Priority Existing Chemical Assessment Report No. 33

Australian Government
Department of Health and Ageing
NICNAS

Diethyl Phthalate

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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME
GPO BOX 58 Sydney NSW 2001 Australia **www.nicnas.gov.au**

Assessing chemicals for your protection

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Preface

This assessment was carried out 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 Australian Government Department of Sustainability, Environment, Water, Population and Communities (SEWPaC), which carries out the environmental assessment for NICNAS.

NICNAS has two major assessment programs: the assessment of human health and safety 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. Chemicals selected for assessment are referred to as Priority Existing Chemicals.

This priority existing chemical report has been prepared by the Director of NICNAS, in accordance with the Act. Under the Act, manufacturers and importers of priority existing chemicals are required to apply for assessment. Applicants for assessment are given a draft copy of the report and 28 days to advise the Director of any errors. Following the correction of any errors, the Director provides applicants and other interested parties with a copy of the draft assessment report for consideration. This is a period of public comment lasting for 28 days during which requests for variation of the report may be made. Where variations are requested, the Director's decision concerning each request is made available to each respondent and to other interested parties (for a further period of 28 days). Notices in relation to public comment and decisions made, appear in the *Commonwealth Chemical Gazette.*

In accordance with the Act, publication of final report revokes the declaration of the chemical as a Priority Existing Chemical, therefore, manufacturers and importers wishing to introduce the chemical in the future need not apply for assessment. However, manufacturers and importers need to be aware of their duty to provide any new information to NICNAS, as required under section 64 of the Act.

Copies of this and other priority existing chemical reports are available on the NICNAS website. Hard copies are available free of charge from NICNAS from the following address:

GPO Box 58

Sydney NSW 2001

AUSTRALIA

Tel: +61 (2) 8577 8800

Fax: +61 (2) 8577 8888

Free call: 1800 638 528

Other information about NICNAS (also available on request and on the NICNAS web site) includes:

- NICNAS Service Charter;
- information sheets on NICNAS Company Registration;
- information sheets on the Priority Existing Chemicals and New Chemical assessment programs;
- safety information sheets on chemicals that have been assessed as Priority Existing Chemicals;
- details for the NICNAS Handbook for Notifiers; and
- details for the Commonwealth Chemical Gazette.

More information on NICNAS can be found at the NICNAS web site:

http://www.nicnas.gov.au

Other information on the management of workplace chemicals can be found at the web site of Safe Work Australia:

http://www.safeworkaustralia.gov.au

Overview

Background and scope of the assessment

Diethyl phthalate (DEP) (CAS No 84-66-2) was one of nine phthalates declared as a Priority Existing Chemical (PEC) for public health risk assessment for use in toys, child care articles and cosmetics under the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) on 7 March 2006. The decision for declaration was based on:

- ubiquitous use of phthalates including DEP as solvents and plasticisers in industrial and consumer products
- consumer products being potentially significant sources of repeated and long-term exposure of the public to DEP through their use in cosmetic and personal care products and toys
- concerns regarding potential adverse health effects, particularly reproductive and developmental effects, from DEP exposure
- current overseas activities including reassessment and review of the use of phthalates including DEP in certain consumer products.

The purpose and scope of this PEC assessment are to determine the health risks to adults and children from the use of DEP in consumer products such as cosmetics, toys and child care articles, particularly after repeated or prolonged exposure.

Manufacture and importation

Data collected through calls for information specific to the assessment of DEP suggest that the total volume of DEP imported annually to Australia for industrial uses is in the range of 100-300 tonnes. The amount of DEP reported for applications with the potential for public exposure such as toys, child care articles and cosmetics was not more than 100 tonnes per annum for 2005 and 2006. With regard to cosmetic application, DEP is imported as a raw material or mixtures for local formulation and in finished (ready-to-use) products at a ratio of 60:40. Manufacture of DEP as a raw material in Australia was not reported.

Uses

The information collected by NICNAS indicated that in Australia DEP is used mainly in epoxy resins, cosmetics, personal care products and perfumes, with a small proportion in children's toys. It can be used as an alcohol denaturant.

The information also suggests that for cosmetic uses, DEP is imported either as cosmetic ingredients or in fragrance bases for use in the formulation of perfumes, household detergents and personal care products. Concentrations of DEP in these products are varied and range from 0.00004% to 34%. The information on the use of DEP in children's toys and child care articles in Australia is limited. However, DEP as a low molecular weight (LMW) phthalate has been reported to be used in conjunction with other phthalates (as a secondary plasticiser or contaminant), including diethylhexyl phthalate (DEHP) or diisononyl phthalate (DINP).

International sources report that DEP is used as a plasticiser in a diverse range of consumer products and applications such as tools, automotive parts, toothbrushes and toys and as a solvent in cosmetics, fragrances and skin care preparations.

Health effects

DEP is rapidly and almost completely absorbed following oral or inhalation exposure. Bioavailability of 100% is assumed for these routes. In contrast, bioavailability via dermal absorption is not likely to exceed 10%. Tissue distribution of DEP is widespread including foetal tissues but there is no evidence of accumulation. DEP is also rapidly metabolised and excreted, predominantly via the urine with monoethyl phthalate (MEP) as the main metabolite.

DEP has low acute toxicity via all routes and low skin and eye irritation potential. There are case reports of sensitisation to perfumes and plastic articles in patients with dermatitis and other skin diseases although DEP is not considered a skin sensitiser.

Repeated exposure to DEP in rodents caused increased liver and stomach weights in a 16-week dietary exposure study. A weak association between liver toxicity and peroxisome proliferation has been reported for DEP in some studies, but the mechanism for digestive organ enlargement is not confirmed in the critical 16-week study. On this basis, these effects could not be excluded from consideration and therefore are relevant to humans for this risk assessment. A conservative NOAEL of 0.2% in the diet (corresponding to 150 mg/kg bw/d) was established based on dose-dependent increased relative liver weight in females and increased stomach weight in males at 1% in the diet (LOAEL of 750-770 mg/kg bw/d).

Available data do not support a genotoxic or carcinogenic potential for DEP.

The low molecular weight phthalate DEP appears not to be a potent testicular toxin in animal studies. Evaluations of potential DEP toxicity to the developing male rat reproductive system have consistently found no effect on testis weight or testis morphology at doses up to 1016 mg/kg bw/d. However, reduced testosterone production and altered Leydig cell ultrastructure following DEP exposure have been reported. In a critical well-conducted two-generation study, reduced testosterone levels were observed in F0 male rats at a dose of 197 mg/kg bw/d. In addition, there was a slight but statistically significant dose-related increase in the frequency of abnormal and tailless sperms in the F0 and F1 generations, although there was no effect on fertility. Based on this study, a NOAEL of 40 mg/kg bw/d was established.

There was no evidence of foetal or neonatal toxicity after perinatal exposure to DEP at oral doses up to 3200 mg/kg bw/d. None of the effects observed with transitional phthalates (C4-6 backbone), such as epididymal malformations or absence of the epididymis, increased incidence of cryptorchidism, hypospadias, decreased anogenital distance (AGD), delayed preputial separation, and retained areolas/nipples were noted. However, decreased pup weight at weaning and developmental delay (delayed onset of vaginal opening and pinna detachment) were reported in high dose rats in the critical two-generation study. The NOAEL for developmental effects was 197 mg/kg bw/d. The NOAEL for maternal liver and kidney effects was 197 mg/kg bw/d.

In other prenatal exposure studies at 3200 or 5600 mg/kg bw/d, an increased frequency of skeletal variations such as rudimentary cervical and/or lumbar ribs was reported, although these effects generally occurred at or above maternally toxic doses. The increase in supernumerary ribs (either cervical or lumbar) is one of the common anomalies seen in

developmental toxicity studies in rodents. In view of the lack of conclusive evidence to assign the skeletal defects to maternal toxicity, these skeletal variations in rodents were interpreted as indicative of slight developmental effects.

There is also some equivocal epidemiological evidence for an association between urinary MEP and the impairment of some reproductive and developmental markers (sperm concentration, motility and morphology, DNA strand breaks in sperm, male reproductive hormones, testicular function, and AGD) in the human male, but the results remain controversial due to limitations of the study design.

Overall, although the available epidemiological studies do not provide sufficient evidence for a causal relationship between exposure to DEP (measured as urinary MEP) and possible health effects, elements of a plausible mode of action for the effects of DEP on the developing male reproductive system (e.g. reduced testosterone and sperm levels and sperm quality) are considered likely to be parallel in rats and humans if the exposure level of DEP is high enough and within a critical window of development. Therefore the effects on reproductive parameters and development in rats are regarded as relevant to humans for risk characterisation.

Public exposure and health risk

Public health risks from DEP exposure were assessed using a margin of exposure (MOE) approach for two exposure scenarios:

a) use of toys and child care articles by children, and

b) use of cosmetic products by the general population.

For exposure scenario (a), two routes of exposure of children to DEP were considered: dermal exposure during normal handling of toys and child care articles and oral exposure during mouthing, sucking and chewing of these products. The rate of phthalates leaching and migration from articles appears largely determined by the magnitude of the mechanical force applied to an article and the properties of the polyvinyl chloride (PVC) grade comprising the article, and less so by the physicochemical characteristics or concentration of the particular phthalate. Therefore, the migration rates determined under chewing condition for diisononyl phthalate (DINP)–the phthalate most frequently found in toy samples, were used to extrapolate to a mixture of phthalate plasticisers which include DEP. The use of DEP as a secondary plasticiser was considered the most likely scenario. Substitution of DEHP or DINP by DEP as a primary plasticiser was not considered likely. Estimates of DEP content as a secondary plasticiser in toys and child care articles are based on the usage and concentration of dibutyl phthalate (DBP)–an alternative secondary plasticiser reported in use in children's toys in Australia.

Studies conducted overseas indicated that children's mouthing behaviour, and therefore the potential for oral exposure, is maximal in the period between 6 and 12 months of age. Based on these studies, for children aged 6-12 months, a reasonable worst-case exposure scenario considered a maximal mouthing time of 3 h/d and a typical exposure scenario considered a mean daily mouthing time of 0.8 h/d.

Given the low acute toxicity, low eye and skin irritation and sensitising potential for DEP, the risk of adverse acute effects for children arising from handling toys is negligible.

Health risks for children were estimated for both systemic toxicity and reproductive/ developmental effects, both of which are potentially associated with repeated handling and mouthing of toys containing DEP. The MOEs were derived by comparing the dose at which

no adverse effects were observed in experimental systems (the NOAEL) with the estimated internal DEP doses for children. In both cases, the MOEs were above 10 000 for both the worst-case and typical exposure scenarios of toy use by children. Therefore, the risk of DEP-induced adverse effects from the use of toys and child care articles by children is considered negligible.

For exposure scenario (b), the main route of exposure to DEP from use of cosmetics in the general population is through dermal contact. Inhalation exposure is also possible from products applied as aerosols. Current information does not indicate use of phthalates in products most prone to accidental oral ingestion such as toothpastes, mouthwashes, lipsticks and lip-glosses. In the absence of Australian specific data, a worst-case exposure scenario of daily use of combined cosmetic products was derived based on European use patterns of cosmetics.

Given the low acute toxicity, low irritation and sensitising potential for DEP, the risk of adverse acute effects for consumers exposed to DEP through cosmetics is very low.

Health risks for the general population were estimated for both systemic toxicity and reproductive/developmental effects, both of which are potentially associated with the repeated use of cosmetic products containing DEP, especially of leave-on products. The MOE derived for general systemic toxicity was greater than 500 indicating low concern in the general population from daily use of combined cosmetic products containing DEP. The MOE for reproductive effects for the general population in the reasonable worst-case scenario was 140, which indicates an adequate safety margin.

As a subset of exposure scenario (b), the health risk to children (12 months or under) was estimated from use of personal care products containing DEP applied over large areas of the body. Based on the estimates for use of body lotions or moisturisers containing 0.25% DEP (the maximum level reported in Australia), the MOE derived for reproductive toxicity was 400 (average), which indicates an adequate safety margin.

The only area of potential concern identified for both adult and children's use of cosmetics was in relation to the use of body lotions or moisturisers. For adults, 0.5% concentration of DEP in body lotions would reduce the MOE for reproductive toxicity in the reasonable worst-case scenario from 140 to 118 which is still an adequate safety margin. For children, 0.5% concentration of DEP in body lotions would reduce the MOE for reproductive toxicity from 400 to 200, which is an adequate, albeit reduced, safety margin.

Overall, the risk estimates for general systemic toxicity indicate low concern for both children and the general population from use of cosmetic products containing DEP at the current reported levels. The risk estimates for reproductive/developmental toxicity also indicate low concern even though the MOEs were lower for these endpoints. A note of caution was identified in relation to the use of one type of cosmetic products used in infants or young children, namely, body lotions or moisturisers, where an increase in the DEP content above 0.5% could reduce the safety margin to unacceptable levels.

The effect of cumulative exposures can arise from use of cosmetics containing multiple phthalates acting on the same biological targets, from the effects of other components in a mixed phthalate used in toys and child care articles, and from the combined exposure scenarios or multiple sources. While cumulative exposures to DEP from multiple sources are addressed under Secondary Notification, the determination of risk from cumulative exposures to multiple phthalates will take into account any risk mitigation measures recommended in each PEC assessment. Risks from cumulative exposure to DEP and DEHP for the two scenarios considered in this assessment is not likely to be higher than that for

DEP alone as risk management measures have been implemented for use of DEHP in toys and cosmetics. Risks from cumulative exposure to DEP and other phthalates will be considered on completion of the other phthalate PEC assessments, and if required, further risk mitigation measures recommended.

Recommendations

This section provides recommendations arising from the assessment of DEP. The recommendation is directed at the appropriate regulatory body with responsibilities for regulating chemicals in consumer products. Implicit in this recommendation is that best practice is implemented to minimise public exposure.

Recommendation 1 - to the Delegate for Chemicals Scheduling

It is recommended that the Delegate for Chemicals Scheduling consider listing DEP in body lotion preparations at greater than 0.5% in Appendix C of the *Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP) to limit the potential exposure of the public, particularly young children to high concentrations of DEP from use in these cosmetics.

Recommendation 1 is based on the following findings of the PEC assessment:

- There is widespread use of body moisturisers in infants or young children who are considered sensitive to the DEP-induced reproductive toxicity.
- Reproductive toxicity induced by DEP may have serious long-term health effects if the exposure to DEP is high and within a critical window of development.
- A cautious approach to the potential risks associated with DEP is warranted, given the level of uncertainty regarding both health effects and the levels of exposure for different population groups.
- The MOE calculation indicates that the use of 0.5% or less of DEP in body lotions would be protective for the public, particularly young children.

Secondary Notification

Under s. 64 of the *Industrial Chemicals (Notification and Assessment) Act 1989*, the secondary notification of a chemical that has been assessed under the Act may be required where change of any circumstances that may warrant a reassessment of its hazards and risks occurs.

In the case of DEP, specific circumstances include the following:

a. Additional information becoming available on the adverse health effects of DEP.

b. Information to indicate that the levels of use of DEP in products in Australia are higher than the estimated levels used in this report.

c. Additional sources of public exposure to DEP giving rise to similar levels as those found in the cosmetics scenario in this report.

The Director of NICNAS must be notified within 28 days of the introducer becoming aware of the above or other circumstances prescribed under s. 64(2) of the Act. It is an offence under s. 64 of the Act if the Director is not notified of the change in circumstances specified above.

Abbreviations and Acronyms

ACP	acid phosphatise
AGD	anogenital distance
AGI	anogenital index
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BBP	butylbenzyl phthalate
BMI	body mass index
d	day
Da	Dalton
DBP	dibutyl phthalate
DCHP	dicyclohexyl phthalate
DEHP	diethylhexyl phthalate
DEP	diethyl phthalate
DIBP	diisobutyl phthalate
DIDP	diisodecyl phthalate
DINP	diisononyl phthalate
DMP	dimethyl phthalate
DNA	deoxyribonucleic acid
DnNP	di-n-nonyl phthalate
DnOP	di-n-octyl phthalate
ESIS	European Chemical Substances Information System
EU	European Union
f	female
FSH	follicle stimulating hormone
g	gram
GD	gestational day
GI	gastro-intestinal
h	hour
HPV	high production volume
HSIS	Hazardous Substances Information System
L	Litre

LD50	median lethal dose
LDH	lactate dehydrogenase
LH	luteinising hormone
LMW	low molecular weight
LOAEL	lowest-observed-adverse-effect level
m	male, or metre
MBP	monobutyl phthalate
MEHP	mono-2-ethylhexyl phthalate
MEP	monoethyl phthalate
MIBP	monoisobutyl phthalate
MMP	monomethyl phthalate
MOE	margin of exposure
MOS	margin of safety
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program (US)
PEC	Priority Existing Chemical
PND	postnatal day
PVC	polyvinyl chloride
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (EU)
SCCP	Scientific Committee on Consumer Products (EU)
SD	Sprague-Dawley
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons
TGD	Technical Guidance Document
TWA	time-weighted average

1. Introduction

1.1 Declaration

Diethyl phthalate (DEP) (CAS No 84-66-2) was one of nine phthalates declared as a Priority Existing Chemical (PEC) under the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) on 7 March 2006 for public health risk assessment of its use in toys, child care articles and cosmetics. The basis for the declaration was the actual and potential use of DEP in toys, child care articles and cosmetics. The declaration notice is available on the NICNAS website at:

http://www.nicnas.gov.au/Industry/Existing_Chemicals/PEC_Declarations.asp

1.2 Objectives

The objectives of this assessment were to:

- characterise the properties of DEP;
- determine the use and functions of DEP in Australia in the specific consumer applications of children's toys, child care articles and cosmetics;
- determine any adverse health effects associated with exposure to DEP;
- determine the extent of exposure of children and adults to DEP from these applications;
- characterise the risks to humans posed by exposure to DEP from use in these applications;
- determine the extent to which any risk is capable of being reduced and recommend appropriate risk mitigation measures.

These consumer applications are as defined below:

- Toys – products or materials designed or clearly intended for use in play by children of less than 14 years of age.
- Child care articles – articles designed to facilitate sleep, relaxation, hygiene, the feeding of children, the teething process or sucking on the part of children e.g. dummies, teething rings, teats, feeding bottles.
- Cosmetics – substances or preparations intended for placement in contact with any external part of the human body including the mucous membranes of the oral cavity and the teeth, with a view to altering the odours of the body, or changing its appearance, or cleansing it, or maintaining it in good condition or perfuming it, or protecting it e.g. soaps, shampoos, face creams and masks, mascara, nail polish.

1.3 Sources of information

Information for this assessment was obtained from various sources including Australian industry and government, overseas regulatory authorities and publicly available literature sources.

Industry

In August 2004, information on the importation and/or manufacture of phthalates as raw materials and information on products imported or manufactured containing phthalates were requested from industry in Australia.

In March 2006, as part of the declaration of certain phthalates including DEP as PECs, importers and manufacturers of DEP as a raw material for use in children's toys, child care articles and cosmetics, and importers of cosmetics containing DEP, were required to apply for assessment and supply information on the use of DEP. Unpublished information on health effects of phthalates including DEP was also requested.

This call for information was followed in July 2006 by a voluntary call for information to importers and manufacturers of toys and child care articles for similar information on phthalates, including DEP, used in these applications. Similarly, unpublished information on health effects and exposure to phthalates from migration and leaching from articles was requested.

Literature review

For this assessment, the International Programme on Chemical Safety's Concise International Chemical Assessment Document 52 on DEP (IPCS, 2003) and Opinions of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers concerning DEP (SCCNFP, 2002; 2003a) were consulted. Information from these documents was supplemented with new relevant data identified from thorough literature searches on Toxnet, Pubmed, ScienceDirect, SciFinder, Embase, CCOH's OSH References and the search engine Google Scholar. The last searches were conducted in April 2011.

In this report, all references, except those marked with an asterisk (*), were reviewed for the purposes of this assessment. Those references marked with an asterisk were not reviewed but were quoted from the key documents as secondary citations.

This assessment also incorporates hazard information from the DEP Hazard Assessment (NICNAS, 2008a) and the Phthalates Hazard Compendium (NICNAS, 2008b) which provides a comparative analysis of key toxicity endpoints for 24 *ortho*-phthalates.

1.4 Peer review

The report has been subjected to internal peer review by NICNAS during all stages of preparation. A final draft was also reviewed by an external expert, Dr Peter Abbott, Biotext Pty Ltd.

1.5 Applicants

Following the declaration of DEP as a Priority Existing Chemical, 30 companies and organisations applied for assessment of this chemical.

