.506, 1.012 or 1.686 mL/kg DEP (purity unknown) on GD 5, 10 and 15 (Singh et al., 1972). Controls were untreated. Dams were terminated on GD 20. The number of resorptions increased at the low dose but not mid or high dose. Pup weight significantly decreased at all doses compared to untreated controls. An increased incidence of skeletal abnormalities (33.3%, 26.3%, 47.1%), not dose-related, was seen. A NOAEL could not be established due to teratogenic effects at the lowest dose tested.

Sprague-Dawley outbred CD rats were treated by gavage daily from GD 12 to GD 19 at 500 mg/kg bw/d. Limited data was presented however, treatment with DEP had no effect on anogenital distance compared to controls (Liu et al., 2005).

### **4.8.5 Developmental/postnatal toxicity studies**

#### **Previous evaluations**

In a study on rats using a range of phthalates (Gray et al., 2000\*), DEP was administered orally to dams at 750 mg/kg bw/d from GD 14 to PND 3. Maternal weight gain was reduced at PND 3 but there was no effect on mean pup weight at birth or weaning, male reproductive organ weight or time of preputial separation.



## 4.8.6 Two-generation reproductive toxicity studies

### Data not reported in previous evaluations

A two-generation reproductive study was conducted in Sprague Dawley rats (24/group) fed diets containing 0, 600, 3000 or 15000 ppm (Fujii et al., 2005). Rats began dosing 10 weeks prior to mating, through mating, gestation, lactation until weaning. F0 and F1 male and female parents in the high-dose group had significantly increased relative liver weights. F1 high-dose females also had significantly increased relative kidney weights. In addition, F0 males had significantly decreased absolute epididymis weight (not dose-related), increased frequency of abnormal sperm in the mid-dose group only and decreased serum testosterone levels in mid and high dose groups (not dose-related). In the F1 parents, there was no effect on reproductive organ weight but there was a dose-related and significant increase in frequency of abnormal and tailless sperm in mid and high-dose groups. There was no effect on the number of implantations, pups delivered or pup weight at birth from the F0 and F1 parents. Pup weight was significantly reduced at PND 21 in high-dose F1 male pups and at PND 4-21 in high-dose F1 female pups (clear dose response at PND 21 only). There was no effect on anogenital distance or age of preputial separation but age of onset of vaginal opening was delayed in high dose F1 females. Developmental delay was also evident in high dose F1 males (significant delay in pinna detachment). Pup weight in F2 offspring was reduced in the high dose groups of both sexes. There was no effect on anogenital distance in F2 male or female pups. The NOAEL for fertility was 600 ppm (estimated 40-56 mg/kg bw/d for males). The LOAEL based on increased abnormal sperm in the F0 and F1 generations was 3000 ppm (197-267 mg/kg bw/d). The developmental NOAEL was 3000 ppm (197-267 mg/kg bw/d). The LOAEL for maternal and developmental toxicity was 15000 ppm based on reduced body weight gain before weaning and developmental delay.

## 4.8.7 Mode of action

DEP did not bind to human oestrogenic receptor (ER) in vitro (Nakai et al., 1999; Toda et al., 2004) and was negative for oestrogenic activity in a yeast two-hybrid assay (Nishihara et al., 2000) and showed weak oestrogenic activity in recombinant yeast assay (Harris et al., 1997). DEP also did not demonstrate oestrogenic activity in human ER $\alpha$  and ER $\beta$  reporter gene assays in CHO-K1 cells transfected with expression vectors for ER $\alpha$ , ER $\beta$  and androgen receptor (AR) (Takeuchi et al., 2005). Expression of CaBP-9k mRNA (a gene highly regulated by 17 $\beta$ -oestradiol) was not increased in 7 day old female SD rats following oral treatment with 600 mg/kg bw/d DEP for 3 days (Hong et al., 2005). DEP increased proliferation of human breast cancer MCF-7 cells in one assay (Hong et al., 2005) but not another (Okubo et al., 2003). MEP induced detachment of germ cells from a Sertoli cell monolayer in vitro but was 10000 fold less potent than MEHP (Gray & Gangoli, 1986).