In accordance with the *Industrial Chemicals (Notification and Assessment) Act* 1989, NICNAS provided the applicants with a draft copy of the report for comment during the corrections/variations phase of the assessment. The applicants were as follows:

Amtrade International Pty Ltd
Level 6, 574 St Kilda Road, Melbourne VIC 3004

Apisant Pty Ltd
Unit 9, 12 Victoria Street, Lidcome NSW 2141

Beiersdorf Australia Ltd
4 Khartoum Road, North Ryde NSW 2113

Chanel (Australia) Pty Ltd
Level 13, 121 Walker Street, North Sydney NSW 2060

Colgate-Palmolive Pty Ltd
Level 14, 345 George Street, Sydney NSW 2000

Combe International Ltd
Level 10, 63 Exhibition Street, Melbourne VIC 3000

Costralia Pty Ltd
119 Foveaux Street, Surry Hills NSW 2010

Coty Australia Pty Ltd
Level 31, 1 Market Street, Sydney NSW 2000

Digital Crown Holdings Pty Ltd
Suite 1103, 370 Pitt Street, Sydney NSW 2000

Drom International Pty Ltd
Unit 7, 7 Jubilee Avenue, Warriewood NSW 2102

Elizabeth Arden (Australia) Pty Ltd
Level 1, 30 Alfred Street, Milsons Point NSW 2061

Firmenich Ltd
73 Kenneth Road, Balgowlah NSW 2093

Frostbland Pty Ltd
Unit 1, 47-53 Moxon Road, Punchbowl NSW 2196

Givaudan Australia Pty Ltd

Unit 36, 5-7 Inglewood Place, Baulkham Hills NSW 2153

International Flavours & Fragrances (Australia) Pty Ltd

310 Frankston-Dandenong Road, Dandenong South VIC 3175

International Sales & Marketing Pty Ltd (formally applied as Nuvo Australia Pty Ltd)

Suite 324, 23 Milton Parade, Malvern VIC 3144

Johnson & Johnson Pacific Pty Ltd

45 Jones Street, Ultimo NSW 2007

La Biosthetique Australia Pty Ltd

Unit 4, 5-15 Epsom Rd, Rosebery NSW 2018

LVMH Perfumes and Cosmetics Group Pty Ltd

Unit 1, 13 Lord Street, Botany NSW 2019

NSW Government Office of Environment & Heritage
(formerly Dept of Environment and Conservation)

59-61 Goulburn Street, Sydney NSW 2000

Plastral Pty Ltd

130 Denison Street, Hillsdale NSW 2036

PZ Cussons Australia Pty Ltd

282-300 Hammond Road, Dandenong VIC 3175

Revlon Australia Pty Ltd

12 Julius Avenue, North Ryde NSW 2113

Sigma-Aldrich Pty Ltd

12 Anella Avenue, Castle Hill NSW 2154

Sucrogen Bioethanol Pty Ltd (formerly applied as CSR Distilleries Operations Pty Ltd)

265 Whitehall Street, Yarraville VIC 3013

Symrise Pty Ltd

168 South Creek Road, Dee Why NSW 2099

Trimex Pty Ltd

5 Crewe Place, Rosebery NSW 2018

2. Background

2.1. International perspective

Diethyl phthalate (DEP) is a member of the group of esters of phthalic acid known as phthalates, used ubiquitously as solvents and plasticisers worldwide.

The US Phthalate Esters Panel High Production Volume (HPV) Testing Group (2001 & 2006) derived three categories of phthalates based on use, physicochemical and toxicological properties. Low molecular weight (LMW) phthalates were defined as those produced from alcohols with straight carbon side-chain of ≤ C3. High molecular weight phthalates were defined as those produced from alcohols with straight carbon side-chain of ≥ C7 or ring structure. A similar definition of high molecular weight phthalates is used by the OECD (OECD, 2004). Transitional phthalates were defined as those produced from alcohols with straight or branched carbon side chain of C4-6.

On the basis of the ester side chain length, DEP belongs to the LMW phthalate group.

DEP is used primarily as a solvent and/or vehicle for fragrance in perfumes, cosmetics, personal care products, and nail polishes, as well as as an alcohol denaturant in toiletries, detergents and insecticides. It is used as a plasticiser in plastic tools, automotive parts, toothbrushes, food packaging and medical tubing, as well as in soft plastic toys and child care articles. It is also employed in non-polymer uses such as dye application agents, adhesives and sealants.

The physicochemical properties of phthalates that impart usefulness as plasticisers also permit their migration and leaching from polymer matrices. Some phthalates such as diethylhexyl phthalate (DEHP) and diisononyl phthalate (DINP) can be present in high concentration (up to approximately 40%-50% w/w) in polymer materials. The potential for leaching from plastics and the widespread use in a variety of consumer products including cosmetics, together with the reproductive toxicity profile for phthalates in general, have led to concerns over the potential for health impacts from exposure to DEP. Particular concerns exist when there is potential for exposure of young children from toys and child care articles or potential prolonged exposure of the general population through the use of cosmetics.

Historically, studies of the health effects of certain phthalate esters have identified reproductive and developmental toxicity to be of particular concern. Accordingly, several overseas jurisdictions have taken regulatory action on a number of phthalates, including DEP, for particular uses.

There are no regulations in the European Union (EU) that restrict the use of DEP in children's toys and child care articles. However, regulatory action has been taken in several EU countries (e.g. Austria, Denmark, Finland, France, Germany, Sweden, etc.) and non-EU countries (e.g. Norway) to prohibit the use of all phthalates, including DEP, in toys and child care articles intended to be placed in the mouth by children under the age of three (Greenpeace International, 2003). The prohibition is a precautionary response based on the risk assessment conducted on other phthalates (DEHP, DBP, BBP, DINP, DIDP, DnOP). Although DEP is included in this general prohibition, it was not included in the toxicological

evaluation of phthalates in toys and child care articles performed by the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (VKM, 2005).

In March 2007, the EU Scientific Committee on Consumer Products was requested to review the safety of phthalates (including DEP) in cosmetic products. The Committee's opinion was that *"new studies on DEP published later than 2003 and reviewed in their assessment, did not provide sufficient new information to change the conclusions given in the safety assessments of the use of DEP in cosmetics adopted by SCCNFP"* (SCCP, 2007). In the opinion of the Scientific Committee on Cosmetics and Non Food Products intended for Consumers (SCCNFP, 2002 & 2003a), the safety profile of DEP supports its use in cosmetic products at current levels based on an evaluation which concluded that an adequate margin of safety (MOS), namely, 161 exists. This MOS was derived using a NOAEL of 150 mg/kg bw (rat, oral, 16 weeks) and a worst-case scenario exposure from the use of 10 mL of a dermally applied cosmetic product containing a maximum of 10% DEP. The SCCNFP did not recommend any specific warnings or restrictions on the use of DEP in cosmetics. However, as for other unregulated or unrestricted cosmetic ingredients, there are obligations on the manufacturer to ensure the safety of the finished product.

DEP has been registered with the European Chemicals Agency (ECHA) under the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) system and no additional authorisation is required for its continued use.

Additional regulatory information on DEP was obtained from the European Chemical Substances Information System (ESIS) (http://ecb.jrc.ec.europa.eu/esis/):

- DEP is not listed in Annex I of Directive 67/548/EEC, relating to the classification, labelling and packaging of substances and mixtures.
- DEP is not listed in a priority list (as foreseen under Council Regulation EEC No 793/93 on the evaluation and control of the risks of existing substances).
- DEP has been reported as a High Production Volume Chemical (HPVC).

Regulatory information on DEP was also available from the USA:

- DEP is neither subject to any restrictions for use in toys, child care articles nor included in the US EPA's phthalate action plan released in December 2009 (US EPA, 2009).

  However, DEP was included in a screening-level hazard characterisation of Phthalate Esters Category released recently in April 2010 under the US EPA High Production Volume (HPV) Challenge Program (US EPA, 2010).
- According to the Public Health Statement for DEP (ATSDR, 1995a), under laws that relate to Superfund[1] sites, US EPA has identified DEP as a hazardous substance, primarily based on the large number of Superfund sites where DEP is found.
- The US Consumer Product Safety Commission is reviewing the potential effects on children's health of all phthalates and phthalate alternatives used in

[1] Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA). CERCLA, also known as Superfund, is the US federal law that concerns the removal or cleanup of hazardous substances in the environment and at hazardous waste sites.

children's toys and child care articles, including DEP, effective 14 April 2010. The reports over the next 18 months will determine whether to continue the interim ban on DINP, DIDP, and DnOP; and whether additional bans on phthalates or phthalate alternatives are needed. The phthalates' endocrine disrupting effects and the cumulative effects of exposure to multiple phthalates from all sources, including personal care products, will also be examined (US CPSC 2009, 2010).

- There are no warnings or restrictions identified for use of DEP in cosmetics in the US.

In Canada, DEP is considered to be of MODERATE priority for further work following Canada's categorization of approximately 23 000 substances on its Domestic Substances List (DSL) (Health Canada, 2008).

2.2 Australian perspective

In 1999, concern over the potential adverse health effects of phthalates, including developmental and reproductive toxicity, led to nomination of phthalates to the NICNAS Candidate List from which chemicals are selected for assessment.

As a result of literature searches and a call for information from industry in 2004 and 2006, one terephthalate and 24 *ortho*-phthalates, including DEP, were identified as currently or potentially in industrial use in Australia. DEP, together with eight other phthalates, was also identified to be in actual or potential use in children's toys, child care articles and/or cosmetics in Australia.

Following public and industry comment, NICNAS in 2008 released a series of hazard assessments on 25 phthalates (http://nicnas.gov.au/Publications/CAR/Other/Phthalates.asp). NICNAS also released a phthalates compendium in which the use and hazards associated with 24 *ortho*-phthalates were summarised and compared (NICNAS, 2008b).

DEP is currently listed with a time weighted average (TWA) exposure standard of 5 mg/m^3 in the Safe Work Australia's Hazardous Substances Information System (HSIS) (Safe Work Australia, 2010) based on an adopted listing of national exposure standards for atmospheric contaminants in the occupational environment (NOHSC, 1995). This hazard is associated with atmospheric contamination only, and the liquid form does not present the same hazard in the absence of further processing to cause aerosol formation or other atmospheric release. The HSIS does not include an occupational hazard classification for DEP.

DEP is included in Appendix C of the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP), also known as the Poison Standard 2010 (Australian Government, 2010), which excludes it from use in sunscreens or personal insect repellents for human use except in preparations containing 0.5% or less of DEP. This decision was made based on concerns about the potential reproductive and developmental toxicity of short-chain or low molecular weight phthalates including DEP in unborn and prepubertal children when applied to large areas of the body.

At the time of this PEC assessment, no other restrictions on the manufacture, import or use of this chemical exist in Australia.

2.3 Assessments by international bodies

DEP has been assessed by several international bodies that have reviewed and evaluated data pertaining to the health and/or environmental hazards posed by the chemical. Of these, the most noteworthy are:

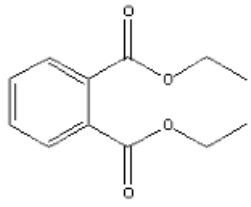
- International Programme on Chemical Safety – Concise International Chemical Assessment Document 52: Diethyl phthalate (IPCS, 2003)
- Cosmetic Ingredient Review – Annual Review of Cosmetic Ingredient Safety Assessments 2002/2003 (CIR, 2005)
- Opinions of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers concerning DEP (SCCNFP, 2002 & 2003a)
- Opinion of the Scientific Committee on Consumer Products on phthalates in cosmetic products (SCCP, 2007)
- US Agency for Toxic Substances and Disease Registry – Toxicological profile for diethyl phthalate (ATSDR, 1995b).

3. Identity, Properties and Analysis

Diethyl phthalate (DEP) is listed on the Australian Inventory of Chemical Substances (AICS) as 1,2-benzenedicarboxylic acid, diethyl ester.

3.1 Chemical identity

Chemical Name	1,2-Benzenedicarboxylic acid, diethyl ester
CAS Nos.	84-66-2
EINECS No.	201-550-6
Synonyms	1,2-Benzenedicarboxylic acid, diethyl ester
	Diethyl phthalate
	Ethyl phthalate
	Neantine
	o-Benzenedicarboxylic acid diethyl ester
	o-Bis(ethoxycarbonyl)benzene
	Palatinol A
	Phthalate, diethyl
	Phthalic acid, diethyl ester
	Solvanol
Molecular Formula	$C_{12}H_{14}O_4$
Molecular Weight	222.30
Structural Formula	



Purity	≥ 99.70% - 99.97% w/w
Impurities	Isophthalic, terephthalic acid and maleic anhydride

3.2 Physical and chemical properties

At ambient conditions, DEP is an oily colourless liquid with slight odour. Table 3.1 summarises the physicochemical properties of DEP.

Table 3.1 - Summary of physicochemical properties (adopted from SCCNFP, 2002; IPCS, 2003)

Property	Value
Melting point	-40.5˚C
Boiling point	298˚C (295˚C - 302˚C)
Density	1120 kg/m^3 (25˚C)
Vapour pressure	2.19 x 10^{-4} kPa (25˚C)
Water solubility	1 g/L (25˚C)
Partition coefficient n-octanol/water (log Kow)	2.47-2.51
Henry's law constant	7.8 x 10^{-7} atm.m^3/mole (25˚C)
Flash point	161˚C

DEP is miscible in all quantities in common organic solvents, e.g. alcohol, acetone, benzene, ether, ketone and vegetable oils.

Conversion factors: 1 ppm = 9.09 mg/m^3

1 mg/m^3 = 0.11 ppm

4. Manufacture, Importation and Use

4.1 Manufacture and importation

DEP is introduced into Australia through importation both in finished products or mixtures and as a raw chemical for local formulation and processing. There are no specific data from calls for information indicating the manufacture of DEP in Australia.

The total volume of DEP imported to Australia for industrial uses, according to responses to a NICNAS call for information in 2004 on phthalates, was in the range of 100-300 tonnes annually. In 2006, the amount of DEP reported for applications with potential public exposure such as uses in children's toys, child care articles and cosmetics was not more than 100 tonnes per annum for 2005 and 2006. The ratio of importation of DEP as a raw material to finished products is approximately 60:40. No further specific information on the introduction volume for these uses of DEP is publicly available.

4.2 Uses of DEP

4.2.1 Use in Australia

According to information collected by NICNAS through calls for information from introducers of DEP in 2004 and 2006, this chemical is used industrially in Australia mainly in epoxy resins, cosmetics, personal care products and perfumes with a small proportion in children's toys. It can be used as an alcohol denaturant. DEP is also imported for distribution to various institutions and laboratories for biotechnological and pharmaceutical research.

Australian companies indicated that DEP is imported either as cosmetic ingredients or in fragrance bases for use in the formulation of perfumes, household detergents and personal care products. Concentrations of DEP in these products or cosmetics in general are highly varied and range from 0.00004% to 25%. Some unspecified cosmetics were reported by industry to contain up to 34% DEP.

Table 4.1 provides a list of different types of skin care products marketed in Australia along with the range of DEP concentrations. Skin care products are divided into rinse-off (i.e. applied and then washed off again) and leave-on (i.e. intended to stay in prolonged contact with the skin, the hair or the mucous membranes).

Table 4.1 - Product types and DEP concentrations reported by the Australian industry in various cosmetics and personal care products

Product Types	DEP Concentrations (%)
Leave-on products	
Deodorant/Antiperspirant roll-on/liquid	0.002-1.13
Antiperspirant spray/Aerosol/Pump	0.008-0.37
Cologne/Aftershave/Splash	0.29-0.97
Nail polish	9.25-25
Face cream/Moisturiser	0.0041-0.42
Body lotion/Oil	0.00009-0.25
Perfume	0.25-2.5
Talc	0.08
Hair colour/Styling	0.04-0.82
Unspecified cosmetics/personal care products	0.1-34
Rinse-off Products	
Soap bars	0.0003-0.15
Hand wash/Hygienic wash	0.00004-0.09
Shower products	0.17-0.48
Face and skin cleanser/Toner/Exfoliant	0.005-0.48
Shampoo/Conditioner	0.012-0.045
Shaving products (cream, gel, stick, lather)	0.0041-0.0045

No data on the levels of DEP found in toys in Australia were provided for the assessment, therefore modelling assumptions and overseas data have been used in exposure estimation.

4.2.2 Uses overseas

Worldwide annual production and/or import volumes of DEP were reported around 700 tonnes in Japan and 10 000 tonnes in EU countries based on 1999 data, and between 4500 to < 23 000 tonnes in US in the calendar year 2005 (IPCS, 2003; US EPA, 2010). More recent aggregated volumes or specific volumes of DEP production, import or export were not identified.

International sources report that DEP is used in a diverse range of consumer products and applications. The European Council for Plasticisers and Intermediates lists DEP as a plasticiser widely used in tools, automotive parts, toothbrushes, food packaging, cosmetics and insecticide (ECPI, 2010). According to IPCS (2003), a common use of DEP as a plasticiser is for cellulose ester plastic films and sheets (photographic, blister packaging, and tape applications) and moulded and extruded articles (toothbrushes, automotive components, tool handles, and toys). For cosmetic applications, DEP is frequently found in skin care preparations, eye shadows, hair sprays, perfumes and other fragrance preparations, in toiletries, soaps, bath preparations, nail polish and enamel removers, and nail extenders. More specifically, DEP is used in perfumes as a fixative and solvent, in toiletries as an alcohol denaturant, in nail polish as a solvent for nitrocellulose and cellulose acetate, and in fingernail elongators as a plasticiser. It is also used in detergents,

insecticide sprays and mosquito repellents, dye applications, adhesives, sealants, surface lubricants for food and pharmaceutical packaging, and medical tubing devices (IPCS, 2003; US EPA, 2010).

Based on a survey of fragrance manufacturers conducted in 1995-1996 by the Research Institute for Fragrance Materials, approximately 4000 tonnes were used in the preparation of fragrance mixtures worldwide (Api, 2001; IPCS, 2003). Concentrations of DEP in cosmetic and fragrance preparations ranged from < 0.1% to 28.6% (97.5th percentile of use based on data from the International Fragrance Association), although < 1% was found in most products. A 2001 survey of fragrance manufacturers in the US indicated maximum concentrations of 1%-11% DEP in perfume and up to 1% in deodorants and other personal care products (Api, 2001; IPCS, 2003). Also, a Greenpeace International report (2005) identified DEP as the most prevalent phthalate found in 34 out of the 36 perfumes tested and with concentrations ranging from below detection to 2.2%. Similarly, DEP was found in 12 out of 17 popular perfumes, colognes and body sprays at concentrations ranging from 0.0098% to 3.2% (The Campaign for Safe Cosmetics, 2010).

In contrast, DEP as a low molecular weight phthalate is not found as the dominant phthalate plasticiser in children's toys and child care articles (see below). Frequently, it is used in conjunction with another plasticiser as a secondary plasticiser or occurs as a minor contaminant of other phthalates, including diethylhexyl phthalate (DEHP) or diisononyl phthalate (DINP). Specific concentrations of DEP in toys were not available.

4.2.3 Uses of phthalates and possibilities for substitution

Phthalates can be substituted for each other in certain applications. However, given the range of phthalate chemicals that exist, there are likely to be limits to substitutability for any particular application. Information on the use patterns of phthalates indicate generally that lower molecular weight phthalates, such as DEP, are used as solvents whilst higher molecular weight phthalates are used as plasticisers (NICNAS, 2008b).

The physicochemical factors expected to affect the choice of specific phthalates for particular uses include viscosity, water solubility and vapour pressure/boiling point. These physicochemical properties alter with increasing molecular weight and side chain length. As side chain length increases from 1 to 13 carbons, phthalates exhibit several orders of magnitude increase in octanol-water partition coefficient (Kow) and an order of magnitude decrease in vapour pressure. Water solubility is also inversely related to molecular weight and side chain length (NICNAS, 2008b). Viscosity varies from 9 mPa.s for DEP to 56 mPa.s for DEHP and up to 190 mPa.s for ditridecyl phthalate (Eastman, 2002).

Thus, a high molecular weight phthalate ester (e.g. DINP) will be quite different to a low molecular weight phthalate ester such as DEP. However, the difference in properties between two phthalates of similar molecular weight, such as dimethyl phthalate (DMP) and DEP, would be expected to be much less. To the extent these are the key considerations, substitution of a particular phthalate with another phthalate of similar molecular weight for any given application, for example substitution of DINP with DEHP as a plasticiser, is more probable than substitution with a phthalate of very different molecular weight, such as DEP.

Little information is available in open literature on the subject of substitutability of phthalates. A number of phthalates and their functions are listed in the International Cosmetic Ingredient Dictionary and Handbook (The Personal Care Products Council, 2010), and DMP, DEP, DBP and DEHP all list functions as fragrance ingredient, plasticiser and solvent. However, the SCCP Opinion on phthalates in cosmetic products (SCCP, 2007) concludes that, among the phthalates found in a study of 36 perfumes, only DEP (up to 2.3%) and DMP (0.3%) are likely to have been deliberately added while DCHP, BBP, DBP, DIBP, DEHP, DINP, DIDP are likely to be present as impurities arising from leaching during manufacture or storage. This information relates to use in a sample of perfumes and there is no information available to extrapolate from perfumes to other cosmetics.