### Conclusion

#### *Fertility effects*

A NOAEL for fertility of 600 ppm (40-56 mg/kg bw/d) was derived from a well-conducted two-generation study in rats (Fujii et al., 2005). There was a dose-related increased frequency of abnormal and tailless sperm in the F0 and F1 generation

exposed to 3000 ppm (197-267 mg/kg bw/d) and testosterone serum levels were reduced in F0 males. Testosterone serum and testicular concentrations were also reduced after treatment with 2% DEP (~ 2000 mg/kg bw/d) for one week in the diet (Oishi & Hiraga, 1980). DEP was associated, in vitro, with reduction in human sperm motility and increased DNA damage in sperm but there was no association with MEP levels and sperm parameters in men attending an andrology clinic.

### ***Developmental toxicity***

Maternal urinary MEP concentration was inversely related to AGI in a group of 85 mother-son pairs. However, the reliability of the measurement of AGD in humans has not been verified in humans and the relationship between urinary MEP and exposure to DEP was not established.

A NOAEL of 3000 ppm (197-267 mg/kg bw/d) was derived from a well-conducted two-generation study in rats (Fujii et al., 2005). There was decreased pup weight at weaning in F1 males and F2 generation and developmental delay (delayed onset of vaginal opening, pinna detachment in the F1 generation at 1016-1375 mg/kg bw/d). There was no difference in pup weight at birth suggesting that these effects may be due to lactational exposure to DEP. At this dose, maternal effects included increased relative liver and kidney weights.

There was no difference in reproductive organ weights or anogenital distance. Pup weight at birth was reduced and frequency of skeletal variations was increased at and above maternotoxic doses.

**Table 4: Effects of DEP on key reproductive toxicity studies**

<b>Study duration</b>	<b>Species, route</b>	<b>Doses (mg/kg bw/d)</b>	<b>NOAEL (mg/kg bw/d)</b>	<b>LOAEL (mg/kg bw/d) &amp; endpoint</b>	<b>References</b>
<b><i>Repeat Dose Toxicity Studies</i></b>					
7 days	Rats, male Wistar Diet	0, 2% (2000)	<u>Fertility</u> : NE	<u>Fertility</u> : 2000; ↓ serum & testes testosterone	Oishi & Hiraga, 1980
4 days; 12/group	Rats, Male Sprague Dawley Gavage	0, 1600	NE	NE	Foster et al., 1980
2 days; 12 males/group	Rats, Wistar Gavage	0, 2000	<u>Fertility</u> : NE	<u>Fertility</u> : 2000; Ultrastructural changes in Leydig cells	Jones et al., 1993
<b><i>Continuous Breeding Studies</i></b>					
1 week pre mating to PND 21; 20/sex/group	Mice, CD1 Diet	0, 0.25, 1.25, 2.5% (0, 340, 1770, 3640)	<u>Fertility</u> : NE <u>Maternal</u> : NE <u>Developmental</u> : NE	<u>Fert</u> : 3640; ↓ epididymal sperm concentration; ↑ prostate wt <u>Maternal</u> : 3640; ↓ body wt, ↑ liver wt <u>Developmental</u> : 3640; ↓ no. of live pups/litter	Lamb et al., 1987
<b><i>Two-Generation Study</i></b>					
10 weeks prior to mating, through mating, gestation, lactation until weaning; 24/group	Rats, Sprague Dawley diet	0, 600, 3000, 15000 ppm (0, 40-56, 197- 267, 1016- 1375) (m-f)	<u>Fertility</u> : 40-56 <u>Maternal</u> : 197- 267 <u>Developmental</u> : 197-267	<u>Fertility</u> : 197-267; ↓ serum testosterone levels (F0), ↑ frequency of abnormal and tailless sperm (F1); <u>Maternal</u> : 1016-1375; ↑ rel liver wt (F0, F1), ↑ rel kidney wt (F1),  <u>Developmental</u> : 1016- 1375; ↓ pup weight at PND 21 (F1 males, F2) and PND 4-21 (F1 females), delayed age of onset of vaginal opening (F1)	Fujii et al., 2005
<b><i>Prenatal Developmental Toxicity Studies</i></b>					
GD 6-13; 50/group	Mice, CD1 gavage	0, 4500	<u>Maternal</u> : 4500 <u>Developmental</u> : 4500	NE	Hardin et al., 1987
GD 6-15; 31-32/group	Rats, SD diet	0, 0.25%, 2.5%, 5% (0, 200, 1910, 3200)	<u>Maternal</u> : 200 <u>Developmental</u> : 1910	<u>Maternal</u> : 1910; significant ↓ body weight gains at 2.5 and 5% <u>Developmental</u> : 3200; ↑ skeletal variations (rudi- mentary lumbar ribs)	Field et al., 1993

<b>Study duration</b>	<b>Species, route</b>	<b>Doses (mg/kg bw/d)</b>	<b>NOAEL (mg/kg bw/d)</b>	<b>LOAEL (mg/kg bw/d) &amp; endpoint</b>	<b>References</b>
GD 0-17	Mice, dermal	0, 500, 1600, 5600	<u>Maternal:</u> NE <u>Developmental:</u> 1600	<u>Maternal:</u> 500; ↓ thymus wt <u>Developmental:</u> 5600; ↓ pup weight; ↑ incidence of skeletal variations (rudimentary cervical and lumbar ribs)	Tanaka et al., 1987*
GD 12-19; 5/group	Rats, Sprague-Dawley gavage	0, 500	<u>Developmental:</u> 500	NE	Lui et al., 2005
GD 5, 10, 15; 5/group	Rats, SD ip	0, 0.506, 1.012 or 1.686 mL/kg	<u>Developmental:</u> NE	<u>Developmental:</u> 0.506 mL/kg; ↓ pup weight	Singh et al., 1972
<b><i>Developmental/Postnatal Toxicity Studies</i></b>					
GD 14 – PND 3; 5/group	Rats, Sprague-Dawley gavage	0, 750	<u>Developmental:</u> 750	NE	Gray et al., 2000

NE: not established; ↓ = decreased; ↑ = increased

## 5. Hazard Characterisation

Toxicity data for DEP 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, 2008), which contains a comparative analysis of toxicity endpoints across 24 phthalates, including DEP. DEP has a straight-chain 2-carbon backbone and is considered to be a low molecular weight phthalate based on the categorization derived by the Phthalate Esters Panel HPV Testing Group (2001).

DEP is readily absorbed and eliminated rapidly, with the urine being the major route of excretion following oral administration to rats and mice. An ester hydrolysis product of the parent compound, monoethyl phthalate (MEP), is the major metabolite present in urine. Percutaneous absorption of DEP through animal skin is significant, although *in vitro* data suggests that absorption through human skin may be significantly less than that of animal skin.

DEP has low acute oral and dermal toxicity. DEP causes minimal skin and eye irritation in animals. DEP is not a skin sensitiser in humans or animals.

Repeated dose toxicity studies indicate that the liver is the primary target organ for DEP. However, hypertrophic effects were also observed in other organs such as kidney, stomach and small intestine. A NOAEL of 1% DEP in the diet (approximately 750 mg/kg bw/d) was determined from a 16 week rat oral study. The LOAEL is based on increased relative liver, kidney, stomach and small intestine weights at 5% (approximately 3200-3700 mg/kg bw/d).

*In vitro* data show equivocal evidence of genotoxic activities for DEP and *in vivo* data are not available, therefore conclusions on genotoxicity cannot be drawn.

There was increased incidence of combined hepatocellular adenomas or carcinomas in mice, however, effects were considered equivocal evidence of carcinogenic activity due to lack of a dose-response relationship in females and a similar incidence of combined hepatocellular adenomas or carcinomas at the high dose males compared to historical controls. No treatment related tumours were reported in a limited 2-year oral carcinogenicity study in rats. DEP was also negative in mouse initiation/promotion study. Taken as a whole, these studies provide equivocal evidence of carcinogenic potential for DEP.

A NOAEL for fertility of 40-56 mg/kg bw/d was derived from a well-conducted two-generation study in rats. There was no effect on male reproductive organ weights up to dose 1016-1375 mg/kg bw/d, however, there was an increased frequency of abnormal and tailless sperm in the F0 and F1 generation exposed to 197-267 mg/kg bw/d and testosterone serum levels were also reduced in F0 males (Fujii et al., 2005). DEP was associated, in *in vitro* studies, with reduction in human sperm motility and increased DNA damage in sperm but there was no association between MEP levels and sperm parameters in men attending an andrology clinic.