Among the phthalate plasticisers, DEHP is largely used in PVC and PVC/polyvinyl acetate copolymers due to high affinity, good solvation and maintaining low temperature flexibility. However DBP is "not convenient" as the primary plasticiser for PVC due to its high volatility (although it may be used as a secondary plasticiser), and is normally used for cellulose nitrate. DEP and DMP are also used in cellulose nitrate systems (Chanda and Roy, 2007).

Therefore, while it is clear that phthalates can be considered to be substitutable by other phthalates of similar properties, there are likely to be limits on the extent to which dissimilar phthalates can be used. DEP is a low molecular weight phthalate and thus it is not likely to substitute for DINP–a high molecular phthalate commonly used in toys and child care articles. However, in the absence of use data of DEP in these scenarios, assumptions may need to be made. In this report, for example, migration or leaching rates reported for DINP are used to undertake an exposure assessment for DEP as part of a mixed phthalate plasticiser (DINP + DEP) in relation to use of toys and child care articles.

5. Public Exposure

Public exposure to DEP is estimated for each of the following consumer applications only:

- Use in children's toys and child care articles; and
- Use in cosmetics

Exposure estimates are derived to allow characterisation of the risks associated with these applications of DEP.

5.1 Methodology for assessing exposure

It is acknowledged that there are always uncertainties in deriving exposure estimates. The use of measured data is always preferred in exposure assessments, however, modelled data may be used if measured data are not available. The use of Australian data is also preferred, however if Australian data are not available, overseas data may be used provided that the scenarios represented by the overseas data are equivalent to Australian exposure scenarios.

In this assessment of specific exposure pathways, the 'reasonable worst-case' approach is used, in which estimates are based on worst-case, but plausible, exposure scenarios. It is believed that this approach will address practically all individuals within the target population. In addition a 'typical' exposure estimate is performed if information is available to determine a use pattern representing an average for the target population.

5.1.1 Model for exposure of children

Exposure to DEP in children's toys and child care articles was estimated for children via both the oral and dermal routes. Insufficient information on the DEP content in toys is available, and therefore the exposure estimate is based on the usage and concentration of an alternative phthalate, dibutyl phthalate (DBP) which, like DEP, has a low molecular weight (i.e. < 250 Da), higher vapour pressure and lower viscosity than the phthalates typically used in PVC. DBP is reported to be used in toys and child care articles in Australia. The usage and concentration data for DBP are considered valid for DEP because of the possibility of substitution of similar phthalates, as discussed in Section 4.2.3.

Oral exposure was modelled using:

- An estimate of highest concentrations of DEP as a component of a mixed plasticiser in toys and child care articles in Australia; and
- An estimate of the available fraction of DEP based on the results of overseas studies of childrens' mouthing behaviour and the extractability of phthalate plasticisers under mouthing conditions.

Dermal exposure was modelled using:

- An estimate of the highest concentrations of DEP in toys and child care articles as a component of a mixed plasticiser in Australia;

- The use of default values for exposed surface area and estimates of dermal contact time with toys; and
- An estimate of the migration rate of the mixed plasticiser from PVC matrix through the skin based on experimental studies (NICNAS, 2010).

5.1.2 Model for exposure of the general population

Exposure of the general population to DEP from cosmetics was estimated for both the dermal and inhalation routes.

Dermal exposure was modelled using:

- The highest concentrations of DEP in cosmetic products for dermal application in Australia; and
- The use of default values for usage volumes and frequency for cosmetic products, and
- An estimate for dermal bioavailability of DEP (see Section 6.1).

Inhalation exposure was modelled using:

- The highest concentrations of DEP in cosmetic products applied by spraying in Australia; and
- The default values for usage volumes and frequency for cosmetic products, and
- The default values for inhalation rate and other parameters related to spray application of cosmetics, and
- An estimate for inhalation bioavailability of DEP (see Section 6.1).

International biomonitoring data provide an estimation of overall exposure of the general population or specific subpopulations to DEP. However biomonitoring data do not allow separate determination of the contributions of specific exposure routes. Therefore the available biomonitoring information was used to check whether the exposure estimates by the different routes were within the range of known population exposures and whether they were likely major contributors to overall exposure.

The uncertainties in the exposure assessment are discussed in the context of the risk characterisation (see Sections 8.3.1 and 8.3.2).

5.2 Children's toys and child care articles

5.2.1 Sources of exposure

According to data provided by local suppliers, several phthalates including DEP are used in children's plastic toys sold in Australia. However, data on the phthalate content of the toys were limited and import volumes relating specifically to toys were not available. Therefore, it was necessary to use overseas data to quantify the presence of phthalates in soft toys and establish possible levels of exposure to children.

The limited Australian information obtained through a voluntary call for information in 2004 showed one company importing toys with a DEP content of 0.02% and another company importing articles of unknown type with a DEP content of 0.06%-2%. Considering that the information collected covers only a small proportion of available toys on the Australian market, available overseas data have also been examined to establish a reasonable worst-case scenario of DEP exposure of children through the use of toys.

Stringer et al. (2000) investigated the composition of a range of plastic children's toys (71 toys, analysed as 76 different plastic components, 88.9% of which were PVC or part-PVC and 11.1% non-PVC) purchased in 17 countries including 5 purchased in Australia. The country of origin was also stated; with 41/71 toys purchased worldwide being made in China, including 4/5 purchased in Australia. For the remaining toy purchased in Australia, the origin was not determined. The country of origin data seen in this 2000 study for the Australian purchased toys was anecdotally confirmed to be relevant for the majority of toys currently being imported to Australia (Australian Toy Association, 2009).

DINP was the phthalate most frequently found in the toy samples (64%) and tended to be present at the highest concentration (up to 51% w/w). DEHP was the next most frequently found in the tested toys (up to 48%) with concentrations ranging from 0.008% to 35.5% w/w. DEP was found in soft PVC toys at a maximum concentration of 0.16% in a teether with 32.3% total phthalate content. DBP was found in 12.5% of the toys tested with concentrations ranging from 0.002% to 0.18%. Variations between batches and the contamination of commercial and industrial mixes with other phthalates or other compounds were noted. Several phthalates were also found in concentrations too low to have a plasticising function. These phthalates may have been present as a constituent or contaminant of other phthalates, constituent of an ink or paint used on the toy or through use as a processing aid or during manufacture of other products. The results indicated that the majority (72%) of soft PVC toys contain substantial proportions of phthalates, and that in all of these, a single phthalate (normally DINP and occasionally DEHP or DIOP–diisooctyl phthalate) was dominant.

Rastogi (1998) performed an analysis of seven PVC toys and 10 non-PVC plastic toys to determine the phthalate content. DINP and DIDP were the predominant phthalates found in all of the seven PVC toys. DEP was found in only one toy (teether) at a maximum level of 0.13%.

The National Environment Research Institute (NERI) in Denmark also investigated the content of phthalates in toys and other articles for children up to 3 years of age (Rastogi et al., 2002 & 2003; Rastogi & Worsoe, 2001). DEP was not found in any of the toys tested. The content of DBP ranged from 0.004% to 0.463% with up to 40% of the tested toys containing DBP. The total phthalate content in the toys was not reported.

In 2006, the Intergovernmental Forum for Chemical Safety (IFCS) published a paper *Toys and chemical safety: a thought starter* (IFCS, 2006) containing information on selected chemicals, including phthalates, in toys available in industrialised countries. The data presented in the report were compiled from a number of available studies on the different types of chemicals found in toys. An illustrative study provided in this review indicated that DEP may be present in certain children's toys (e.g. ice teether) at weight concentrations up to 53 ppm

(0.0053%) and DBP levels of up to 380 ppm (0.038%). Most of toys containing DEP or DBP also contain a mixture of phthalates with high concentrations of DINP and DEHP.

The phthalate levels of toys available in the Indian market were investigated with most of the toys analysed for children aged 3 years and below. A total of 15 soft and 9 hard toys were tested and all the samples were reported to contain phthalates. The predominant phthalates in the soft toys were DINP and DEHP, with concentrations of up to 16.2% DINP and 2.6% DEHP. DEP was not detected in any of the toys analysed. DBP was found in 3 out of the 15 soft toys with levels of up to 0.1% (Johnson et al., 2011).

Chen (1998) conducted a study to identify phthalate-containing products (total of 35 samples) that are likely to be mouthed by children in the USA, and to determine the amount of phthalate migration from these products using in vitro and in vivo tests. The products include soothers, teethers, nipples, pacifiers, books, handbag, and a variety of toys. In vitro tests were conducted either by shaking a PVC sample in a saliva stimulant or subjecting cut samples of PVC to impaction applied by a piston. For in vivo tests, human volunteers gently chewed/mouthed a polyethylene disk from a toy duck for four 15 minutes intervals and saliva was collected after each chewing period. The study reported DINP to be the predominant phthalate found in children's toys with content ranging from 15%-54% by weight. DEHP and other phthalates, DIOP and di-n-nonyl phthalate (DnNP), were also found. DEP and DBP were not found in any of the samples tested.

Health Canada (Canada Gazette, 2009) analysed 100 toys for phthalate content during 2007 and, of these, 72 toys had parts made of PVC. Among the 72 PVC-containing toys, 17 contained non-phthalate plasticisers only, while 54 contained phthalates at above 0.1%. Of these 54 toys, 33 (61%) contained DEHP, 35 (65%) contained DINP and 4 (7%) contained DBP, while none contained DEP, BBP, DIDP or DnOP. The average concentrations were 12.5% (DEHP), 21.9% (DINP) and 0.08% (DBP). Concentrations in individual toys were not reported. The results of this study were consistent with the results from Stringer et al. (2000), confirming that both DEHP and DINP were widely used, but with overall higher levels of DINP.

The overall findings from the above studies indicated that DEP was infrequently found in toys and, where present, is at very low concentrations (up to 0.2%) and in conjunction with higher levels of the predominant phthalates DINP and DEHP. The pattern of usage of DEP in toys, based on these findings, was similar to the pattern of usage of DBP (at up to approximately 0.5%), although DEP was less commonly observed.

5.2.2 Concentration estimates for use in exposure assessment

Australian information on the concentrations of DEP in toys and child care articles is restricted to one company that provided information that DEP is imported as a component of toys at a concentration of 0.02%. The limited reporting of DEP in toys and child care articles and the low concentrations reported are consistent with the available published information above that DEP is not normally used as a plasticiser in PVC (Wypych, 2003; Chanda & Roy, 2007), and that the main plasticisers used are DEHP and DINP, both of which have lower volatility. However, Chanda & Roy (2007) also indicated that the more volatile DBP has an

application in PVC as a secondary plasticiser, and is used as a small component of a mixture of plasticisers as a processing aid.

The use of DEP as a secondary plasticiser, similar to the known use of DBP, is more probable than the substitution of DEP for DEHP or DINP as a primary plasticiser, due to the closer similarity of DEP to DBP. This use scenario is consistent with the findings of the analytical studies described above (Section 5.2.1).

Therefore, the calculation of exposures to DEP is based on the assumption that DEP completely substitutes for DBP as a secondary plasticiser, and the maximum DBP level observed in the analytical studies of the toys of 0.5% (w/w), as a component of a mixture of plasticisers will be used.

5.2.3 Routes of exposure

Two routes of exposure to DEP are considered likely during use of plastic toys and child care articles. Firstly, dermal exposure may occur during normal handling and, secondly, oral exposure may occur through chewing, sucking and biting of these products, regardless of whether the products are intended to be mouthed. Inhalation exposure to DEP from these products is considered negligible due to the low vapour pressure of DEP.

When children mouth or chew child care articles or toys, phthalate plasticisers can migrate into the saliva and be swallowed and absorbed in the GI tract, or can be absorbed directly through the buccal mucosa. The amount of phthalate released from a product when it is mouthed or chewed is determined by the amount of time the product is in the child's mouth and the migration rate of phthalate from the product. The studies used for estimation of mouthing times and migration rates of phthalates from plastic articles under mouthing conditions have been mostly performed on PVC that contains DINP and are summarised in the NICNAS PEC assessment of DEHP (NICNAS, 2010). The results demonstrate that migration rate of phthalate plasticisers from plastic toys into saliva through biting and chewing is the combined effect of molecular diffusion and mechanical action with the latter the likely dominating factor. The phthalate migration rate from articles appears largely determined by the magnitude of the mechanical force applied to an article and the properties of the PVC grade comprising the article, and less so by the physicochemical characteristics or concentration of the particular phthalate. Therefore, although migration data specific for DEP and most phthalates are not available, the migration rates determined for DINP under chewing condition can be extrapolated to other phthalates such as a mixture of phthalate plasticisers (i.e. primary and secondary plasticisers) which include DEP.

5.2.4 Estimates of oral exposure for children from toys and child care articles

Oral exposure of children to DEP from mouthing of toys was estimated by assuming that DEP is present in the toys as part of a phthalate plasticiser mixture at a maximum concentration of 0.5% based on the weight of the toy in conjunction with a higher concentration of a primary phthalate plasticiser such as DINP. A detailed calculation of exposure of children to DINP under this scenario explaining the derivation of all of the relevant parameters is given in the NICNAS PEC assessment of DEHP (NICNAS, 2010), where DINP exposure is calculated as a surrogate for DEHP. The exposure estimate was made for a 6-month old infant

(mean bodyweight of 7.5 kg) based on studies that demonstrate that maximum mouthing behaviour occurs at this stage.

The parameters considered in estimating the oral DEP exposure from mouthing toys and child care articles were the following:

- the surface area of the child's open mouth (10 cm^2);
- the time the child spends mouthing toys and child care articles (typical value is 0.8 h/d and worst-case value is 3 h/d);
- phthalate oral bioavailability (100%); and
- the migration rate of DINP from the toys and child care articles under mouthing conditions (typical value is 26 $\mu g/cm^2/h$ and worst-case value is 58 $\mu g/cm^2/h$, based on studies using adult volunteers).

The calculated internal doses for the typical and worst-case scenarios for total phthalate and DEP are shown in Table 5.1. The assessment of exposure to total phthalate is based on the following assumptions:

- reasonable worst-case extraction data from a well-conducted study for DINP at a measured plasticiser concentration of 43% (w/w) (NICNAS, 2010);
- the extractability data for 43% DINP are also applicable where the total phthalate concentration in the toys and child care articles of 43% (w/w) is comprised of 0.5% (w/w) DEP and 42.5% (w/w) DINP, i.e. 43% of a mixed phthalate containing 1.16% DEP and 98.84% DINP; and
- the mixed phthalate is extracted under mouthing conditions without change in composition.

The estimates for DEP are derived by multiplying the internal exposures from the total mixed phthalates by the proportion of the DEP content (1.16%) in the mixed phthalates based on the parameters and assumptions stated above.

Table 5.1 - Estimated daily internal dose for total phthalate and DEP from oral exposure to children mouthing toys and child care articles

	Total phthalate $\mathbf{D_{int.dermal}}$ (μg/kg bw/d) (NICNAS, 2010)	**DEP** $\mathbf{D_{int.dermal}}$ (μg/kg bw/d)
Typical Exposure Scenario	27.8	0.32
Worst-case Exposure Scenario	231.7	2.69

5.2.5 Estimates of dermal exposure for children from toys and child care articles

Dermal exposure of children to DEP from mouthing of toys can be estimated by assuming that DEP is present in the toys as part of a mixed phthalate plasticiser at a maximum concentration of 0.5% based on the weight of the toy. A detailed

calculation of exposure of children to DEHP under this scenario explaining the derivation of all of the relevant parameters is given in the NICNAS PEC assessment of DEHP (NICNAS, 2010), and this calculation is assumed to be applicable for a mixed phthalate containing DEP. The estimate is made for a 6-month old infant (mean bodyweight of 7.5 kg), as the combined dermal and oral exposure is expected to be highest for this age group.

The parameters considered in estimating the dermal DEP exposure from toys and child care articles were the following:

- the contact surface area based on exposure to lips and hands (100 cm^2);
- the time the child spends handling the toys (typical value is 0.8 h/d and worst-case value is 3 h/d); and
- the dermal absorption rate of DEHP in the skin (0.24 $\mu g/cm^2/h$).

The calculated internal doses for the typical and worst-case scenarios for total phthalate and DEP are shown in Table 5.2. The assessment of exposure to total phthalate is based on the following assumptions:

- reasonable worst-case extraction data from a well-conducted study for DEHP at a plasticiser concentration of 40.4% (w/w) (NICNAS, 2010);
- the extractability data for 40.4% DEHP are applicable where the total phthalate concentration in the toys of 40.4% (w/w) is comprised of 0.5% (w/w) DEP and 39.9% (w/w) DEHP, i.e. 40.4% of a mixed phthalate containing 1.24% DEP and 98.76% DEHP; and
- the mixed phthalate migrates from the toys and is absorbed through the skin without change in composition.

The estimates for DEP are derived by multiplying the internal exposures from the mixed phthalates by the proportion of the DEP content (1.24%) in the mixed phthalates based on the parameters and assumptions stated above.

Table 5.2 - Estimated daily internal dose for total phthalate and DEP from dermal exposure to children from toys and child care articles

	Total phthalate $D_{int.dermal}$ **(μg/kg bw/d)** (NICNAS, 2010)	**DEP** $D_{int.dermal}$ **(μg/kg bw/d)**
Typical Exposure Scenario	2.6	0.03
Worst-case Exposure Scenario	9.6	0.12

5.2.6 Combined exposure estimates for children from contact with toys and child care articles

The combined exposure arising from both dermal and oral contact with children's toys and child care products is summarised in Table 5.3.

Table 5.3 - Estimated total internal exposure for children

Route of Exposure	Typical D_{int} (μg/kg bw/d)	Worst-case D_{int} (μg/kg bw/d)
Oral	0.32	2.69
Dermal	0.03	0.12
Combined	0.35	2.81

5.3 Cosmetics and personal care products

5.3.1 Sources of exposure

In addition to their use as plasticisers, phthalates also have applications in cosmetic and personal care formulations as humectants (skin moisturisers), emollients (skin softeners), skin penetration enhancers, agents to prevent brittleness and cracking in nail polishes and sealants, antifoaming agents in aerosols, and solvents (Hubinger & Havery; 2006*; US FDA, 2008).

DEP is the predominant phthalate used in cosmetics with current Australian data (2004 and 2006) showing the presence of DEP in all cosmetic product types. DMP, DBP and DnOP are also currently used, or have the potential for use in these applications.

Worldwide, the phthalates predominantly found in personal care and cosmetic products are DEP and DBP (Hubinger & Havery, 2006*; US FDA, 2008). A survey of 2000 perfume products found that the 97.5th percentile concentration of DEP was 28.6% (Api, 2001). Analysis of 48 cosmetic products available to consumers in the US showed that DEP was the most frequently found phthalate at concentrations up to 38 663 ppm (3.9%) (Hubinger & Havery, 2006*). A follow-up survey of 84 cosmetic and personal care products available in the US market showed DEP levels as high as 36 006 μg/g (3.6%) mostly in fragrances and DBP at a maximum level of 62 607 μg/g (6.3%) in nail polish (Hubinger, 2010). In cosmetic products available in Korea, DEP has been detected in 24 out of 42 perfumes and 2 out of 8 deodorants at concentrations of up to 12 402 ppm (1.2%) (Koo & Lee, 2004). DEP has been found in 34 out of 36 perfumes tested in the EU with concentrations of up to 22 299 ppm (2.2%) (Peters, 2005).

A more recent analysis of 252 cosmetic and personal care products, 98 of which were baby care products, collected from retail stores in Canada detected DEP, DMP, DIBP, DnBP, and DEHP. DEP was detected in 103 products with a maximum concentration of 25 542 μg/g (2.6%) in fragrances. DEP was the only phthalate detected in the baby care products (33 out of 98) with a maximum level of 2 566 μg/g (0.26%) in diaper cream (Koniecki et al., 2011).

Plasticised containers for cosmetic and personal care products may also represent a source of exposure to phthalates, including DEP, through leaching of plasticiser from the container into the product. Unfortunately, no data are currently available for leaching of DEP or phthalates in general, from plastic containers used for storage and dispensing of cosmetics and personal care products.

Mitani et al. (2003) analysed the amount of DEP, DPP, DBP and DEHP in samples of syrup, lotion and four types of eye drops packaged in plastic containers available in Japan. For most of the tested phthalates, the levels were well below the

limits of detection. DEP was detected in only one of the four eye drops samples at 178.6 ± 17.2 ng/mL.

Given that it is considered unlikely that DEP is present at high concentrations in PVC packaging (Section 5.2.2) and that the concentrations of DEP deliberately added to cosmetic products are well above the single measured value for DEP from packaging, it is considered that the contribution of packaging to DEP concentrations in cosmetics is negligible.

5.3.2 Concentration estimates for use in exposure assessment

Sufficient Australian information is available for this assessment on the concentrations of DEP in cosmetic products. These values are used in the calculation of exposures for the different cosmetic product types (see Table 5.4).