Reduced testicular and serum testosterone levels were reported in rats after 7 days diet containing 2% (2000 mg/kg bw/d) DEP (Oishi and Hiraga, 1980) but no effect on testicular zinc levels were reported after 4 days dosing with 1600 mg/kg bw/d DEP (Foster et al., 1980). Changes in Leydig cell ultrastructure by DEP has also been demonstrated in rats treated for only 2 days (Jones et al., 1993\*). This suggests that the observed reduction in testosterone levels seen after administration of DEP (Oishi and Hiraga, 1980) might be due to its direct effects on the Leydig cells of the testes despite a lack of testicular atrophy.

The mechanism for the reduced testosterone levels observed with DEP is uncertain. Induction of CYP 4 isozymes (a group of testosterone metabolising enzymes), as shown by DEP in rat liver microsomes, is a possible explanation for the effect. However, there are studies that showed a more complex testosterone regulatory system in which change in testosterone metabolising enzymes of P450 might not translate into changes in circulating testosterone levels.

Human data are limited and hampered by measurements of multiple phthalates in small sample sizes. Urinary MEP concentration was inversely associated with anogenital distance in a study of 85 mother-sons pairs. The reliability of the measurement of anogenital distance in humans has not been verified. In another study, no association was found between breast milk MEP levels and cryptorchidism but MEP showed positive correlations with sex-hormone binding globulin and LH:free testosterone ratio.

In a two-generation reprotoxicity study, the main effects included reduced pup weight at weaning and delayed onset of vaginal opening and pinna detachment at the highest dose (maternal effects were also observed at this dose). There was no difference in pup weight at birth suggesting that these effects may be due to lactational exposure to DEP. In developmental studies, effects in offspring (increased skeletal variations, primarily rudimentary extra lumbar ribs) occurred above maternotoxic doses. The NOAEL for development effects was 197-267 mg/kg bw/d and the LOAEL was 1016-1375 mg/kg bw/d based on decreased pup weight and developmental delay (Fujii et al., 2005). There was little evidence that DEP has oestrogenic activity in vivo or in vitro.

DEP, unlike “transitional” phthalates of 4-6 carbon backbones which have been identified as being associated with reproductive and developmental toxicity (Phthalate Esters Panel HPV Testing Group, 2001; NICNAS, 2008), showed no effect on relative testis weight and does not demonstrate the typical pattern of malformations including decreased anogenital distance, delayed preputial separation and retained thoracic nipples in male pups.

## 6. Human Health Hazard Summary Table

Phthalate	Acute Toxicity	Irritation & Sensitisation	Repeated Dose Toxicity	Genetic Toxicity	Carcinogenicity	Fertility	Developmental Toxicity
Diethyl phthalate (DEP)	<p>Oral Rat: LD50 &gt;5600 to 31000 mg/kg bw</p> <p>Dermal Rat: LD50 &gt;11000 mg/kg bw</p> <p>Inhalation Rat: LC50 = 7.50 mg/L</p>	<p>Skin Irritation: minimal effects</p> <p>Eye Irritation: minimal effects</p> <p>Skin Sensitisation: negative</p>	<p>Rat: NOAEL = 750-770 mg/kg bw/d (m-f)</p> <p>LOAEL = 3200-3700 mg/kg bw/d (m-f)</p> <p>↑ in relative liver, kidney, stomach and small intestine weight (no histopathological abnormalities)</p>	<p>In vitro: Negative in majority bacterial mutation assays (±S9)</p> <p>Negative in chromosomal aberrations assays</p> <p>Positive in sister chromatid exchange assay (+S9)</p> <p>Association between human urinary MEP levels and increased DNA damage (using in vitro comet assay) in sperm (sample size = 141)</p> <p>In vivo: No data</p>	<p><i>Two year dermal study</i> F-344/N rat: no treatment-related carcinogenicity; ↓ fibroadenomas of the mammary glands (f)</p> <p><i>Two year dermal study</i> B6C3F1 mouse: ↑ hepatocellular adenomas or carcinomas combined (dose-related in males only from 280 mg/kg bw/d and similar incidence in high dose and controls)</p> <p><i>Initiation/promotion study</i> Swiss CD-1 mouse (m): negative</p>	<p>Rat: NOAEL = 40-56 mg/kg bw/d (m-f)</p> <p>LOAEL = 197-267 mg/kg bw/d (m-f)</p> <p>↓ serum testosterone levels, ↑ frequency of abnormal and tailless sperm</p>	<p><i>Two generation study</i> Rat: NOAEL Maternal = 197-267 mg/kg bw/d (m-f) Developmental = 197-267 mg/kg bw/d (m-f)</p> <p>LOAEL Maternal = 1016-1375 mg/kg bw/d (m-f) ↑ rel liver wt (F0, F1), ↑ rel kidney wt (F1), Developmental = 1016-1375 mg/kg bw/d (m-f) ↓ pup weight at PND 21 (F1 males, F2) and PND 4-21 (F1 females), delayed age of onset of vaginal opening (F1).</p> <p><i>Developmental study</i> Rat: NOAEL Maternal = 200 mg/kg bw/d Developmental = 1910 mg/kg bw/d</p> <p>LOAEL Maternal = 1910 mg/kg bw/d ↓ body weight Developmental = 3200 mg/kg bw/d ↑ skeletal variations (supernumerary ribs)</p>