5.3.3 Routes of exposure

Considering the range of cosmetic and personal care products that may contain phthalates, the main route of public exposure to phthalates is through dermal contact. Dermal exposure to phthalates may occur during use of creams or liquid products. Inhalation exposure may occur through breathing overspray from products applied as aerosols. Due to the low vapour pressure of DEP, inhalation exposure to DEP from cream or liquid products applied on the skin is likely to be negligible.

Accidental oral exposure to phthalates via cosmetic and personal care products is unlikely to occur frequently and would involve only very small amounts of phthalates. Current information does not indicate use of phthalates in oral cosmetics products that are likely to be subject to inadvertent ingestion, such as toothpastes, mouthwashes, lipsticks and lip-glosses. Therefore, the potential for public exposure via this route is expected to be negligible and, hence, is not characterised further.

5.3.4 Estimates of dermal exposure

Dermal exposure in adults – deterministic approach

Depending on the type of product, dermal contact with cosmetics and personal care products can be limited to specific areas of the body such as the eye region, face, hands, nails, or feet, or it can be more extensive, covering large areas of the trunk as well as the face. In addition, the duration of exposure for various products may differ substantially. For rinse-off products such as soaps or shampoos, exposure may only be for a few minutes, although some residual product may remain. In contrast, for leave-on products, exposure may last for several hours.

Dermal exposure to DEP was calculated as an internal dose which is proportional to the use volumes, product retention factors (reflecting proportions of product remaining on the skin during normal use), phthalate concentrations per product type and dermal bioavailability of DEP. The rate of absorption was not used as it is considered that the total dermal bioavailability better reflects the absorption for a single dose over a prolonged exposure period.

No data on Australian use patterns (for example, typical amount used per application, frequency of use and exposure duration) were available for cosmetics

or personal care products. However, data collected on typical use patterns of some classes of these products in Europe are provided in the *Technical guidance document on risk assessment* (TGD) of the European Chemicals Bureau (EC, 2003) and the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers' *Notes of guidance for the testing of cosmetic ingredients and their safety evaluation* (SCCNFP, 2003b & SCCP, 2006).

For the purposes of this assessment, Australian use patterns for these products are considered similar to those in Europe and, consequently, data from these overseas sources have been used in determining Australian phthalate exposures.

The bioavailability of DEP via the dermal route was assessed to be 10% (based on a number of studies discussed in Section 6.1.1 and 7.1). The internal dose arising from dermal exposure to cosmetic and personal care products was estimated using Equation 1 below:

$$D_{int,derm} = \frac{A_{prod} \bullet n \bullet \frac{C}{100} \bullet \frac{B_{derm}}{100} \bullet RF \bullet CF}{BW} \qquad \text{Equation 1}$$

Where:

$D_{int,derm}$	=	Internal dose via the dermal route, μg/kg bw/d
A_{prod}	=	Amount of cosmetic and personal care product applied to skin, mg/event
n	=	Frequency of product application, event/d
C	=	Concentration of DEP in product, % (w/w)
B_{derm}	=	Bioavailability via the dermal route, %
RF	=	Retention factor
CF	=	Conversion factor, 1000 μg/mg
BW	=	Adult bodyweight, 70 kg

The calculated daily internal DEP doses from the use of different product types are shown in Table 5.4.

Table 5.4 - Typical use pattern and calculated daily internal dose from dermal exposure to various cosmetic and personal care products in adults

Product Type	A_{prod}[a] (mg/event)	n[a] (events/d)	RF[a]	C (% w/w)	$D_{int,derm}$ (µg/kg bw/d)
Leave-on products					
Body antiperspirant roll-on / liquid	500	1	1	1.13	8.07
Cologne / aftershave / Splash	1200	2	1	0.97	33.26
Nail polish	250	0.43[c]	1	25	38.26
Face cream / Moisturizer	800	1	1	0.42	4.80
Body lotion	7500	2	1	0.25	53.57
Perfume spray	637.5[b]	5	1	2.5	113.84
Rinse-off products					
Soap bars	800	6	0.01	0.15	0.102
Shower products	5000	2	0.01	0.48	0.68
Shampoo / conditioner	12 000	1	0.01	0.05	0.086
Shaving products (cream, gel, stick, lather)	2000	1	0.01	0.005	0.0014

[a]Typical values for use parameters derived from EU TGD (EC, 2003) or the SCCP (2006). The higher value from the two references is chosen for the calculation of internal dermal exposure.
[b] Typical amount is 750 mg/event and assuming 85% of the spray product amount ends up on the skin (Bremmer et al., 2006).
[c] three events per week or 3/7 events per day.

Not all product types reported by the Australian industry to contain DEP (summarised in Table 4.1) have been included in the calculation. Some of the cosmetic and personal care products have interchangeable uses (e.g. hand wash and bar soaps), and, in these categories, only the product types with the higher DEP concentration have been used for the calculation.

Dermal exposure in adults - probabilistic approach

The internal dermal exposures calculated using Equation 1 are frequently referred to as point estimates from a deterministic approach, using single values to represent each exposure variable to produce a single exposure estimate.

An alternative method used in the exposure calculations is a probabilistic modelling approach, which uses the distributions around each variable as inputs, rather than single values, to generate an exposure distribution. Calculations therefore account for all the possible values of a variable in relation to the probability of each value occurring, generating a range of risk estimates (WHO, 2005).

In the case of the estimates for internal exposure to DEP, the probabilistic approach was not conducted since the implementation of this distribution-based approach requires data obtained from a large sample size (IGHRC, 2004) and distribution data for the exposure variables for typical use levels of cosmetics (i.e. amount used and frequency of use) are not currently available in Australia. Hall et al. (2007) investigated the probabilistic analysis of the use pattern based on distribution values from actual monitoring of the use of some cosmetic products by 44 100 households and 18 057 individual consumers in five European countries. The amounts (95th percentile) of cosmetic products used per day in the Hall et al. (2007) study were: 8.651 g/d for body lotion, 1.806 g/d for liquid deodorant, 1.801 g/d for facial moisturiser and 12.181 g/d for shampoo. These probabilistic estimates are comparable to the amount of product applied as reported in the EU TGD (EC, 2003) and SCCP (2006).

The internal DEP dose was calculated using the probabilistic estimates (95th percentiles) for the externally applied doses of 4 types of cosmetic products calculated by Hall et al. and the bioavailability and concentration of DEP in these cosmetic product types from Table 5.4. This approach estimated the internal DEP dose from liquid deodorant as 29.2 μg/kg bw/d, from face moisturiser as 10.81 μg/kg bw/d, from body lotion as 61.79 μg/kg bw/d and from shampoo as 0.087 μg/kg bw/d. These data for DEP exposure derived from probabilistic estimates of product exposures are comparable to the data derived from point estimates in Table 5.4.

For the worst-case scenario estimation under these assumptions, if a person were a simultaneous user of all the products listed in Table 5.4, the combined internal dose from dermal exposure is determined to be 252.68 μg/kg bw/d.

The daily DEP dermal exposure from the cosmetic and personal care products analysed in Canada (Koniecki et al., 2011) was estimated as 78 μg/kg bw/d for female adults. The dermal bioavailability used in the estimation was 5%. Taking into account the difference in the assumption of dermal bioavailability (5% vs. 10%) this figure is consistent with the estimate in this assessment.

Dermal exposure in children

Using the model developed by NICNAS (NICNAS, 2009), the quantity of whole body product applied to a child or infant can be estimated from the ratio of body surface area of the child or infant compared with the adult. The systemic dose depends on the body weight of the child or infant, and therefore the systemic dose for any product used similarly in children and adults will vary according to the ratio of surface area to body weight, if the skin permeability is the same in adults and children. An estimate of the magnitude of the difference can be made using data issued by the SCCNFP on the Margin of Safety calculation for children (SCCP, 2006). For children from 0 to 10 years, the difference between surface area to bodyweight (SA/BW) ratio is as follows: 2.3 fold at birth, 1.8 fold at 6 months, 1.6 fold at 12 months, 1.5 fold at 5 years and 1.3 fold at 10 years (SCCP, 2006). However, there are no available data on the usage of cosmetic products in children by age or of differences in permeability of skin between children and adults.

One type of cosmetic product potentially containing DEP and used in infants or children is body lotions or creams. These would have use equivalent to the body lotion scenario for adults. The maximum concentration for DEP in lotions and creams is 0.25% and if the same number of applications per day as in adults is

assumed then the internal doses for infants by age can be calculated as shown in Table 5.5.

Table 5.5 - Calculated daily internal dose for infants from dermal exposure to baby lotions or creams

Infant Age	Adult $D_{int,derm}$ (µg/kg bw/d)	SA/BW ratio	$D_{int,derm}$ (µg/kg bw/d)
Newborn	53.57	2.3	123.2
6 months	53.57	1.8	96.4
12 months	53.57	1.6	85.7

The daily DEP dermal exposures from the cosmetic and personal care products analysed in Canada (Koniecki et al., 2011) were estimated as follows:
20 µg/kg bw/d for children 6 months to 4 years and 42 µg/kg bw/d for children 0-6 months. The dermal bioavailability used in the estimation was 5%. As for the estimate of adult exposure above, the results of the Canadian exposure assessment differ from those estimated in this assessment primarily due to the different assumed dermal bioavailability.

5.3.5 Estimates of inhalation exposure

Inhalation exposure to DEP from cosmetic and personal care products can occur via inhalation of spray aerosols such as antiperspirant body sprays and/or perfume sprays.

In order to estimate the internal dose from the use of these products, the following parameters/assumptions were used in the calculations:

- Adult inhalation rate is 22 m^3/d (enHealth, 2003);
- Phthalate bioavailability via the inhalation route is 100%;
- The average body weight is 70 kg (ABS, 2005);
- Room volume of 2 m^3 to represent the volume of air immediately surrounding the user (EC, 2003); and
- Assumed exposure duration is 3.17 minutes, consisting of 10 seconds for actual spraying of the product and a further 3 minutes exposure after spraying (Bremmer et al., 2006).

The equation used in the calculations of the internal dose via the inhalation route is shown below:

$$D_{int,inh} = \frac{A_{prod} \bullet n \bullet \frac{C}{100} \bullet \frac{B_{inh}}{100} \bullet t \bullet IR_{air} \bullet CF_1 \bullet CF_2}{BW \bullet V_{room}} \qquad \text{Equation 2}$$

Where:

$D_{int,inh}$	=	Internal dose via the inhalation route, µg/kg bw/d
A_{prod}	=	Amount of perfume spray, mg/event
n	=	Frequency of spray application, event/d
C	=	Concentration of DEP in product, %

B_{inh}	=	Bioavailability via the inhalation route, %
t	=	Time of contact (spray and exposure duration), minute
IR_{air}	=	Inhalation rate of person, m^3/d
CF_1	=	Conversion factor (time), 1 d/1440 minutes
CF	=	Conversion factor (amount), 1000 μg/mg
V	=	Room volume, m^3
BW	=	Adult body weight, kg

Data on typical use pattern of these products can be found in the *Technical guidance document on risk assessment* (TGD) of the European Chemicals Bureau (EC, 2003). For the purposes of the exposure assessment via inhalation exposure, Australian use patterns for these products are assumed to be similar to those in Europe (at the maximum daily usage rate) and the concentrations of DEP are the maximum concentrations reported in these products in Australia. The typical use pattern and calculations of DEP internal oral doses for the deodorant and perfume spray are shown in Table 5.6.

Table 5.6 - Exposure parameters and calculated daily internal dose from inhalation exposure to cosmetic and personal care products

Product Type	A_{prod}* (mg/event)	n* (events/d)	C (%)	$D_{int,inh}$ (μg/kg bw/d)
Perfume spray	750	1-5	2.5	32.4
Antiperspirant / deodorant spray	3000	1-3	0.37	11.5

*Typical values for use parameters derived from EU TGD (EC, 2003).

For a worst-case scenario estimation, the internal dose from inhalation exposure is determined to be 32.4 μg/kg bw/d. It is considered likely that only one of these two types of products would be used by an individual on a single day. However, even if both products are used by the same individual the internal dose will be low because of low concentrations of DEP in these products.

5.3.6 Combined exposure from contact with cosmetic products

The systemic exposure to DEP, internal dose (D_{int}), arising from the combined use of cosmetic products containing DEP at the assumed maximum levels is summarised in Table 5.7.

Table 5.7 - Total estimated exposure to DEP from cosmetic use

Route of Exposure	D_{int} (μg/kg bw/d)
Dermal	252.7
Inhalation	32.4
Combined	285.1

5.4 Comparison with biomonitoring data

There have been some attempts to use biomonitoring data to estimate exposure to DEP as a result of the use of cosmetic and personal care products. However, DEP is ubiquitous and it is very difficult to assess DEP exposure specifically through these products unless there is available information on their phthalate content and use rates. One US study (Sathyanarayana et al., 2008a) monitored the presence of

metabolites of 9 phthalates, including DEP, in the urine of 163 infants in relation to mother's reported use of 5 types of baby care products within the 24 h prior to urine collection. The urine measurements were not used to determine doses. The study suggested that the level of DEP, DMP and DIBP metabolites in the infant's urine could be associated with the use of baby care products and significant association was observed in younger infants (Sathyanarayana et al., 2008b). However, no information was available on the phthalate content of the products used in the study (tested or manufacturer-reported) and information on use was derived from self-reporting by the mothers which did not include reporting on the amount of product used.

Biomonitoring data for a particular chemical or its metabolites represent exposure to the chemical from all sources and pathways. The toxicokinetics of DEP demonstrates that DEP is rapidly excreted and does not appear to accumulate in tissues (Section 6.1), and therefore single day measurements approximate the daily dosing. The analytical approaches and uncertainties associated with biomonitoring data limit their use in exposure and human health risk assessments (Albertini et al., 2006). It is not possible to determine the relative contribution of different exposure routes directly from population biomonitoring data and, for this purpose, modelling is the most suitable method. However, population biomonitoring data are useful in determining whether the exposures calculated through modelling are within the observed range of exposure, and their magnitude compared with the integrated exposure of the population.

Biomonitoring data for the Australian general population or specific subpopulations are not available. Several international biomonitoring investigations are available for providing exposure estimates for DEP as determined from the concentrations of the urinary metabolites of DEP, which is monoethyl phthalate (MEP). These studies are summarised in Table 5.8.

Table 5.8 - Summary of biomonitoring data estimating exposure to DEP

Study	Population Group	Exposure (µg/kg bw/d)		
		Mean	Median	95th Percentile
Calafat & McKee (2006)	2772 people from the American population (6 - >20 years old)	5.5		61.7
Marsee et al. (2006)	214 mother-infant pairs observed for MEP levels		6.64	112.3
Wormuth et al. (2006)	Compilation of several German studies for the general population		3.9 (females) 1.4 (males)	32.6 (females) 28.1 (males)
Kho et al. (2008)	60 Korean children	0.8		
Frederiksen et al. (2011)	129 Danish children and adolescents		1.09	8.04
Guo et al. (2011)	Adults (21-49 years old) from 7 Asian countries		64-3900 µg/day	

Calafat & McKee (2006) estimated the daily DEP exposure to children from the cumulative biomonitoring data in the US based on the Third National Report on Human Exposure to Environmental Chemicals (CDC, 2005). The published Fourth National Report on Human Exposure to Environmental Chemicals (CDC, 2009) reported an increase in the mean serum levels by approximately 7% in children. Corresponding dose estimates have not yet been published, however, assuming the parameters used by Calafat & McKee (2006) in estimating exposure levels from the serum concentrations are the same, a corresponding increase in the daily exposures could be assumed. The resulting exposure at the 95th percentile would be expected to lie between the values reported by Calafat & McKee (2006) and Marsee et al. (2006).

The wide range between the measure of central tendency (mean or median) and the outliers in these large studies indicate that some members of the population have been exposed to much higher DEP doses than the population average. For example, the maximum calculated exposure from biomonitoring data was 96.9 µg/kg bw/d, for one female participant, compared with a median dose of 3.9 µg/kg bw/d for female adults (Wormuth et al., 2006). This indicates that there are likely to be high exposure scenarios applicable to a subset of the population.

The calculated worst-case DEP exposure to cosmetics and personal care products is greater than the biomonitoring data of the DEP metabolite, due to the worst-case assumptions used. However the estimates for cosmetic use for a single product such as body lotion are close to the 95th percentile and maximum concentrations measured in these large biomonitoring studies. This indicates that the worst-case exposure scenarios considered in this assessment are applicable for highly exposed individuals. The results seen in the biomonitoring studies are also consistent with the basis of the exposure assessment of DEP, as they indicate that the general population exposure is much lower than the individual exposure which can arise from these specific high exposure scenarios. In comparison, the adult biomonitoring values for DEP were up to 12 times higher than the DEHP concentrations in the Marsee et al. (2006) study and consistent with the expectation that DEP is more widely used in cosmetic products than DEHP.

5.5 Cumulative exposure to multiple phthalates

Cumulative exposures can arise from exposure to multiple phthalates used in cosmetics and/or toys and child care articles. Co-exposure to DEP and DEHP in these two scenarios is not likely to occur as risk mitigation measures have been introduced in Australia for DEHP.

6. Human Health Hazard Assessment

The Existing Chemical Hazard Assessment Report on DEP was published by NICNAS in June 2008 (NICNAS, 2008a) using as data sources the International Programme on Chemical Safety's Concise International Chemical Assessment Document 52 (IPCS, 2003) and Opinions of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers concerning DEP (SCCNFP, 2002 & 2003a). This chapter of the PEC assessment report is largely based on the Existing Chemical Hazard Assessment Report (NICNAS, 2008a), but has been supplemented with an evaluation of new relevant data identified from comprehensive searches of DEP-related literature up to April 2011.

The recently evaluated studies (since the release of the DEP Hazard Assessment in 2008) are marked with 'ND' for 'new data' (e.g. 2009 **ND**). References marked with an asterisk (*) were not reviewed but were quoted as secondary citations from the key documents listed in Section 1.3.

6.1 Kinetics and metabolism

The toxicokinetics of DEP have been studied in experimental animals following oral and dermal exposure. No data are available for inhalation exposure. A limited number of studies have also examined the toxicokinetics of DEP in humans.

6.1.1 Absorption

Absorption via the oral route

Available data indicate that the oral absorption of DEP is extensive and rapid based on measurement of urinary and faecal excretion. Following oral administration of ^{14}C-DEP to rats and mice (doses not stated), much of the radioactivity from the administered dose (90%) was excreted in the urine within 48 h, with the majority (82%) being eliminated during the first 24 h. Approximately 3% of the radioactivity was found in the faeces over the same period of time (Ioku et al., 1976*; Api, 2001).

Following administration of DEP (10 or 100 mg) by stomach intubation in rats, 85%-93% of the administered dose was excreted in the urine within 7 d as measured by gas chromatography - mass spectroscopy (Kawano, 1980*; IPCS, 2003). For both dose levels, approximately 78% of the administered dose was excreted in urine within 24 h as monoethyl phthalate (MEP) (~70%), phthalic acid (~9%) and parent compound (0.1%-0.4%).

No information is available concerning differences in absorption and bioavailability of orally administered DEP between adult and immature animals or between animals and humans. The oral bioavailability of diethylhexyl phthalate (DEHP) appears to be higher in young rats (Sjöberg et al., 1985). The higher proportion of intestinal tissue in relation to body weight (Younoszai & Ranshaw, 1973), and the relatively higher blood flow through the gastro-intestinal (GI) tract

(Varga & Csaky, 1976) have been suggested as the likely factors causing an increased absorption in young animals. Therefore, for the purposes of this assessment, bioavailability of DEP via the oral route is assumed to be 100% for both children and adults.

Absorption via the dermal route

When ^{14}C-DEP was applied to male rat skin at 5-8 mg/cm^2 under occlusion, 24% and 1% of the applied dose was excreted in the urine and faeces respectively, within 24 h (Elsisi et al., 1989; IPCS, 2003). In a similar experiment where ^{14}C-DEP (dose not stated) was applied to female rabbit skin, around 49% and 1% of the dose was excreted in the urine and faeces respectively, after 4 d (RIFM, 1973*; Api, 2001). The metabolites were not characterised for these studies.

In an in vitro study, the comparative percutaneous absorption of DEP between human and rat skin was evaluated in flow-through diffusion cells. Results showed that dermal absorption of ^{14}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 h under occlusive conditions (Mint et al., 1994*; IPCS, 2003). Scott et al. (1987; 1989 Errata) using a similar experimental system reported that the in vitro absorption of DEP through rat skin was more than 30 times higher than through human skin with the steady state absorption rate of 413.7 vs. 12.8 µg/cm^2/h for rat and human skin respectively.

In another in vitro diffusion cell study, permeability coefficients and lag time measurements for six industrial chemicals, including DEP (in saturated aqueous solutions) were highly correlated between human and hairless guinea pig skin (Frasch & Barbero, 2009 **ND**). However, the steady state absorption was double (up to 23.9 µg/cm^2/h) for DEP applied via a saturated aqueous solution compared with its pure or neat form (Frasch et al., 2007 **ND**).