m-f: male-female; ↑: increase; ↓: decrease.

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# References

- Api AM (1997) In vitro assessment of phototoxicity. *In vitro Toxicology* 10: 339-350.
- Api AM (2001) Toxicological profile of diethyl phthalate: a vehicle for fragrance and cosmetic ingredients. *Food and Chemical Toxicology* 39:97-108.
- ATSDR (1995) Toxicological profile for diethylphthalate. Atlanta, GA, US Department of Health and Human Services, Agent for Toxic Substances and Disease Registry.
- Brown D, Butterworth KR, Gaunt IF, Grasso P, Gangoli SD (1978) Short-term oral toxicity study of diethyl phthalate in the rats. *Food and Cosmetics Toxicology* 16:415-422.
- Buehler EV (1996) Non-specific hypersensitivity: false positive responses with the use of Freud's complete adjuvant. *Contact Dermatitis* 34:111-114.
- David RM, Lockhart LK, & Ruble KM (2003) Lack of sensitization for trimellitate, phthalate, terephthalate and isobutyrate plasticizers in a human repeated insult patch test. *Food Chemical Toxicology* 41:589-593.
- Draize JH et al. (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *Journal of Pharmacology and Experimental Therapeutics* 82:377-390.
- Duty S, Silva M, Barr D, Brock J, Ryan L, Chen Z, Herrick R, Christiani D, & Hauser R (2003a) Phthalate Exposure and Human Semen Parameters. *Epidemiology* 14: 269 –277
- Duty SM, Singh NP, Silva MJ, Barr DB, Brock JW, Ryan L, Herrick RF, Christiani DC, & Hauser R (2003b) The relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral comet assay. *Environmental Health Perspectives* 111: 1164-1169.
- ECPI (2006) Diethyl phthalate Information Centre [on line]. Available from URL: <http://www.dep-facts.com/> [Assesses 2006 Oct 31].
- Elsisi AE et al. (1989) Dermal absorption of phthalate diesters in rats. *Fundamental and Applied Toxicology* 12:70-77.
- Field EA, Price CJ, Sleet RB, George JD, Marr MC, Myers CB, Schwetz BA, & Morrissey RE. (1993) Developmental toxicity evaluation of diethyl and dimethyl phthalate in rats. *Teratology* 48: 33-44.
- Foster P, Thomas L, Cook M, & Gangoli S (1980) Study of the testicular effects and changes in zinc excretion produced by some n-alkyl phthalates in the rat. *Tox Appl Pharm* 54: 392-398.
- Fredricsson B, Møller L, Pousette Å, & Westerholm R (1993) Human sperm motility is affected by plasticisers and diesel particle extracts. *Pharmacology and Toxicology* 72:128-133.
- Fujii S, Yabe K, Furukawa M, Hirata M, Kiguchi M, & Ikka T (2005). *J Tox Sc* 30: 97-116.
- Gray Jr LE, Ostby J, Furr J, Price M, Veeramachaneni DNR, & Parks L (2000) Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats, DMP, or DOTP, alters sexual differentiation of the male rat. *Toxicological Sciences* 58: 350-365.
- Gray T & Gangoli S (1986) Aspects of the testicular toxicity of phthalate esters. *Environ Health Perspect* 65: 229-235.
- Hardin BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, MacKenzie KM, Piccirillo VJ, & Smith KN (1987) Evaluation of 60 chemicals in a preliminary developmental toxicity test. *Teratogenesis, Carcinogenesis and Mutagenesis* 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.
- Hong EJ, Ji YK, Choi KC, Manabe N, & Jeung EB (2005) Conflict of estrogenic activity by various phthalates between in vitro and in vivo models related to the expression of Calbindin-D9k. *Journal of Reproduction & Development* 51(2): 253-63.
- Hotchkiss SAM (1994) Metabolism of phthalic acid esters during percutaneous absorption through rat and human skin in vitro. *Journal of Investigative Dermatology* 102:647.
- Ioku T, Mukaide A, Kitanaka H, Sakagami Y, & Kamevama T (1976) In vitro distribution of drugs. Labeled compounds. *Yakuri To Chiryō* 4:510-514.
- IPCS (2003) Diethyl phthalate: Concise International Chemical Assessment Document 52. World Health Organisation.
- Jones HB, Garside DA, Liu R, & Roberts JC (1993) Influence of phthalate esters on Leydig cell structure and function in vitro and in vivo. *Experimental and Molecular Pathology* 58:179-193.
- Jonsson B, Richthoff J, Rylander L, Giwercman A, & Hagmar L (2005) Urinary phthalate metabolites and biomarkers of reproductive function in young men. *Epidemiology* 16(4): 487-493.
- Kawano M (1980) Toxicological studies on phthalate esters. Metabolism, accumulation and excretion of phthalate esters in rats. *Japanese Journal for Hygiene* 35:693-701.
- Klecak G (1979) The opening epicutaneous test (OET), a predictive test procedure in the guinea pig for estimation of allergenic properties of simple chemical compounds, their mixtures and of finished cosmetic preparations. *International Federation Societies Cosmetic Chemists*, 18 September.
- Klecak G, Geleick H, Frey JR (1977) Screening of fragrance materials for allergenicity in the guinea pigs: Comparisons of four testing methods. *Journal of the Society of Cosmetic Chemists* 28:53-64.