In a 2-week single-blinded study, 26 healthy male Caucasians were given a whole body topical application (5 d/week) of 2 mg/cm^2 basic cream without (week 1– control week) and with (week 2) DEP, dibutyl phthalate (DBP), and butyl paraben at 2% w/w each. Two hours after the first cream application containing approximately 800 mg DEP, serum concentrations of MEP peaked at 1000 µg/L (corresponding to 6.9 mg or ~10% of absorbed DEP) and decreased to 23 µg/L after 24 h just before the second application, but did not reach the baseline levels observed in the first week. Average daily recovery of DEP excreted in urine as MEP was 5.8% (Janjua et al., 2007 **ND**; 2008 **ND**).

In conclusion, based on the use of urinary and faecal excretion as an index of absorption, DEP appears to be well absorbed via the skin with around 25% to 50% of administered doses excreted within 24 h and 4 d respectively in rats and rabbits. Recent human studies indicated a lower dermal absorption than that seen in rats, with approximately 10% and 5.8% of dermally applied DEP found in serum and urine, respectively within 24 h. The difference in dermal absorption between rats and humans may reflect species differences, differences in vehicle (alcohol vs. skin cream), and/or differences in application (occlusive vs. non-occlusive) (Janjua et al., 2008 **ND**). On a weight of evidence basis, a dermal bioavailability for DEP of 10% in humans is assumed for the purposes of this risk assessment.

6.1.2 Distribution

Following oral administration of ^{14}C-DEP (doses not stated) to rats and mice, the radioactivity was widely distributed with the highest concentrations observed in kidney and liver, 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 h (Ioku et al., 1976*; Api, 2001).

In female rabbits, when ^{14}C-DEP (dose not stated) was applied to the skin, very little radioactivity was found in tissues 4 d after exposure with the amounts as follows: liver (0.004% of dose), kidney (0.003% of dose) and blood (less than 1% of dose) (Api, 2001; RIFM, 1973*). When a single dose of DEP was applied to male rat skin, very little radioactivity was found in the tissues after 7 d of exposure. The amounts of radioactivity in the adipose tissue, muscle, skin, brain, lung, liver, spleen, small intestine, kidney, testis, spinal cord and blood were each less than 0.5% of the dose (Elsisi et al., 1989; IPCS, 2003).

Following intraperitoneal (ip) injection of ^{14}C-DEP in pregnant rats on gestational day (GD) 5 or 10, radioactivity was detected in amniotic fluid, as well as in maternal, placental, and foetal tissues, indicating that the compound can pass through the placenta to the developing foetus. The half-life of the compound in foetal tissue was approximately 2.2 d (Singh et al., 1975*; IPCS, 2003).

6.1.3 Metabolism

Following oral dosing of rats and mice, MEP was the major urinary metabolite with phthalic acid as a minor secondary metabolite (Ioku et al., 1976*; Api, 2001). Similarly, in another study, approximately 70% of the dose administered by stomach intubation in rats was excreted in urine within 24 h as MEP (Kawano, 1980*; IPCS, 2003). Hydrolysis to the monoester by skin was also demonstrated in vitro for both rats and humans (Hotchkiss and Mint, 1994*; Api, 2001).

6.1.4 Elimination and excretion

In experimental animals, DEP is rapidly eliminated and does not accumulate in tissues. The urine appears to be the major route of DEP excretion. Most (90%) of the oral dose (doses not stated) administered to rats and mice was excreted in the urine within 48 h post-dosing, with the majority (82%) being eliminated during the first 24 h (Ioku et al., 1976*; Api, 2001). Administration of DEP by stomach intubation resulted in 85%-93% of the administered dose being excreted in the urine in rats 7 d post-dosing (Kawano, 1980*). When applied to skin, 24% and 1% of the administered 14C-DEP dose was excreted in the urine and faeces respectively after 24 h in rats (Elsisi et al., 1989) and 49% and 1% in the urine and faeces respectively after 4 d in rabbits (RIFM, 1973*).

Following daily whole body dermal applications of DEP in humans over one treatment week, 24-h urine samples were collected and analysed by liquid chromatography - Tandem Mass Spectroscopy. During the treatment week, the mean recovery rate of DEP in the urine was 5.8% as MEP with an unconjugated (free) fraction of up to 78%. The majority of MEP was excreted within the first 8 h after application. The recovery rates recorded daily were between 0.3%-13.9%, indicating large intra-individual variations (Janjua et al., 2008 **ND).** An earlier study also showed that in humans, almost three quarters (71%) of the total amount

of MEP excreted in the urine was in the form of free monoester, the rest being MEP glucuronide (Silva et al., 2003).

Air levels of DEP were also found statistically significantly positively correlated with the levels of MEP in 48-h spot urine samples collected from pregnant women living in New York (Adibi et al., 2003 **ND**; 2008 **ND**). The correlation suggests DEP may be absorbed via inhalation prior to excretion via urine.

6.2 Effects on laboratory animals and other test systems

6.2.1 Acute toxicity

The acute toxicity of DEP has been evaluated in a number of species after oral, dermal and inhalation administration.

DEP has low acute oral and dermal toxicity. LD50 values were reported in the range of 1-31 g/kg bw by the oral route in mice, rats, rabbits, dogs and guinea pigs. Clinical signs included CNS depression, convulsion and respiratory paralysis prior to death.

After a single dermal application of DEP in rats (3/sex) under occluded patches (1, 2, 5, and 10 mL/kg, corresponding to up to 11 000 mg/kg), no deaths or gross changes at necropsy were noted. Slight redness of the skin at the site of application was observed at 24 h for all concentrations (RIFM, 1978*). A dermal LD50 of 3000 mg/kg bw in guinea pig was also reported.

LD50 values derived from these studies are shown in Table 6.1.

Table 6.1 - Acute animal toxicity studies (adapted from SCCNFP, 2002)

Study Type	Species	Results (LD50/LC50) mg/kg bw
Oral	Mouse	6200
	Rat	>5600 - 31 000
	Rabbit	1000
	Dog	5000
	Guinea pig	>4000 - 8600
Dermal	Rat	>11 000
	Guinea pig	3000
Inhalation	Mouse	4.9 mg/L
	Rat	7.5 mg/L

6.2.2 Skin and eye irritation

Skin irritation

Several studies have been conducted in rats and rabbits.

Application of undiluted DEP (purity and duration not stated) on intact and abraded rabbit skin (6 animals) in a closed patch test caused slight to moderate

irritation at both sites after 24 h. Irritation was reduced by 40% at 72 h (RIFM 1974*; Api, 2001). In contrast, in two other 4-h semi-occlusive patch tests in rabbits, 0.5 mL of undiluted DEP did not cause skin irritation (RIFM, 1984* and 1985*; Api, 2001).

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

Overall, the data indicate that DEP causes minimal skin irritation.

Eye irritation

Application of undiluted DEP (0.1 mL) into the conjunctival sac of the rabbit eye caused minimal irritation after 1 h. No reactions were noted at 24, 48 or 96 h (Draize, 1944*; Api, 2001). Similarly, undiluted DEP (0.1 mL) resulted in transient slight redness of the conjunctivae (RIFM, 1978*; Api, 2001) and minimal eye irritation (ATSDR, 1995b).

Overall, the studies in rabbits show that DEP causes minimal eye irritation.

6.2.3 Sensitisation

The skin sensitising potential of DEP has been investigated using a number of standardised guinea pig test methods and a local lymph node assay. Data for respiratory sensitisation are not available.

Skin sensitisation

One Buehler study (RIFM, 1978*; Api, 2001) and two Magnusson and Kligman maximisation studies (Klecak et al., 1977*; Buehler, 1996*; Api, 2001), using 50% aqueous solution and undiluted DEP respectively, did not show any skin sensitisation effect. Also, no dermal sensitisation responses were observed with undiluted DEP in an open epicutaneous test, the Draize intradermal test and the Freund's complete adjuvant test (Klecak et al., 1977*; Klecak, 1979*; Api, 2001).

In a local lymph node assay, DEP (25 µL of 25%-100% DEP in acetone-olive oil) did not induce significant increases in thymidine incorporation into lymph nodes (Ryan et al., 2000*; IPCS, 2003).

Overall, data indicate that DEP is not a skin sensitiser.

6.2.4 Repeated dose toxicity

Several studies have been conducted with DEP in rats and mice via the oral and dermal routes.

Oral route

Sprague-Dawley (SD) rats (15/sex) were fed with DEP in the diet at 0, 0.2, 1, or 5% for 16 weeks (approximately 0, 150, 770-750 or 3160-3710 (m-f) mg/kg bw/d). After 16 weeks, body weights were statistically significantly reduced in both sexes

at 5% (20%-23% reduction) and in females at 1% (8% reduction). Concurrent paired-feeding experiments indicated that the decrease in body weight gain was primarily attributable to lower food consumption and/or poorer food utilisation. After 16 weeks, both sexes at 5% showed significant increases in relative liver (31%-33%) and kidney weights (11%-17%). In females, increases in relative liver weights at 0.2% and 1% doses (6.5% and 8.3% increases respectively) were also statistically significant and dose-dependent. There were also significant dose-dependent increases in the relative weights of stomach and small intestine in female rats at all doses at week 16. The authors considered that unusually low control values compared to historical control data (not supplied) for stomach and small intestine weights in the female rats confound the significance of weight changes in these organs in females. Also at this time, in male rats, relative small intestine weights were increased at 5% only, whereas relative stomach weights were significantly increased at 1% (10% increase) and 5% (41% increase). There were no effects on the gross or microscopic pathology of the lungs, trachea, or thymus, or abnormal histopathology of the liver, kidney or digestive organs. Also, no significant effects on haematology, serum enzyme levels or urinary parameters were reported. Relative organ weight changes in this study are likely, to some extent, to be linked to body weight changes. However, the extent to which organ weight changes at multiple doses can be discounted is not certain. A conservative NOAEL of 0.2% DEP in the diet (approximately 150 mg/kg bw/d) was therefore identified based on dose-dependent increased relative liver weights in females and increased relative stomach weights in males at 1% (750 mg/kg bw/d) and above (Brown et al., 1978).

Ten male Wistar rats were given 2% DEP (equivalent to 2000 mg/kg bw/d) in the diet for 1 week. A significant increase (12%) in relative liver weight was observed, with no changes in kidney and testis weights (Oishi & Hiraga, 1980).

The inductive effect of five phthalates on the microsomal levels of laurate hydroxylase (a marker for peroxisome proliferation) was examined. Administration by oral intubation of DEP to SD rats (5 males) at approximately 1200 mg/kg bw/d for 3 d increased the laurate hydroxylase activity by 1.6-fold compared to that induced in control rats but 6.9-fold less than that induced by DEHP. In addition, whereas DEHP increased peroxisomal palmitoyl-CoA oxidation by 6-fold, DEP increased this activity by only 1.3 fold compared to control. The authors concluded that DEP and DEHP do not share similar inductive properties on peroxisome proliferation (Okita & Okita, 1992).

In a 150-d repeated dose toxicity study, DEP was administered in the diet at 0, 0.57, 1.43, or 2.85 mg/kg bw/d to young male Wistar rats (6/group). 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 treated groups, however only increases for glycogen and cholesterol showed dose-dependence. Liver and serum levels of acid phosphatase (ACP), alanine and aspartate aminotransferases (ALT and AST), and lactate dehydrogenase (LDH) were also increased. Electron micrographs of liver from low dose animals showed severe intra- and intercellular vacuolation, loss of hepatic 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 the data were noted which hampered the interpretation of the results from this publication (Pereira et al., 2006). Furthermore, the increased cholesterol and liver changes induced by DEP were not entirely reproducible in a three-generation study (same dosing, species, and also 150 d of treatment for the parental generation) and in a 180-d study at the same laboratory to evaluate gender-specific toxicity of DEP (only one dose of 2.5 mg/kg bw/d used) (Pereira et al., 2007 **ND**; Sinkar & Rao, 2007 **ND**).

Toxic effects of DEP on thyroid glands have also been observed from another three-generation study in Wistar rats (F0 dosed at 0.57, 1.43, or 2.85 mg/kg bw/d whereas F1 and F2 at a single dose of 1.43 and 0.57 mg/kg bw/d respectively for 150 d each generation). In F2, thyroid glands showed follicular shrinkage, loss of thyroglobulin and fibrosis of the interfollicular epithelium with a lesser intensity seen in F0 and F1 rats (Pereira et al., 2008a **ND**).

The toxicity of DEP was also evaluated in a 90-d dietary study in female Swiss mice (5/group). DEP dissolved in corn oil was administered at 0, 10, 25, and 50 ppm in the diet (approximately 0, 1.25, 3.13, and 6.25 mg/kg bw/d). Another group of mice was fed with corn oil only as vehicle control. No significant changes in body and liver weights were recorded, but a significant dose-dependent increase across all groups was observed in serum levels of ACP, ALT and AST while LHD was significantly increased only in the 25 and 50 ppm dose groups. Liver glycogen, cholesterol and triglycerides were increased in all treated groups. Intracellular hepatocytic vacuolations were seen in all treated groups with additional degeneration and hypertrophy of the hepatocytes being evident at 50 ppm. Proliferation of mitochondria and peroxisomes was also evident in all treated mice and in the 25 and 50 ppm DEP-treated mice, mitochondrial hypertrophy became severe with increased accumulation of lipid droplets (Mapuskar et al., 2007 **ND**).

Dermal route

Two 4-week studies of dermal exposure to undiluted DEP in rats and mice show increases in kidney and liver weights without significant histological findings.

Rats (10/sex) treated dermally with 0, 37.5, 75, 150, or 300 µL DEP (approximately 0, 200-300, 400-600, 800-1200, or 1600-2500 (m-f) mg/kg bw/d) for 4 weeks exhibited no clinical signs of toxicity. Increased relative liver weights were observed in 300 µL male (9%) and female rats (7%) and 150 µL female rats (10%) as compared to controls. Relative kidney weights of 150 µL and 300 µL males and 150 µL females were also greater than those of controls. No gross or microscopic lesions were observed (NTP, 1995*).

Mice (10/sex) were treated dermally, 5 d per week for 4 weeks, with 0, 12.5, 25, 50, or 100 µL DEP (approximately 0, 560-630, 1090-1250, 2100-2500, or 4300-5000 (m-f) mg/kg bw/d). There was no histological evidence of damage to any organ. Absolute and relative liver weights were greater than those of the controls in female mice treated with 25 µL and 100 µL DEP (NTP, 1995*).

Overall, in both short- and medium-term repeated dose toxicity studies the liver appears to be the primary target organ for DEP. Observed effects were increased organ weight, vacuolation, elevated serum and liver enzyme levels, and

proliferation of mitochondria and peroxisomes. Increased weights of other organs such as kidney, stomach and small intestine were also reported.

6.2.5 Genotoxicity

Only in vitro genotoxicity studies are available for DEP. No in vivo studies have been conducted.

In vitro

Studies conducted by the National Toxicology Program demonstrated an absence of mutagenic responses with DEP (up to 10 mg/plate) in S. typhimurium strains TA100, TA1535, TA98, and TA1537 with or without activation (NTP, 1995*). MEP also showed no mutagenic effect when tested with S. typhimurium strains TA100 and TA98 and E. coli WP2 strains uvr A^+ and uvr A^+, with or without rat liver S9 (Yoshikawa et al., 1983*; Api, 2001). An earlier study reported that DEP was weakly mutagenic for S. typhimurium strains TA100 and TA1535 in the absence of metabolic activation (Kozumbo et al., 1982*).

No chromosomal aberrations were induced by DEP in Chinese hamster ovary cells, with or without rat liver S9, at concentrations up to 324 µg/mL. However, DEP was reported to induce sister chromatid exchanges at concentrations 167-750 µg/mL in the presence of S9 (NTP, 1995*).

The data on DEP are limited, but the available studies provide little evidence of genotoxicity. Overall, on a weight-of-evidence basis, DEP is not considered genotoxic.

6.2.6 Carcinogenicity

The carcinogenicity of DEP has been evaluated in rats and mice by the oral and dermal routes.

Oral route

Rats (15/sex) were fed with DEP in the diet at 0, 0.5, 2.5 or 5% (approximately 0, 250, 1250 or 2500 mg/kg bw/d) for 2 years. Decreased body weight gain without decreased food consumption was observed in both sexes at 5% throughout the study. There were no treatment-related effects on haematology, blood sugar, nitrogen levels, urinalyses or gross or microscopic pathology (RIFM, 1955*; Api, 2001).

Dermal route

F344/N rats (60/sex) were treated dermally with 0, 100, or 300 µL undiluted DEP (approximately 0, 320-520, or 1010-1560 (m-f) mg/kg bw/d), 5 d per week for 2 years. Survival rates of treated animals were similar to control. The mean body weights of 300 µL males were 4%-9% less than those of the controls throughout the study. No evidence of skin neoplasia was found in both sexes except for a treatment-related increase of minimal to mild epidermal acanthosis at the site of application, which was considered an adaptive response to irritation. Female treated rats showed decreased incidence of fibroadenomas of the mammary glands. 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 (NTP, 1995*).

B6C3F1 mice (60/sex) were treated dermally with 0, 7.5, 15, or 30 µL DEP dissolved in acetone to a total of 100 µL (approximately 0, 280, 520-550, or 1020-1140 (m-f) mg/kg bw/d), 5 d per week for 2 years. Survival and mean body weights of the dosed animals were similar to control throughout the study. A statistically significant increase of non-neoplastic proliferative lesions (basophilic foci) in the liver was reported in the 15 µL dosed males, but not females. This effect was not dose-related. Incidences of combined hepatocellular adenomas and carcinomas were increased in both sexes at all doses but they were statistically significantly dose-related only in males. Effects were considered equivocal evidence of carcinogenic activity due to lack of dose-response relationship in females and similar incidence of combined hepatocellular adenomas and carcinomas in males at the highest dose compared to historical controls (NTP, 1995*).

The National Toxicology Program also evaluated the capability of DEP to initiate or promote tumourigenesis using TPA (12-O-tetradecanoylphorbol-13-acetate) and DMBA (7,12-dimethylbenz[a]anthracene) as positive controls of a promoter and initiator, respectively. DEP applied dermally to male Swiss CD-1 mice for one year demonstrated no promotion activity after DMBA initiation, and when applied once as initiator showed no activity when followed by one year of TPA dosing. The promoting activity of TPA following DMBA initiation was confirmed in this study (NTP, 1995*).

Overall, the available data do not indicate a carcinogenic potential for DEP.

6.2.7 Reproductive toxicity

Reproductive toxicity associated with DEP has been examined in multigeneration studies in rats and mice, in specific studies on testicular function, in prenatal and postnatal developmental toxicity studies, and in studies which focus on possible modes of action. They are presented below in chronological order for each type of study.

Multigenerational reproductive toxicity studies

These studies are designed to examine the effects of DEP on the integrity and performance of the male and female reproductive systems, and on the growth and development of the offspring. DEP is administered daily in graduated doses to several groups of males and females during growth, mating, gestation, lactation and through weaning over two or more successive generations.

In a two-generation continuous breeding study, CD-1 mice (20/sex/group) were fed diets containing DEP at 0, 0.25, 1.25, or 2.5% (equivalent to 0, 340, 1770 or 3640 mg/kg bw/d) for a total of 18 weeks (commencing one week before and continuing for three weeks after individual males and females were co-habited for 14 weeks) In this protocol, offspring were removed within 12 h of delivery except for the final litters which remained with the mother until weaning. At maturity (approximately 10 weeks of age), pairs of the same treatment group (0% or 2.5% DEP, 20 pairs/group) were mated and the F2 litters were examined for litter size, survival, sex, and pup weight. There were no adverse effects of DEP on the physiology, fertility or reproductive performance of the F0 generation (hence the

NOAEL in F0 was ≥ 2.5%). In the F1 generation, DEP-exposed mice showed reduced body weight (12-8% m-f), decreased number of live pups per litter (14% when sexes were combined, but not when analysed by males and females separately), decreased sperm concentration (30% but no change in sperm motility or abnormal sperm rate), increased prostate weight in males (32%), increased liver weight (15%) and decreased pituitary weight (17%) in females. Proportion of pups born alive, sex and pup weight were not affected. As only one dose (2.5% DEP) was used in the F1 generation study, a NOAEL could not be established for reproductive effects on F1 male mice while the reproductive NOAEL for F1 female mice was 3640 mg/kg bw/d. The LOAEL for systemic effects in male and female mice and reproductive/developmental toxicity in male mice was 3640 mg/kg bw/d, based on changes in body, liver and prostate weights and reproductive/developmental effects in the F1 animals (Lamb et al., 1987).

In a two-generation reproductive study, SD rats (24/sex/group) were fed diets containing DEP at 0, 600, 3000 or 15 000 ppm (equivalent to 40-56, 197-267, 1016-1375 (m-f) mg/kg bw/d). Dosing began 10 weeks prior to mating, and then through mating, gestation and lactation until weaning (totalling approximately 15 weeks for males and 17 weeks for females). F1 parents were reared for 10 weeks and bred to obtain F2 offspring in a similar manner as for F0 animals. High dose F0 and F1 animals of both sexes had statistically significantly increased absolute and/or relative liver weights (7%-14%). High dose F1 females also had significantly increased absolute and relative kidney weights (7%-9%). For F0 males, there were statistically significantly but not dose-related decreases in absolute epididymis weight (5%) in the high dose group, increases in the number of abnormal and tailless sperms in the mid dose group (73%-85%), and decreases in serum testosterone levels in the mid and high dose groups (80% and 50% respectively). In the F1 parents, there was no effect on reproductive organ weight but there was a dose-related and significant increase in abnormal and tailless sperms in mid and high dose groups (115%-150%). The numbers of implants, pups delivered and pup weights were unaffected at birth from the F0 and F1 parents. In the high dose group, F1 and F2 pup weights were significantly reduced on postnatal day (PND) 21 (12%-19%) in both sexes with a dose-related response seen in F1 females from PND 4-21. 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. Significant delay in pinna detachment was also evident in F1 high dose males (Fujii et al., 2005). Delayed pinna detachment and vaginal opening are assessed as adverse developmental effects occurring concurrently with maternal toxicity (identified by increased liver and kidney weights) given that no evidence of a causal relationship between maternal toxicity and these developmental effects has been previously established (ECETOC, 2004).

For F0 and F1 male rats, the NOAEL for fertility-related parameters was 600 ppm (40 mg/kg bw/d) and the LOAEL was 3000 ppm (197 mg/kg bw/d) based on increased abnormal and tailless sperms in both F0 and F1 generations and decreased testosterone levels in F0 parents at the mid and high doses. The developmental NOAEL was 3000 ppm (197 mg/kg bw/d) and the LOAEL was 15 000 ppm (1016 mg/kg bw/d) based on reduced body weight gain before weaning and delayed pinna detachment at the high dose. For female rats, the NOAEL for fertility-related parameters was the highest dose tested, i.e. 15 000 ppm (1375 mg/kg bw/d). The NOAEL for developmental effects was 3000 ppm (267 mg/kg bw/d) and the LOAEL was 1375 mg/kg bw/d based on reduced body

weight gain before weaning and delayed vaginal opening at the high dose. The NOAEL for systemic toxicity was 3000 ppm (197-267 (m-f) mg/kg bw/d) and the LOAEL was 15 000 ppm (1016-1375 (m-f) mg/kg bw/d) based on increased liver and kidney weights (Fujii et al., 2005).

It was reported that this study complied with the OECD two-generation reproductive toxicity test (Yamasaki et al., 2005 **ND**).

Studies on testes and testicular function

Groups of young male SD rats (12/group) were dosed by oral intubation with 1600 mg/kg bw/d DEP for 4 d. There was no significant effect on food intake, body weight gain, or weight and zinc content of testes, kidney or liver. Histological examination did not reveal any testicular lesions (Foster et al., 1980).

Groups of young male Wistar rats (5 weeks old, 10/group) were fed a diet containing 2% DEP (equivalent to 2000 mg/kg bw/d) for 7 d. The concentration of testosterone in both serum and testes was significantly decreased by approximately 40%. Testis weights and zinc levels in the testes were unaffected. Zinc is thought to be essential for the maintenance of testicular function (Oishi & Hiraga, 1980).

In an investigation of ultrastructural changes of Leydig cells, male Wistar rats were treated with each of four phthalate esters, including DEP, by oral gavage at 2000 mg/kg bw/d for 2 d. DEP induced significant Leydig cell ultrastructural alterations, characterised by smooth endoplasmic reticulum focal dilation and vesiculation, and mitochondrial swelling associated with reduced or loss of matrix granules. Increased interstitial macrophage activity was also seen with the Leydig cells showing substantial cytoplasmic alterations such as swollen mitochondria. The histological effects were not replicated in vitro when Leydig cells were cultured with 1000 µM MEP (Jones et al., 1993).

In an experiment to examine the long-term effects of DEP on the rat testicular antioxidant system, male Wistar rats were fed a diet containing DEP dissolved in corn oil at 0, 10, 25, and 50 ppm (approximately 0, 0.57, 1.43, and 2.85 mg/kg bw/d) for 150 d. Control rats were fed either a normal diet or a normal diet mixed with corn oil. Body weight, testis weight, epididymis weight and the serum testosterone and androstenedione levels were significantly decreased in all treated groups. Testicular lipid peroxidation showed a significant dose-dependent increase and was observed in parallel with a dose-dependent decrease in testicular antioxidant enzymes (such as superoxide dismutase, glutathione peroxidase and reductase). This suggests an impairment of the testicular defence system following chronic exposure to DEP (Pereira et al., 2008b **ND**).

MEP (but not DEP) when given via gavage to male SD rats at 250 mg/kg bw/d for 4 weeks induced decreased sperm counts and sperm motility. No decrease in testis weight was observed with either MEP or DEP (Kwack et al., 2009 **ND**).

Prenatal developmental toxicity studies

These studies are designed to examine the effects of prenatal exposure to DEP on the pregnant test animal and on the developing foetus. DEP is administered to pregnant animals only during gestation.

Intraperitoneal (ip) injection to pregnant SD rats (5/dose) on GD 5, 10 and 15 with 0, 0.51, 1.01 or 1.69 mL/kg bw (equivalent to 500, 1000, 1500 mg/kg bw) DEP

caused a dose-dependent increased incidence (0%, 26.3%, 47.1%, or 81.3% respectively) of skeletal abnormalities such as elongated and fused ribs, curved and elongated upper and lower jaw bones, and incomplete skull bones. The number of resorptions increased at the low dose but not mid and high doses. Pup weight significantly decreased at all doses compared to controls. A NOAEL could not be established due to the developmental effects at the lowest dose tested (Singh et al., 1972). Although incomplete skull bones could represent a developmental defect, the authors also note that the delayed ossification may be secondary to general retardation of growth of pups. This study is limited as it uses a small sample size (5 dams/dose), does not provide a statistical analysis of the results, and uses the injection route rather than oral or dermal routes of administration.

Groups of pregnant mice (17-20/dose) were treated with DEP percutaneously during GD 0-17 at levels of 0, 500, 1600, 5600 mg/kg bw/d. Maternal body and liver weights were not affected. While adrenal and kidney weights increased in high dose animals compared to the controls, reduced thymus and spleen weights (7%) were observed at all doses. There was no effect on number of implantations, live born foetuses, visceral or skeletal malformations. Lower foetal weight and increased incidence of variations, primarily cervical and lumbar ribs were observed at 5600 mg/kg bw/d probably related to maternal toxicity. However, in view of the lack of conclusive evidence that the skeletal defects are consequential to maternal toxicity, these skeletal variations were interpreted as indicative of slight developmental effects (Chernoff & Rogers, 2004; Daston & Seed, 2007; NICNAS, 2008b). The NOAEL was 1600 mg/kg bw/d and LOAEL was 5600 mg/kg bw/d for effects on the offspring. A small reduction in thymus and spleen weights was not considered adverse, and thus the NOAEL for maternal effects was 1600 mg/kg bw/d based on increases in adrenal and kidney weights at the high dose (Tanaka et al., 1987*). Only the study's summaries from SCCNFP (2002) and IPCS (2003) were available for review.

In another developmental toxicity study, 50 pregnant CD-1 mice received DEP at 0 or 4500 mg/kg bw/d by oral gavage during GD 6-13. No effect on body weight of dams, litter size, birth weight, neonatal growth or survival was noted. The NOAEL was 4500 mg/kg bw/d (Hardin et al., 1987).

Groups of pregnant SD outbred CD rats (27-32/dose) were fed a diet containing DEP at levels of 0%, 0.25%, 2.5%, and 5.0% (equivalent to 0, 200, 1900, and 3200 mg/kg bw/d) during GD 6-15. The rats were sacrificed on GD 20. Maternal body weights of mid and high dose groups were significantly lowered on GD 9 and from GD 9-18, respectively. Decreased food and water consumption at mid and high dose were also observed during GD 6-9 but when treatment ended consumption had risen. Although high dose animals appeared to recover from the weight loss when normal feeding resumed, their body weights were statistically significantly lowered than controls. Weights of uterus, liver or kidney were unaffected. There was no effect on resorption incidence, live litter size, mean pup weight or frequency of malformations. The only effect was an increased incidence of supernumerary lumbar ribs in the high dose group (21% vs. 8.8% in the control), which could be related to maternal toxicity. The reduced maternal body weight on only GD 9 of mid dose dams was considered transient, and thus the maternal and developmental NOAEL was 1900 mg/kg bw/d. A developmental LOAEL of 3200 mg/kg bw/d was based on significantly increased frequency of skeletal variations (Field et al., 1993).

In a study of gene expression, pregnant CD rats were treated with corn oil vehicle (10 animals) or 500 mg/kg bw/d DEP (5 animals) by gavage from GD 12-19. Neither significant changes in gene expression in the foetal testes nor effects on anogenital distance (AGD) were observed in treated animals compared to controls (Liu et al., 2005).

SD pregnant rats (5/dose) were treated with DEP by gavage on GD 8-18 at dose levels of 0 (corn oil vehicle), 100, 300, 600, and 900 mg/kg bw/d. There was no effect on foetal testosterone production. Maternal body weight gains were similar to controls over the dose range tested (Howdeshell et al., 2008 **ND**).

Postnatal developmental toxicity studies

The postnatal developmental toxicity studies examine the in utero and early postnatal developmental effects of DEP administered daily to females through gestation, lactation and weaning.

In a one-generation study using a range of phthalates, DEP was administered by gavage to SD dams (5/dose) at 0 or 750 mg/kg bw/d in corn oil from GD 14 to PND 3. There was no overt maternal or neonatal toxicity nor reduced litter sizes, shortened AGD or increased incidence of developmental malformations (Gray et al., 2000).

However, in multigenerational reproductive toxicity studies described previously (Section 6.2.7), postnatal developmental effects such as reduced number of live pups per litter, reduced pup weight at weaning and delayed onset of pinna detachment and vaginal opening have been reported (Lamb et al., 1987; Fujii et al., 2005).

Mode of action studies

DEP did not bind to human oestrogen receptor (hER) in vitro (Nakai et al., 1999; Toda et al., 2004) and showed extremely weak oestrogenic activity in both recombinant and two-hybrid yeast assays (Harris et al., 1997; Nishihara et al., 2000). DEP also did not demonstrate hERα- and hERβ-mediated oestrogenic activities, nor antiandrogenic activity in reporter gene assays using CHO-K1 cells transfected with respective expression vectors (Takeuchi et al., 2005). DEP increased proliferation of human breast cancer MCF-7 cells in one assay (van Meeuwen et al., 2008 **ND**) but not in others (Okubo et al., 2003; Hong et al., 2005). There was a moderate correlation between DEP and 17β-oestradiol (endogenous oestrogen) in gene expression profiles of MCF-7 cells using a DNA microarray assay (EstrArray) (Parveen et al., 2008 **ND**).

MEP induced detachment of germ cells from a Sertoli cell monolayer in vitro, but was 10 000-fold less potent than mono-2-ethylhexyl phthalate (MEHP–a metabolite of DEHP) (Gray & Gangolli, 1986).

In vivo, expression of CaBP-9k mRNA (a gene highly regulated by 17β-oestradiol) was not increased in immature female Sprague-Dawley rats following oral treatment with 600 mg/kg bw/d DEP for 3 d (Hong et al., 2005). DEP showed negative endocrine-mediated effects in rats dosed at 0, 40, 200, and 1000 mg/kg bw/d for 28 d (using a draft protocol for "enhanced OECD Test Guideline 407 – Repeated dose toxicity study") (Shiraishi et al., 2006 **ND**), but was positive in rats

dosed at 197 and 1016 mg/kg bw/d continuously for 15 weeks (i.e. in the OECD two-generation reproductive toxicity study) (Fujii et al., 2005).

In conclusion, the results on the oestrogenic or anti-androgenic potency of DEP are inconsistent and limited, and hence the exact mechanism of DEP effects on the male reproductive system such as reduced testosterone, sperm concentration and sperm quality cannot be determined although it appears to interfere with endocrine function.

The DEP effects on reproductive endpoints in rats and mice are summarised in Table 6.2.

Table 6.2 - Summary of the fertility and developmental effects of DEP

Study design	Species & Route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & Endpoint	Reference
Multigenerational reproductive toxicity studies					
18 weeks (1 week prior to mating till weaning) 20/sex/group	Mice CD-1 Diet	0, 0.25, 1.25, 2.5% (0, 340, 1770, 3640)	*Maternal:* 3640 (F0) NE (F1) *Fertility-related parameters:* 3640 (F0) NE (m, F1) 3640 (f, F1) *Developmental:* 3640 (F1) NE (F2)	*Maternal:* 3640 (F1): ↓body weight (m-f); ↑ liver & ↓ pituitary weights (f) *Fertility-related parameters:* 3640 (m, F1): ↓sperm counts, ↑ prostate weight *Developmental:* 3640 (F2): ↓no. of live pups/litter (combined sexes)	Lamb et al., 1987
15-17 weeks per generation (10 weeks prior to mating till weaning) 24/sex/group	Rats SD Diet	0, 600, 3000, 15 000 ppm (0, 40-56, 197-267, 1016-1375) (m-f)	*Maternal:* 197-267 (m-f, F0, F1) *Fertility-related parameters:* 40 (m, F0, F1) 1375 (f, F0, F1) *Developmental:* 197-267 (m-f, F1, F2)	*Maternal:* 1016-1375 (m-f): ↑ liver weight (F0, F1); ↑ kidney weight (f, F1) *Fertility-related parameters:* 197 (m): ↓serum testosterone (F0), ↑ abnormal and tailless sperms (F0, F1) *Developmental:* 1016-1375 (m-f): ↓pup weight on PND 21 (F1, F2) and PND 4-21 (f, F1), delayed pinna detachment (m, F1) & vaginal opening (f, F1)	Fujii et al., 2005
Studies on testes and testicular function					
4 days 12/group	Rats Male SD Intubation	0, 1600	*Fertility-related parameters:* 1600	NE	Foster et al., 1980

7 days 10/group	Rats Male Wistar Diet	0, 2% (~2000)	NE	*Fertility-related parameters:* 2000: ↓ serum and testis testosterone	Oishi & Hiraga, 1980
2 days 12/group	Rats Male Wistar Gavage	0, 2000	NE	*Fertility-related parameters:* 2000: ultrastructural changes in Leydig cells	Jones et al., 1993
150 days 6/group	Rats Male Wistar Diet	0, 10, 25, 50 ppm (0, 0.57, 1.43, 2.85)	NE	*Fertility-related parameters:* 0.57: ↓ testis weight, testicular antioxidant enzymes, serum testosterone and androstenedione	Pereira et al., 2008b **ND**
28 days 6/group	Rats Male SD Gavage	0, 250 (MEP)	NE	*Fertility-related parameters:* 250: ↓ sperm counts & motility	Kwack et al., 2009 **ND**
Prenatal developmental toxicity studies					
GD 5, 10, 15 5/group	Rats SD ip	0, 0.51, 1.01, 1.69 mL/kg (0, 500, 1000, 1500)	NE	*Developmental:* 500: ↓ pup weight, ↑ skeletal abnormalities	Singh et al., 1972
GD 0-17 17-20/group	Mice Jcl:ICR Dermal	0, 500, 1600, 5600	*Maternal:* 1600 *Developmental:* 1600	*Maternal:* 5600: ↑ adrenal and kidney weights *Developmental:* 5600: ↓ pup weight, ↑ skeletal variations (rudimentary cervical and lumbar ribs)	Tanaka et al., 1987* (reviewed by SCCNFP, 2002; IPCS, 2003)
GD 6-13 50/group	Mice CD-1 Gavage	0, 4500	*Developmental:* 4500	NE	Hardin et al., 1987
GD 6-15 27-32/group	Rats CD Diet	0, 0.25, 2.5, 5% (0, 200, 1900, 3200)	*Maternal:* 200 *Developmental:* 1900	*Maternal:* 1900: ↓ body weight & food consumption *Developmental:* 3200: ↑ skeletal variations (rudimentary lumbar ribs)	Field et al., 1993
GD 12-19 5/group	Rats CD Gavage	0, 500	*Developmental:* 500	NE	Liu et al., 2005
GD 8-18 5/group	Rats SD Gavage	0, 100, 300, 600, 900	*Maternal:* 900 *Developmental:* 900	NE	Howdeshell et al., 2008 **ND**

Postnatal developmental toxicity study (one-generation study)					
GD 14 - PND 3 5/group	Rats SD Gavage	0, 750	*Developmental:* 750	NE	Gray et al., 2000

F0 = parental generation; F1= first filial/offspring generation; F2 = second filial/offspring generation; m-f = male-female; ip = intraperitoneal; no. = number. ↓ = decreased; ↑ = increased; GD = gestational day; NE = not established; PND = postnatal day; SD = Sprague-Dawley
* Quoted as secondary citations from the key documents listed in Section 1.3;
ND = new data since the release of the NICNAS DEP Hazard Assessment in 2008.

6.3 Effects observed in humans

6.3.1 Acute poisoning

The SCCNFP (2002) cited a lethal dose of 0.5 g/kg for oral exposure and an LD50 of 1 g/m^3 for inhalation exposure in humans.

6.3.2 Irritation, sensitisation and phototoxicity

The Research Institute for Fragrance Materials Inc (RIFM) database contains occluded/closed patch test reports of 576 human volunteers exposed to undiluted DEP with no adverse dermal reactions (Api, 2001).

There was also no evidence of irritation or sensitisation following a 3-week application of DEP (2% v/v) to the skin of 203 volunteers under semi-occlusive patches including a challenge patch application following a 2-week rest period (David et al., 2003). Slight erythema (score less than 1) was observed in only one subject at 96 h after challenge. In addition, DEP has not been reported to be a dermal sensitiser in a number of studies in healthy volunteers although sensitisation has been reported in individual case reports of patients with dermatitis from perfume products and plastic articles and with other skin diseases (Api, 2001, IPCS, 2003; Politano & Api, 2008 **ND**).

Phototoxicity studies including human repeated insult patch tests by RIFM (cited by Api, 2001 and SCCNFP, 2002) also demonstrated that DEP (2.5% in ethanol) had no phototoxicity or photoallergenicity potential in humans.

6.3.3 Human studies

Genotoxicity and carcinogenicity

Duty et al. (2003a) reported a significant positive association between human urinary MEP (median of 160 ng/mL) from environmental exposures (unidentified sources) and increased DNA damage in sperm as measured by a neutral comet assay in 141 volunteers. Subjects were male partners of subfertile couples attending an andrology clinic in Boston for semen and urine analyses between January 2000 and October 2001 as part of an infertility investigation. The finding was reconfirmed in another study using a larger sample size (379 volunteers presenting at the same clinic between April 2000 and May 2004) (Hauser et al., 2007 **ND**).

Urinary MEP concentrations were also positively associated with breast cancer incidence among a group of northern Mexican women (233 cancer cases vs. 221

healthy women) (Lopez-Carrillo et al., 2010 **ND**). This association became stronger when estimated for premenopausal women. However, this is the first time that this effect has been reported and requires confirmation and clarification of mode of action.

Fertility-related parameters

When human sperm suspensions were incubated with DEP (33, 330, 3300 µmol/L), sperm motility was dose-dependently decreased with a statistically significant difference from the control (approximately 10%) observed at 3300 µmol/L (Fredricsson et al., 1993; IPCS, 2003).

In a 2-week single-blind study, 26 healthy young men were given one week of daily whole body topical applications of a cream containing 2% w/w of DEP vs. a similar one week application of vehicle cream. There were no statistically significant differences in serum levels of reproductive hormones (follicle stimulating hormone (FSH), luteinising hormone (LH), testosterone, oestradiol and inhibin B) or thyroid hormones (thyroid stimulating hormone, free thyroxine, total triiodothyroxine and total thyroxine) (Janjua et al., 2007 **ND**).

In a series of related studies in humans, Duty et al. (2003b; 2004 **ND**; 2005 **ND**) examined the relationship between urinary levels of phthalate metabolites and semen/sperm qualities and reproductive hormones in men attending an andrology clinic in Boston. Eight phthalate monoesters, including MEP, were measured in a single spot urine sample collected on the same day as the semen sample. There was no dose-response relation between MEP and serum reproductive hormone levels, sperm concentration, motility or morphology. Through a computer-aided sperm analysis (CASA) of sperm motion parameters, however, MEP was found to be associated positively with straight-line velocity and curvilinear velocity and negatively with linearity although they were not statistically significant.

Jonsson et al. (2005) also analysed semen parameters and urinary phthalate monoester levels in 234 Swedish military recruits. The highest quartile for MEP levels was weakly associated with low sperm motility and low LH levels. Sperm concentration and other reproductive hormones such as FSH, sex hormone-binding globulin, testosterone and oestradiol in serum were unaltered.

There was no relationship between urinary MEP and sperm concentration, motility or morphology in a study of 463 male partners of subfertile couples (Hauser et al., 2006 **ND**). In contrast, Wirth et al. (2008 **ND**) found men recruited at a Michigan infertility clinic (45 men) with above median concentrations of urinary MEP had significantly lower sperm concentrations.