- Kozumbo WJ, Kroll R, Rubin RJ (1982) assessment of the mutagenicity of phthalate esters. *Environmental Health Perspective* 45:103-109.
- Lamb JC, Chapin RE, Teague J, Lawton AD & Reel JR (1987) Reproductive effects of four phthalic acid esters in the mouse. *Toxicology and Applied Pharmacology* 88:225-269.
- Lawrence WH, Malik M, Turner JE, Singh AR, & Autian J (1975) A toxicological investigation of some acute, short-term and chronic effects of administering DEHP and other phthalate esters. *Environmental Research* 9:1-11.
- Liu K, Lehmann KP, Sar M, Young SS, & Gaido KW (2005) Gene expression profiling in utero exposure to phthalate esters reveals new gene targets in the etiology of testicular dysgenesis. *Biol Reprod* 73:180-92.
- Main KM, Mortensen GK, Kaleva MM, Boisen KA, Damgaard IN, Chellakooty M, Schmidt IM, Suomi A-M, Virtanen HE, Petersen JH, Andersson A-M, Toppari J, & Skakkebaek NE (2006) Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. *Env. Health Environ* 114: 270-276.
- Marsee K, Woodruff TJ, Axelrad DA, Calafat AM, & Swan SH (2006) Estimated daily phthalate exposures in a population of mothers of male infants exhibiting reduced anogenital distance. *Environ. Health Perspect* 114: 805-809.
- Marsman DS, Herbert RA, & Haseman JK (1994) Dermal carcinogenesis studies of diethylphthalate (DEP) and dimethylphthalate (DMP) in F344/N rats and B6C3F1 mice with initiation/promotion studies in male CD-1 mice. *Toxicologist* 14: 302.
- 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 (2008) Phthalate hazard compendium: a summary of physicochemical and human health hazard data for 24 *ortho*-phthalate chemicals. Sydney, National Industrial Chemicals Notification and Assessment Scheme.
- Mint A, Hotchkiss SAM, & Caldwell J (1994) Percutaneous absorption of diethyl phthalate through rat and human skin in vitro. *Toxicology in vitro* 8: 251-156.
- Nakai M, Tabira Y, Asai D, Yakabe Y, Shimyoze T, Noguchi M, Takatsuki M, & Shimohigashi Y (1999) Binding characteristics of dialkyl phthalates for the estrogen receptor. *Biochem Biophys Res Comm* 254: 311-314.
- 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 (1995) National Toxicology Program. Toxicology and carcinogenesis studies of diethyl phthalate in F344/N rats (gavage studies) and B6C3F<sub>1</sub> mice (dermal studies) with dermal initiation/promotion study of diethyl phthalate and dimethyl phthalate in male Swiss (CD-1) mice. Technical report series number 429.
- Oishi S & Hiraga K (1980) Testicular atrophy induced by phthalic acid esters effect on testosterone and zinc concentration. *Toxicology and Applied Pharmacology* 53:35-41.
- Oishi S (1985) Reversibility of testicular atrophy induced by Di(2-ethylhexyl) phthalate in rats. *Environ Res* 36:160-9.
- Okita R & Okita JR (1992) Effects of diethyl phthalate and other plasticisers on laurate hydroxylation in rat liver microsomes. *Pharmaceutical Research* 9:1648-1653
- Okubo T, Suzuki T, Yokoyama Y, Kano K, & Kano I (2003) Estimation of estrogenic and anti-estrogenic activities of some phthalate diesters and monoesters by MCF-7 cell proliferation assay in vitro. *Biol Pharm Bull* 26(8): 1219-1224.
- Pereira C, Mapuskar K & Rao V (2006) Chronic toxicity of diethyl phthalate in male Wistar rats – A dose-response study. *Regulatory Toxicology and Pharmacology*, 45: 169-177.
- Phthalate Esters Panel HPV Testing Group (2001) High production volume (HPV) chemical challenge programme test plan for the phthalate esters category. December 10, 2001. Prepared by:ExxonMobil Biomedical Sciences, Inc. for the Phthalate Esters Panel HPV Testing Group of the American Chemistry Council.  
<http://www.epa.gov/hpv/pubs/summaries/benzene/c13467tc.htm> (Accessed 2007)
- Phthalate Esters Panel HPV Testing Group (2001) High production volume (HPV) chemical challenge programme test plan for the phthalate esters category. December 10, 2001.
- RIFM (Research Institute for Fragrance Materials Inc.) (1955) Toxicological studies of diethyl phthalate. Document number 23199, December 23.
- RIFM (1973) Tissue distribution of diethyl phthalate following percutaneous administration to female Albino rabbits. RIFM report number 9984, January 12.
- RIFM (1974) Primary skin irritation tests with diethyl phthalate in rabbits. RIFM report number 14300, December 10.
- RIFM (1978a) Primary eye irritation study in the Albino rabbits. RIFM report number 12327, December 3.
- RIFM (1978c) Acute dermal toxicity (LD50) study of diethyl phthalate in albino rats. Unpublished report from International Flavours & Fragrances, Inc., 31 March. Report number 14302.
- RIFM (1978d) Report on human maximisation studies. RIFM report number 1698, June 5.
- RIFM (1984) Acute dermal irritation study. RIFM report number 1795, June 1.
- RIFM (1985) Acute dermal irritation study. RIFM report number 3099, June 1.