Also, a statistically significant negative correlation between semen DEP levels (0.64-3.11 µg/mL) and sperm concentration was reported in a population of 300 healthy men from rural/urban areas of Lucknow, India. Other measured parameters such as sperm motility, abnormal sperm, depolarized mitochondria, reactive oxygen species, lipid peroxidation and DNA fragmentation index showed no correlation with DEP (Pant et al., 2008 **ND**).

Prolonged semen liquefaction time could indicate changes to sperm plasma components that are necessary for sperm movement and maturation. A small study (n = 52) conducted in China reported a statistically significant positive association

between semen liquefaction time and semen concentrations of MEP (mean = 0.47 µg/mL) (Zhang et al., 2006 **ND**).

Overall, inconsistent results are observed in these studies. Some studies report adverse effects associated with DEP exposures (reflected by MEP levels) on particular adult human sperm parameters, whilst other studies fail to find such effects. Inconsistent findings could be due to different study populations with potentially different genetic susceptibilities to the phthalate effects as well as different sampling designs (Wirth et al., 2008 **ND**). In comparative human studies, MEP was found to be at the highest urinary concentration compared to other phthalate metabolites, ranging from 5- to 32-fold higher than MEHP or MBP (Duty et al., 2003a; Wirth et al., 2008 **ND**).

The relationship between adverse reproductive health effects in women and exposure to DEP has been poorly studied and mostly limited to cases of endometriosis. Plasma DEP was not detected in either endometriosis cases or controls in an Indian study (Rozati et al., 2008 **ND**) and creatinine-adjusted levels of urinary MEP showed no significant association with endometriosis (Itoh et al., 2009 **ND**). Also, no associations between urinary MEP and endometriosis or uterine leiomyomata (fibroids) were found in a large cross-sectional study among 1227 women aged 20-54 from three cycles of the US National Health and Nutrition Examination Survey (NHANES 1999-2004) (Weuve et al., 2010 **ND**).

Developmental effects

Associations between maternal urinary phthalate monoester concentrations and genital parameters such as anogenital index (AGI = anogenital distance (AGD)/weight) and testicular descent were investigated in 85 mother-son pairs (Swan et al., 2005). Urinary MEP concentration was statistically significantly and inversely related to AGI. This study has been criticised by McEwen and Renner (2006) from the Cosmetic and Fragrance Associations of America and Europe. They suggested that the AGD is likely to be proportional to infant length rather than weight and that maternal urinary phthalate concentration was not normalised for urine volume. Also, the reliability of AGD measurement in humans has not been verified. One study of 87 neonates found that in males the correlation of AGD with body weight was 0.48 and that body length may be a slightly better predictor for AGD than weight (Salazar-Martinez et al., 2004).

Swan subsequently replicated and extended the finding of a significant association between maternal phthalate exposure and AGI in a cohort of 106 mother-son pairs. In this larger cohort, AGD measurements were taken over two visits for more than half of the offspring and AGDs were corrected not by weight, but using weight percentiles (weight for age) data from US population datasets. Urinary concentrations of MEP were statistically significantly and inversely related to corrected AGD (Swan, 2008 **ND**).

In a follow-up study to Swan et al. (2005), Marsee et al. (2006) estimated the daily phthalate exposure using two different pharmacokinetic models and urinary phthalate monoester concentrations from a Swan study population of 214 pregnant women. The estimated median and 95th percentile of daily exposures to DEP was 6.64 and 112.3 µg/kg bw/d, respectively.

Breast milk samples were analysed for six different phthalate monoesters in a Danish-Finnish cohort study in which serum measurements for gonadotropins (e.g.

FSH and LH), inhibin B, sex hormone-binding globulin and testosterone were also taken from newborn 3-month old boys (62 cryptorchid and 68 healthy boys). No associations between any phthalate monoesters and cryptorchidism (testis maldescent) were found, but MEP showed positive, statistically significant correlations with levels of sex hormone-binding globulin and with LH:free testosterone ratio–a measure of Leydig cell function (Main et al., 2006).

Wolff et al. (2008 **ND**) investigated associations between prenatal phthalate exposures and birth outcomes in a multiethnic cohort of 352 mother-infant pairs. Maternal urinary MEP concentrations (median of 380 µg/L) showed positive, statistically significant associations with gestational age and infant head circumference, but not with birth weight or length. The extent to which these associated parameters were related to maternal anthropometry was not known.

The relationships between prenatal exposure to urinary phthalate metabolites, including MEP (mean of 9.76 µg/g creatinine), and birth outcomes however were not significant in a study of 149 Japanese pregnant women and their newborns by Suzuki et al. (2010 **ND**).

Wolff et al. (2010 **ND**) also examined associations of concurrent exposures from three chemical classes (phenols, phthalates, and phytoestrogens) with female pubertal development. Associations were positive, albeit very weak, for low molecular weight (LMW) phthalates (i.e. sum of urinary metabolites MEP, MBP–monobutyl phthalate, and MIBP–monoisobutyl phthalate) with both breast and pubic hair development (assessed over 2 years) in a multiethnic cohort of 1 151 girls aged 6-8 years living in New York City. However, the authors noted that the peripubertal period was probably not the only critical window of exposure for pubertal development.

Non-reproductive effects

Hoppin et al. (2004 **ND**) found that urinary MEP concentrations (median of 0.61 µg/g creatinine) were statistically significantly inversely associated with particular pulmonary function parameters, e.g. forced vital capacity (FVC) and forced expiratory volume at 1 second (FEV_1) in men but not in women. However, statistical significance was lost when the two men with non-detectable levels of phthalates were excluded or when the analysis was limited to 37 men who never smoked. The study used a small subset of 100 male and 140 female participants in the NHANES III (1988-1994).

In a separate NHANES (1999-2002) cross-sectional study (Stahlhut et al., 2007 **ND**), several urinary phthalate metabolites including MEP showed statistically significant correlations with male abdominal obesity (waist circumference) and insulin resistance (that can be associated with androgenic effects). There were also positive associations between MEP quartile and body mass index (BMI) and waist circumference in adult males (aged 20-59 and 60-80), and adolescent (12-19) and adult females (20-59) in another cross-sectional study of NHANES data covering 4369 participants (Hatch et al., 2008 **ND**). No relationship was found for adolescent males and an inverse correlation was found for older females.

Urinary MEP concentrations (creatinine-uncorrected only) were also found statistically significantly positively correlated with maternal BMI in a cohort of 352 mother-infant pairs (Wolff et al., 2008 **ND**). Similar correlations between creatinine-corrected urinary MEP concentrations and BMI were found also in 75

Taiwanese pregnant women who were recruited to study associations between phthalate exposure and thyroid hormones that are essential for growth, metabolism and mental development in children (Huang et al., 2007 **ND**). MEP did not affect thyroid activity in these pregnant women.

However, creatinine-uncorrected urinary MEP (measured among other 11 phthalate metabolites) were negatively associated with serum levels of thyroid hormones (i.e. total T3, free T3 and total T4), but not with free T4 or insulin-like growth factor I, in 845 Danish children 4-9 years of age, although statistically significant primarily in girls. MEP was also significantly negatively associated with absolute values of height, weight, body surface area, and BMI in this cohort (Boas et al., 2010 **ND**).

Engel et al. (2010 **ND**) reported an inverse association between childhood cognition and behaviour and maternal (creatinine-corrected) urinary levels of LMW phthalate metabolites (i.e. log sum of MMP–monomethyl phthalate, MEP, MBP, and MIBP). Spot urinary samples were obtained between 25-40 weeks of gestation from a multiethnic cohort of 177 women living in New York City who returned for follow-up visits when their children were 4-9 years of age. Assessment of childhood cognition and behaviour were based on single parent-rated reports for a total of 188 children. A different pattern of association was reported in a study by Engel et al. (2009 **ND**), indicating better motor performance among newborn boys with increasing maternal LMW phthalate metabolites. Neurobehaviour showed no correlation with LMW phthalate metabolites in newborn girls.

In another cohort of 137 women who returned for follow-up visits when their children were 7-9 years of age, greater childhood social deficits (i.e. more autistic-like behaviours) were also found associated with increasing log concentrations of similarly collected and creatinine-corrected maternal LMW phthalate metabolites (Miodovnik et al., 2011 **ND**).

Overall, until the mechanism underlying a possible association between DEP or MEP with these non-reproductive effects are better understood, the implications of these findings are unclear.

7. Human Health Hazard Characterisation

This section provides a brief overview of the main features of the toxicity data, identifies the critical endpoints and the no observed adverse effect levels (NOAELs), and discusses the relevance of the effects observed in animal studies to humans.

Given that there is limited information available from human studies on the potential health effects associated with exposure to DEP, the hazard profile is based principally on animal data. In addition, for those toxicological endpoints where the data are incomplete or unavailable, information from structurally similar phthalates was used to examine the potential toxicity. This information was obtained from other NICNAS assessment reports for relevant phthalates. The NICNAS Phthalates Hazard Compendium (NICNAS, 2008b) contains a comparative analysis of toxicity endpoints across 24 *ortho*-phthalates, including DEP. DEP has a straight-chain 2-carbon backbone and is considered to be a low molecular weight phthalate (Phthalate Esters Panel HPV Testing Group, 2001 & 2006; OECD, 2004).

7.1 Toxicokinetics

Orally administered DEP in animals is rapidly and almost completely absorbed from the GI tract. No information is available concerning differences in oral absorption between adult and immature animals or between animals and humans. Based on data for another phthalate DEHP (NICNAS, 2010), oral absorption may be higher in young rats given their relatively higher proportion of intestinal tissue in relation to body weight and higher blood flow through the GI tract. For the purposes of this review, the oral bioavailability of DEP is considered to be 100% for both adults and children.

The available data suggest that dermal absorption of DEP through human skin may be significantly less than that of animal skin. This may reflect species differences, differences in vehicle and/or differences in application (Janjua et al., 2008). On the basis of the data available, a dermal bioavailability of 10% is estimated for DEP in humans.

Quantitative information on inhalation absorption of DEP is not available. A significant positive correlation between personal air and urine measurements for DEP reported in cohorts of pregnant women in New York (Adibi et al., 2003; 2008) indicates inhalation may also be an important route of exposure for DEP. Inhaled DEP is not subject to first pass metabolism in the liver and so a significant proportion of inhaled DEP is likely to be available systemically. On this basis, the inhalational bioavailability of DEP is estimated to be similar to the oral bioavailability of 100%.

Following oral and/or dermal administration, DEP is widely distributed to tissues with no evidence of accumulation. Highest concentrations are observed in kidney, liver, and blood, which rapidly decrease to trace amounts after 24 h. DEP and its

metabolites have been detected in amniotic fluid, as well as maternal, placental and foetal tissues, indicating that the compound can cross the placenta.

DEP is rapidly metabolised and excreted predominantly via the urine in animals. The monoester MEP is the major urinary metabolite with phthalic acid as a minor secondary metabolite. In humans, almost three quarters (71%) of the total amount of MEP excreted in the urine is in the form of free monoester, the rest being MEP glucuronide (Silva et al., 2003).

7.2 Acute toxicity, irritation and sensitisation

In experimental animals, DEP has low acute oral, dermal and inhalation toxicity.

It causes minimal skin and eye irritation in animals and did not induce skin irritation in human volunteers.

DEP has not been reported to be a skin sensitiser in animals or humans although sensitisation was reported in case reports of patients with dermatitis and other skin disease from perfume products and plastic articles.

No data are available for the respiratory irritant or sensitising potential of DEP.

7.3 Repeated dose toxicity

The repeated dose toxicity of DEP has been evaluated in rats and mice after oral and dermal routes of exposure. The liver appears to be the primary target organ for DEP in both short- and medium-term studies. Observed effects were increased organ weight, vacuolation, elevated serum and liver enzyme levels, and proliferation of mitochondria and peroxisomes. Hypertrophic effects have also been reported in other organs such as kidney, stomach and small intestine.

Based on the available data, the 16-week dietary study in rats by Brown et al. (1978) is considered the critical study in identifying a NOAEL for repeated dose effects of DEP. In this study, relative kidney and liver weights were increased significantly in both sexes at a dose of 5% (w/w) in the diet. In females, increases in relative liver weights were dose-dependent and statistically significant at all doses. In male rats, small intestine weights were increased at the 5% dose only, whereas stomach weights were increased at both the 1% and 5% dose levels. There was no abnormal histopathology of the liver, kidney or digestive organs. Neither were there significant effects on haematology, serum enzyme levels or urinary parameters. A conservative NOAEL of 0.2% (corresponding to 150 mg/kg bw/d) was established from this study based on dose-dependent increased relative liver weight in females and increased stomach weight in males at 1% (LOAEL of 750-770 mg/kg bw/d).

In other studies in rats and mice by Pereira et al. (2006) and Mapuskar et al. (2007) respectively, the reported liver effects of DEP were accompanied by evidence of peroxisome proliferation. This mechanism of hepatotoxicity is well known with the phthalate esters and has been discussed extensively in the literature, including the NICNAS Phthalates Hazard Compendium (NICNAS, 2008). In general, phthalate-induced hepatomegaly in rodents, when related to peroxisome proliferative effects, is not considered relevant to humans.

In the Brown et al. (1978) study no histological or biochemical evidence of peroxisome proliferation was found and so the extent to which this may explain the observed liver hypertrophy is unclear. There was also no histological or biochemical evidence to explain the mechanism of hypertrophy in other organs. Overall, although some organ weight changes could be at least partially explained on the basis of inconsistencies in control data, from a mechanistic perspective other organ effects (stomach and small intestine) could not be discounted and therefore are regarded conservatively as a basis for effect levels.

No human studies relating to DEP-induced hypertrophic effects of digestive organs are available.

7.4 Genotoxicity and carcinogenicity

DEP was negative in a majority of in vitro bacterial mutagenic assays as well as a chromosome aberration assay but positive in a sister chromatid exchange assay. No in vivo animal data were available.

In human volunteers, significant positive associations have been reported between urinary levels of MEP and DNA damage in sperm as measured by the neutral comet assay (Duty et al., 2003a; Hauser et al., 2007). However, no significant associations were found between comet assay parameters for sperm damage and any other urinary phthalate metabolites, including MEHP, MBB, MBP and MEP (Duty et al., 2003a; Hauser et al., 2007).

Overall, these data do not support a genotoxic potential for DEP.

With regards to carcinogenicity, 2-year dermal studies by NTP (NTP, 1995*) reported a statistically significant (but not dose-related) increase in basophilic foci in the liver in male mice dosed with 520 mg/kg bw/d. This effect was not reported in female mice. In addition, marginally increased incidences of combined hepatocellular adenomas and carcinomas were noted in both sexes but they were statistically significantly dose-related only in male mice. Due to lack of dose-response relationship in female mice and similar incidences of hepatocellular neoplasms between the high dose male mice and historical controls, these increases were considered equivocal evidence of carcinogenic activity for DEP.

In similar dermal studies in rats (NTP, 1995*), no evidence of increased neoplasia was found other than treatment-related epidermal acanthosis at sites of DEP application, which was considered an adaptive response to irritation. No other lesions or neoplasms were noted in these 2-year studies both in mice and rats. In additional separate studies, DEP also did not demonstrate any initiating or promoting activity.

Overall, the available data do not support a carcinogenic potential for DEP.

7.5 Reproductive toxicity

Following repeated dose and multigenerational exposure (including perinatal exposure) of rodents to DEP, effects on testosterone production, sperm concentration, sperm motility and quality were observed.

7.5.1 Parameters related to fertility

Reduced serum and testicular testosterone levels and altered Leydig cell ultrastructure were reported in Wistar rats at 2000 mg/kg bw/d DEP (Oishi & Hiraga, 1980; Jones et al., 1993), but no effect on testicular zinc levels were reported after 4-d dosing with 1600 mg/kg bw/d DEP (Foster et al., 1980). Serum testosterone and testicular antioxidant enzymes were also reduced in rats fed a diet containing 0.57 mg/kg bw/d DEP for 150 days (Pereira et al., 2008b). In addition, MEP reduced sperm counts and motility in male SD rats after oral gavage of 250 mg/kg bw/d for 4 weeks (Kwack et al., 2009). NOAELs for these studies could not be determined as effects were seen at the lowest doses tested.

In a well conducted two-generation dietary study in rats, there was no effect on testis weight at doses up to 1016 mg/kg bw/d. However, reduced testosterone levels were observed in F0 males from 197 mg/kg bw/d. In addition, there was a slight but statistically significant and dose-related increase in the frequency of abnormal and tailless sperms in the F0 and F1 generations although it did not affect fertility outcomes. Based on this study, a NOAEL of 40 mg/kg bw/d was established for fertility-related parameters based on the reduced testosterone levels and the increased incidence of abnormal sperms at 197 mg/kg bw/d (Fujii et al., 2005).

In mice, although sperm motility and the percentage of abnormal sperms were not affected following DEP exposure (in diets for 18 weeks) the sperm concentration was decreased in treated F1 mice at 2.5% (3640 mg/kg bw/d)–the only dose tested (Lamb et al., 1987).

In human sperm in vitro, DEP elicited a reduction in motility (Fredricsson et al., 1993) and induced DNA damage (Duty et al., 2003a). However, there was no association with urinary MEP levels and sperm concentration or morphology in men attending an andrology clinic (Duty et al. 2003b; Hauser et al., 2006 & 2007). In contrast, in another smaller clinic study, lower sperm concentrations were associated with elevated urinary MEP levels (Wirth et al., 2008), and in a study of 234 Swedish military recruits, Jonsson et al. (2005) found that men in the highest quartile for MEP had fewer motile sperms than men in the lowest MEP quartile.

With regard to potential female fertility effects, levels of DEP or MEP in plasma or urine were not associated with risk of endometriosis or uterine leiomyomata (fibroids) in limited human studies.

In summary, associations have been drawn between exposure to DEP and abnormal sperm parameters in both animals and humans, but there is no evidence that the observed effects lead to decreased fertility in either animals or humans (Lamb et al., 1987; Fujii et al., 2005; Jonsson et al., 2005; Wirth et al., 2008).

Overall, the effects on testosterone and sperm levels and sperm quality observed in several rodent studies are regarded as relevant to a human risk assessment.

7.5.2 Developmental toxicity

Perinatal exposure to DEP at oral doses up to 3200 mg/kg bw/d showed no foetal or neonatal toxicity in a number of rat studies (Hardin et al., 1987; Field et al., 1993; Gray et al., 2000; Howdeshell et al., 2008). DEP also did not alter male rat sexual differentiation and/or AGD (Gray et al., 2000; Liu et al., 2005). Changes in

pup weight or early body weight in rodents after prenatal exposure to DEP have been reported in some but not all studies.

In a well-described two-generation reproductive toxicity dietary study in rats (Fujii et al., 2005), the main developmental effects of DEP were reduced pup weight at weaning, delayed onset of pinna detachment and vaginal opening in the high dose rats (1016-1375 m-f mg/kg bw/d). The developmental NOAEL was determined to be 197 mg/kg bw/d and the LOAEL was 1016 mg/kg bw/d based on decreased pup weight and developmental delay.

After prenatal exposure in rats and mice at higher doses (3200 mg/kg bw/d orally and 5600 mg/kg bw/d dermally, respectively), an increased frequency of skeletal variations such as rudimentary cervical and/or lumbar ribs was reported but no dose response was evident and these effects generally occurred at or above maternally toxic doses (Tanaka et al., 1987; Field et al., 1993). The increase in supernumerary ribs (either cervical or lumbar) is one of the common anomalies seen in developmental toxicity studies in rodents (Chernoff & Rogers, 2004; Daston & Seed, 2007; NICNAS, 2008b). In view of the lack of conclusive evidence to assign the skeletal defects to maternal toxicity, these skeletal variations in rodents were interpreted as indicative of slight developmental effects.

Singh et al. (1972) reported some skeletal malformations (not skeletal variations) such as incomplete skull bones from gestational exposure at a lower dose of 500 mg/kg bw/d administered intraperitoneally in rats, however the effects were considered inconsistent with findings in the above studies that used a larger sample size and oral and dermal routes of administration.

In humans, several studies have explored the relationship between DEP exposures and developmental outcomes. Maternal urinary MEP levels have been reported to be inversely related to offspring AGI (Swan et al. 2005) and AGD corrected by weight percentiles (Swan, 2008) and positively correlated with gestational age and infant head circumference (Wolff et al., 2008). Also, breast milk levels of MEP were reported to be positively correlated with levels of sex hormone-binding globulin and LH: free testosterone ratio (Main et al., 2006).

These human study findings are limited by questions regarding the significance of AGD measurements, the reliability of spot urine/breast milk samples as indicators of DEP exposures and by other confounding factors such as the measured presence of other phthalate metabolites. The current human data provide contradictory evidence of developmental effects from DEP exposure.

Overall, the effects on developmental toxicity such as decreased pup weight, delayed onset of vaginal opening and pinna detachment in rodent studies are regarded as relevant to a human risk assessment.