- RIFM (1994) Two-week dermal dose range-finding study in rats. Acute dermal irritation study. RIFM report number 23238, February 24.
- Ryan CA, Gerberick GF, Cruse LW, Basketter DA, Lea L, Blaikie L, Dearman RJ, Warbrick EV, & Kimber I (2000) Activity of human contact allergens in the murine local lymph node assay. *Contact Dermatitis*, 43, 95-102.
- 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.
- Scientific Committee on Cosmetic Products and Non-food products (SCCNFP) (2002) Opinion of the scientific committee on cosmetic products and non-food products intended for consumers concerning Diethyl phthalate, report adopted during the 20<sup>th</sup> Plenary meeting of 4 June 2002.
- Scott RC, Dugard PH, Ramsey JD, & Rhodes C (1987) In vitro absorption of some phthalate diesters through human and rat skins. *Environmental Health Perspective* 74:223-227.
- Silva MJ, Barr DB, Reidy RA, Kato K, Malen NA, Hodge CC, et al. (2003) Glucuronidation patterns of common urinary and serum monoester phthalate metabolites. *Arch Toxicol* 77, 561-567.
- Singh A, Lawrence W, & Autian J (1972) Teratogenicity of phthalate esters in rats. *J Pharm Sci* 61: 51-55.
- Singh AR, Lawrence WH, & Autian J (1975) Maternal-foetal transfer of <sup>14</sup>C-di-2-ethylhexyl phthalate and <sup>14</sup>C-diethylphthalate in rats. *J Pharmaceutical Sci* 64 (8): 1347-1350.
- Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, Mao CS, Redmon JB, Ternand CL, Sullivan S, Teague JL, & 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.
- Tanaka C, Siratori K, Ikegami K, & Wakisaka Y (1987) A teratological evaluation following dermal application of diethyl phthalate to pregnant mice. *Oyo Yakuri* 33:387-392.
- Toda C, Okamoto Y, Ueda K, Hashizume K, Itoh K, & Kojima N (2004) Unequivocal estrogen receptor-binding affinity of phthalate esters featured with ring hydroxylation and proper alkyl chain size. *Arch Biochem Biophys* 431: 16-21.
- Yoshikawa K, Tanaka A, Yamaha T, & Kurata H (1983) Mutagenicity study of nine monoalkyl phthalates and a dialkyl phthalate using *S. typhimurium* and *Escherichia coli*. *Food and Chemical Toxicology* 21: 221-223.

# Appendix - Robust Study Summaries

## Developmental toxicity/ teratogenicity

<b>Test substance</b>	: Diethyl phthalate (99.8%)
<b>Species</b>	: Rat, Crj:CD (SD) IGS strain, 24/sex/dose, 4 weeks, Tsukuba Breeding Center, Charles River Japan Inc.
<b>Route of administration</b>	: diet
<b>Exposure period</b>	: 10 weeks pre-mating, mating, gestation, weaning (~15 weeks, males; 17 weeks, females)
<b>Study Duration</b>	: 2 generations
<b>Frequency of treatm.</b>	: Ad libitum
<b>Doses</b>	: 0, 600, 3000, 15000 ppm (0, 40-56, 197-267, 1016-1375 mg/kg bw/d)
<b>Control group</b>	: Basal feed
<b>NOAEL maternal tox.</b>	: 197-267 mg/kg bw/d
<b>NOAEL teratogen.</b>	: 197-267 mg/kg bw/d
<b>Guidelines</b>	: Yes
<b>GLP</b>	: Yes
<b>Method</b>	: Additional measurements included ano-genital distance, timing of sexual maturation, P450 isozyme liver contents, serum testosterone and progesterone levels,
<b>Result</b>	: F0 and F1 male and female parents in the high dose group had significantly increased relative liver weights. F1 high dose females also had significantly increased relative kidney weights.  F0 males had significantly decreased absolute epididymis weight, increased frequency of abnormal sperm in the mid-dose group only and decreased serum testosterone levels in mid and high dose groups. In the F1 parents, there was no effect on reproductive organ weight but there was a dose-related and significant increase in frequency of abnormal and tailless sperm in mid and high dose group.  There was no effect on reproductive parameters such as number of implantations and number of pups delivered in the F0 and F1 generations. Pup weight was significantly reduced at PND 21 in high dose F1 males and at PND 4-21 in high dose F1 female pups. There was no effect on ano-genital distance or age of preputial separation but age of onset of vaginal opening was delayed in high dose F1 females. Pup weight in F2 offspring was reduced in the high dose groups of both sexes. There was no effect on ano-genital distance in F2 male or female pups.
<b>Conclusion</b>	: DEP caused reproductive effects
<b>Reliability</b>	: 1
<b>Reference</b>	Fujii S, Yabe K, Furukawa M, Hirata M, Kiguchi M, & Ikka T (2005) A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats. J Tox Sc, 30: 97-116.