7.5.3 Mode of action

Historically, health impacts associated with phthalates have been linked most strongly to reproductive effects. The majority of data on the mode of action of phthalates in inducing reproductive effects involve studies of mid molecular weight (so-called ‘transitional’) phthalates such as DEHP (reviewed by Foster, 2005; Ge et al., 2007; Hu et al., 2009). These studies support a mode of action for transitional phthalates in rodents involving effects on steroidogenesis and expression of genes critical for development of the reproductive system. The extent

to which this mode of action for transitional phthalates is reflective of the mode of action for low molecular weight phthalates such as DEP is not certain. Compared to certain transitional phthalates, there is a paucity of information to examine the mode of action of DEP with respect to reproductive effects.

Effects of DEP on the male reproductive system such as reduced testosterone, sperm concentration and sperm quality have been demonstrated in rodents. Changes in Leydig cell ultrastructure from DEP has also been reported in rats. This might suggest that the observed testosterone reductions after administration of DEP may be due to direct effects on Leydig cells (Jones et al., 1993).

In other in vitro studies, DEP was shown not to display affinity for oestrogen or androgen receptors. MEP was shown to induce detachment of germ cells from a Sertoli cell monolayer in vitro although this effect was 10 000-fold less potent than with MEHP–a metabolite of a known active testicular toxin DEHP (Gray & Gangolli, 1986).

In OECD compliant toxicity tests, DEP was positive for endocrine-mediated effects (such as reduced testosterone, abnormal sperm, and delayed physical and sexual post-natal development) in rats exposed to DEP continuously for 15 weeks at 197 and 1016 mg/kg bw/d (Fujii et al., 2005), but negative in rats dosed up to 200 mg/kg bw/d for 28 days (Shiraishi et al., 2006).

Overall, although there are considerable uncertainties with respect to the exact mechanism of DEP effects on fertility-related parameters and development in rodents, the mechanism appears to involve alterations of endocrine function.

7.6 Non-reproductive effects

Recent human studies suggest some statistical correlations between MEP found in urine and possible adverse changes on lung function, increased adiposity and insulin resistance that may be related with low testosterone in adult males. Some effects on childhood cognition and behaviour were also found correlated with maternal urinary metabolites of LMW phthalates, including MEP, MMP, MBP, and MIBP. These findings are preliminary and provide insufficient basis for risk assessment.

7.7 Summary

The critical toxicity endpoints for DEP in animal studies are repeated dose toxicity (dose-dependent increase in liver and stomach weights) and reproductive and developmental toxicity (reduced testosterone, increased abnormal and tailless sperm, decreased pup weight and developmental delays). The available human studies with DEP indicate a need to examine the potential risk associated with reproductive and developmental effects.

Although some studies reported the association between liver toxicity and peroxisome proliferation, there is no histological or biochemical evidence to explain the mechanism of digestive organs enlargement seen in the critical study following repeated DEP dietary exposure. On this basis, these organ effects could not be excluded and therefore are considered relevant to humans for this risk assessment.

The low molecular weight phthalate DEP appears not to be a potent testicular toxin in animal studies. Evaluations of potential DEP toxicity to the developing male rat reproductive system have consistently found no effect on testis weight or testis atrophy at doses up to 1016 mg/kg bw/d. There was also no foetal or neonatal toxicity (e.g. epididymal malformations or absence of the epididymis, increased incidence of cryptorchidism, hypospadias, decreased AGD, delayed preputial separation, and retained areolas/nipples as commonly noted with the transitional phthalates of C4-6 backbone) after perinatal exposure to DEP at oral doses up to 3200 mg/kg bw/d.

However, reduced testosterone production and altered Leydig cell ultrastructure by DEP has been reported. In addition, the increase in frequency of abnormal and tailless sperms in the F0 and F1 generations (although that did not alter reproductive performance) was statistically significant and dose-dependent. Decreased pup weight at weaning and developmental delay (delayed onset of vaginal opening and pinna detachment) were also observed in the high dose rats.

There is also some equivocal evidence for DEP or MEP of impairment of some reproduction markers (sperm concentration, motility, morphology, DNA strand breaks in sperm, male reproductive hormones, testicular function, and the AGD) in the human male, but the results are limited and remained controversial due to limitations of the study design.

Overall, the epidemiological studies available do not provide sufficient evidence for a causal relationship between exposure to DEP and adverse health effects in humans. However, elements of the plausible mode of action for DEP effects on the developing male reproductive system are considered likely to be parallel in rats and humans if the exposure level to DEP is high and within a critical window of development. Therefore, the effects observed in animal studies are regarded as relevant to a human risk assessment.

Table 7.1 lists the critical studies for DEP, the health effects observed and the effect levels selected for risk characterisation.

Table 7.1 - Endpoints selected for risk characterisation of DEP

Toxicity	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Effects at LOAEL	Species & age at treatment	Reference
Repeated dose (organ weights)	150	750	↑ relative weights of liver (f) & stomach (m)	CD rat, adults	Brown et al., 1978
Reproductive (effects on testosterone and sperm)	40	197	↓ serum testosterone (F0), ↑ abnormal and tailless sperms (F0, F1)	SD rat, adults	Fujii et al., 2005
Reproductive (post-natal development effects)	197	1016	↓ pup weight on PND 21 (m-f, F1, F2) and PND 4-21 (f, F1), delayed	SD rat, adults	Fujii et al., 2005

		pinna detachment (m, F1) and vaginal opening (F1)

F0 = parental generation; F1= first filial/offspring generation; F2 = second filial/offspring generation; m-f = male-female; ↓ = decreased; ↑ = increased; PND = postnatal day; SD = Sprague-Dawley

8. Human Health Risk Characterisation

8.1 Methodology

A margin of exposure (MOE) methodology is used frequently in international assessments to characterise risks to human health associated with exposure to chemicals (EC, 2003). The risk characterisation is conducted by comparing quantitative information on exposure to the NOAEL/NOAEC and deriving a margin of exposure as follows:

1. Identification of critical health effect(s)
2. Identification of the most appropriate/reliable NOAEL (if available) for the critical effect(s).
3. Where appropriate, comparison of the estimated or measured human dose or exposure (EHD) to provide an MOE:

   MOE = NOAEL/EHD

4. Characterisation of risk, by evaluating whether the MOE indicates a concern for the human population under consideration.

The MOE provides a measure of the likelihood that a particular adverse health effect will occur under the conditions of exposure. As the MOE increases, the risk of potential adverse effects decreases. In deciding whether the MOE is of sufficient magnitude, expert judgment is required. Such judgments are usually made on a case-by-case basis, and should take into account uncertainties arising in the risk assessment process such as the completeness and quality of the database, the nature and severity of effect(s) and intra/inter species variability.

In this assessment, the MOE methodology was used for characterising the public health risks from DEP exposure through use of:

- toys and child care articles for children, and
- cosmetic products for the general population

8.2 Critical health effects

Key animal studies and toxicological effects for DEP have been described in Section 6 - Health Hazard Assessment. The critical studies and health effects relevant to humans have been evaluated in Section 7 - Health Hazard Characterisation.

The critical health effects of DEP identified for risk characterisation are repeated dose toxicity (dose-dependent increased liver and stomach weights) and reproductive and developmental toxicity (reduced testosterone, increased abnormal and tailless sperm, decreased pup weight and developmental delays) observed in rodents. The NOAELs for risk characterisation are 150 mg/kg bw/d (repeated dose

toxicity), 40 mg/kg bw/d (reproductive toxicity) and 197 mg/kg bw/d (developmental toxicity) (Table 7.1).

8.3 Risk estimates

8.3.1 Risk estimate related to use of toys and child care articles

The two dominant routes of exposure to DEP through the use of plastic toys and child care articles are dermal exposure during normal handling of toys and child care articles and oral exposure during chewing, sucking and biting of these products.

The combined internal dose for children, arising from contact with toys and child care articles is discussed in Section 5.2.5 and summarised in Table 8.1. Two exposure scenarios are considered for children using toys and child care articles, a "typical" and a reasonable "worst-case" scenario. The reasonable worst-case scenario takes into account the maximal mouthing time of 3 h/d identified for children aged 6-12 months. The typical scenario considers the mean daily mouthing time of 0.8 h/d calculated as an average across several studies examining mouthing behaviours in the same age group. These scenarios are based on international literature examining mouthing behaviour in children in different age groups from 0 to 36 months of age. Overall, these studies demonstrate that mouthing times are highest for children aged 6-12 months and they decrease with increasing age. In the absence of Australian information, these mouthing behaviours are assumed applicable to Australian children.

Additional assumptions considered are as follows:

- Maximal and typical migration rate for DEP as part of a mixed phthalate plasticiser from plastic toys into saliva through biting and chewing is similar to that determined for DINP in a study conducted with adult volunteers (Chen, 1998).
- The highest migration rate, which is applied to the worst-case exposure scenario, is 58 μg/cm^2/h. The mean migration rate, which is applied to the typical exposure scenario, is 26 μg/cm^2/h (Chen, 1998).
- Bioavailability of DEP via the oral route is assumed to be 100%.
- Dermal absorption of DEP from PVC matrix is 0.24 μg/cm^2/h.

Table 8.1 - Estimated total internal exposure for children

Route of Exposure	Typical D_{int} (μg/kg bw/d)	Worst-case D_{int} (μg/kg bw/d)
Oral	0.32	2.69
Dermal	0.03	0.12
Combined	0.35	2.81

Estimation of margin of exposure

Risk estimates take into account the likelihood for adverse effects on digestive organs and reproduction/development at future life stages related to long-term exposure through repeated handling and mouthing of toys. Table 8.2 provides the margins of exposure (MOE) estimated from the internal DEP dose in children and the dose at which no adverse effects were observed on the liver, stomach and the reproductive system in experimental animals, i.e. the NOAEL.

Table 8.2 - Calculated MOE in children for critical health effects of DEP from estimated exposure to toys and child care articles

Toxicity endpoints	NOAEL mg/kg bw/d	MOE for typical exposure scenario	MOE for worst-case exposure scenario
Reproductive (effects on testosterone & sperm)	40	114 000	14 000
(post-natal development effects)	197	563 000	70 000
Repeated dose (liver & stomach effects)	150	429 000	53 000

The risk estimates for DEP-induced effects on the liver, stomach and the reproductive system in both scenarios of toy use by children derive MOEs above 10 000 (Table 8.2) and hence indicate extremely low risk of adverse effects on these organs and/or systems.

An MOE of greater than 100 in risk characterisation is usually regarded as an indication of low concern as it encompasses the conservative default uncertainty factors of 10 each for intraspecies and interspecies variability (IPCS, 1994; ECETOC, 2003).

Uncertainties in the risk estimate

Uncertainties in any risk characterisation process arise from inadequate information, assumptions made during the process and variability in experimental conditions. The uncertainties inherent in the characterisation of risk for DEP arise mainly from inadequate data and include:

- absence of DEP-specific data for migration from PVC
- absence of Australian-specific data on DEP content in toys and child care articles
- absence of Australian-specific data on children's mouthing behaviours
- the significance of the observed toxicity in animals, particularly the reproductive effects, to the human population and
- lack of adequate epidemiological studies for determining the health effects of DEP in children following repeated exposure.

Areas of concern

The risk estimates above do not indicate particular areas of concern from exposure of children to DEP via handling/mouthing of toys and child care articles. It should be noted that exposure of children to DEP can also occur from application of personal care products such as baby lotions and creams (Table 5.5). This additional source of exposure to DEP is discussed below. However, the contribution of exposure through handling/mouthing toys and child care articles to the combined exposures is unlikely to represent a concern, given the magnitudes of MOE for toys and child care articles (Table 8.2).

8.3.2 Risk estimate related to use of cosmetics

The main route of exposure to DEP from use of cosmetics in the general population is through dermal contact. Inhalation exposure is also possible from products applied as aerosols. Oral exposure is considered negligible as current information does not indicate use of phthalates in products prone to accidental oral ingestion such as toothpastes, mouthwashes, lipsticks and lip-glosses.

Given the low acute toxicity, low skin and eye irritation and skin sensitising potential for DEP, the risk of adverse acute effects for consumers arising from use of DEP-containing cosmetics is very low.

The potential risks from cosmetic use are related to long-term exposure through repeated use, especially of leave-on products. The internal dose of DEP from daily use of various DEP-containing cosmetic products is estimated to be 285.1 μg/kg bw/d (Section 5.3.6) considering a "worst-case" scenario of daily use of all (leave-on, wash-off and spray applications) cosmetic products, as outlined in the *Guidance for the testing of cosmetic ingredients and their safety evaluation* (SCCNFP, 2003b & SCCP, 2006) and the EU TGD (EC, 2003). Additional assumptions are as follows:

- DEP content in cosmetics is equivalent to that reported in a limited number of cosmetic products in Australia
- Bioavailability of DEP via the dermal route is 10%, and via the inhalation route is 100%.

Estimation of margin of exposure

Table 8.3 - Calculated MOE in the general population for critical health effects of DEP from estimated aggregate exposure to cosmetic products

Toxicity endpoints	NOAEL mg/kg bw/d	MOE for reasonable worst-case exposure scenario
Reproductive (effects on testosterone & sperm)	40	140
Repeated dose (liver & stomach effects)	150	526

The risk estimate for chronic effects on the liver and stomach derives a MOE above 500 indicating low concern in the general population from simultaneous use of multiple cosmetic products containing DEP. The MOE for reproductive effects in the reasonable worst-case scenario is 140 (Table 8.3).

As the DEP concentration in body lotions increases the MOE for adults reduces, as shown in Table 8.4. At 0.5%, the MOE for risk of reproductive effects in the reasonable worst-case scenario reduces from 140 to 118, without any other changes in assumptions.

Table 8.4 - Calculated MOE in the general population for reproductive effects (reduced testosterone and abnormal sperm) from estimated aggregate exposure to cosmetic products

	Varying concentrations of DEP in body lotions			
	#0.25%	0.5%	0.75%	1%
$D_{int,derm}$ (μg/kg bw/d)	252.7+32.4	306.3+32.4	359.8+32.4	413.4+32.4
MOE	140	118	102	90

the upper limit of DEP reported for body lotions in Australia

From Table 5.4, two types of cosmetic products such as body lotion and perfume spray were considered significant contributors to the derived daily internal DEP doses. While perfume spray is unlikely to be used on children, body lotions could be applied repeatedly on large areas of the body of infants or young children. Thus, exposure to DEP from use of body lotions was also estimated specifically for children of three different age groups based on the surface area to bodyweight ratios estimated by SCCP (2006) (Section 5.3.4, Table 5.5). Based on the estimates for use of body lotions containing 0.25% DEP (the maximum level reported for this type of product in Australia), the MOE for reproductive effects of DEP, using the NOAEL of 40 mg/kg bw/d, was also found to be well above 100. At higher DEP concentrations in body lotions, the MOE for children would be lower, as shown in Table 8.5. At 0.5%, MOE for risk of reproductive effects is well above 100. However, at 0.75%, the MOE in newborns is marginally above 100 and is of concern. The MOE at 0.75% for 6-12 months age group gives rise to concern especially if there is co-exposure to DEP and other phthalates acting on the same biological targets from mouthing toys and child care articles.

Table 8.5 - Calculated MOE in children for reproductive effects (reduced testosterone and abnormal sperm) from estimated exposure to varying concentrations of DEP in body lotions

Infant Age	**$D_{int,derm}$ at 0.25% (μg/kg bw/d)**	**MOE**			
		#0.25%	0.5%	0.75%	1%
Newborn	123.2	324	162	108	81
6 months	96.4	414	207	138	104
12 months	85.7	466	233	155	117

the upper limit of DEP reported for body lotions in Australia

Uncertainties in the risk estimate

Uncertainties in the risk characterisation for the general population from cosmetic use result from database limitations. Australian data on the use patterns of consumer products are not available to allow a precise exposure assessment for cosmetics. Given the limited available data, conservative plausible assumptions such as daily use of all cosmetics containing DEP have been used to determine the risk to consumers.

The exposure and MOE estimates assume a reasonable but worst-case scenario where all possible DEP-containing cosmetic products are used daily. However, use patterns of cosmetic products are likely to vary greatly among individuals. For many adult consumers, this assumption will lead to an overestimation of risk. In addition, the MOE estimate does not consider the use pattern of cosmetics by specific subpopulations such as children and teenagers, who may differ significantly in their use of cosmetics products. Use of several products of a specific brand containing DEP may also contribute to increased exposure in subpopulations inclined to brand loyalty.

There is a high degree of uncertainty associated with the exposure estimates in newborn and infant, as it is not known whether DEP has been used in baby lotions or creams. However, it is possible that general moisturisers may also be used in newborns and infants. In addition, information related to use pattern and/or DEP levels in personal care products for babies and children is not available.

The inadequate human data on the health effects of DEP in young and/or adult humans following repeated exposure also represents an additional uncertainty factor in these risk estimates.

Areas of concern

The risk estimates for general systemic toxicity above indicate low risk for both children and the general population from use of cosmetic products containing DEP at the current reported levels and is of low concern. However, for one type of cosmetic products that could be used in infants or young children and applied on large areas of the body, body lotions, there is a concern if the concentration at which DEP is used in these products increases. Under these circumstances, the MOE for reproductive effects from aggregate exposure to cosmetic products in adults could be reduced to close to or below 100 (Table 8.4) and is of concern. This concern is particularly significant in newborn babies up to 6 months of age when the use of body lotion alone with the DEP content above 0.5% could result in unacceptable MOEs (Table 8.5).

As discussed above, use patterns of cosmetic products are likely to vary among individuals and even subpopulations in the general population (e.g. women, men, young adults/teenagers) and the assumptions used in the exposure scenario may lead to overestimation of risk for certain individuals. However, a separate determination of the level of exposure to DEP for the different subpopulations that may be at highest risk in the cosmetic use scenario is difficult. The results of the large biomonitoring studies (Section 5.4) where substantial difference was detected between the average levels of DEP for the population (mean or median) compared to the level measured for the outliers clearly indicate that some members of the population have been exposed to much higher DEP doses than the population average. In particular, a maximum exposure has been calculated for female adults (Wormuth et al., 2006). This indicates that there are specific high exposure scenarios such as through the repeated application of body lotions with high DEP concentrations. Increases in concentrations of DEP in these products above those currently stated to be used in Australia, especially in infants or young children is of concern as these subgroups of the population are considered most sensitive to the reproductive toxicity of phthalates including DEP.

In addition, effects due to cumulative exposures can arise from use of cosmetics containing multiple phthalates acting on the same biological targets, from the effects of other components in a mixed phthalate used in toys and child care articles, and from the combined exposure scenarios or from multiple sources. While cumulative exposures to DEP from multiple sources are addressed under Secondary Notification, the determination of risk from cumulative exposures to multiple phthalates will take into account any risk mitigation measures recommended in each PEC assessment. The cumulative risk estimates will be then considered in determining the need for further risk mitigation measures for each phthalate so that the effect of cumulative exposures does not lead to an unacceptable risk.

Risks from cumulative exposure to DEP and DEHP for the two scenarios considered in this assessment is not likely to be higher than that for DEP alone as risk management measures have been implemented for use of DEHP in toys and cosmetics. The cumulative risks from exposure of infants to DEP in cosmetics (e.g. body lotions) along with exposure to DINP in toys and child care articles will be considered on completion of the DINP PEC assessment.

9. Current Human Health Risk Management

This section discusses current regulatory controls and risk management practices in place in Australia to protect the public from exposure to DEP.

9.1 Current public health risk standards

9.1.1 Toys and child care articles

There are currently no restrictions on the use of DEP in toys and child care articles in Australia. DEP is not included in the Australian/New Zealand Standard AS/NZS ISO 8124 – *Safety of toys.*

In Australia, DEP was identified as being in use or with the potential for use in children's toys and child care articles. One toy company specified that the maximum DEP content in their products designed for children aged 4 years and older is 0.02% and another company importing articles of unknown type reported a DEP content of 0.06%-2%.

9.1.2 Cosmetics

In Australia, the current listing of DEP in Appendix C of the SUSMP excludes it from use in sunscreens or personal insect repellents for human use except in preparations containing 0.5% or less.

Limited Australian information shows that DEP is introduced as a raw material with potential downstream use in the cosmetic and perfume industry. It is also imported as a component of finished cosmetic products and fragrances with typical concentrations of 0.5% and 2.5% respectively.

Labelling for consumer products

There are currently no specific labelling requirements for consumer goods that contain DEP. However, disclosure of the presence of DEP is required on the packaging or on the product itself for cosmetics and toiletries in accordance with the Trade Practices (Consumer Product Information Standard) (Cosmetics) Amendment Regulations 1998 (no. 1) (the mandatory information standard) made under the *Competition and Consumer Act 2010.*

The current Australian/New Zealand Standard AS/NZS ISO 8124 for toy safety, parts of which are mandatory under the Trade Practices Regulations, does not include labelling or testing requirements for toys with regards to DEP content.

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