Australian Government

Department of Health and Ageing

NICNAS

Diethylhexyl Phthalate

Priority Existing Chemical Draft Assessment Report

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Contents

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 the Environment, Water, Heritage and the Arts, 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.

For the purposes of Section 78(1) of the Act, copies of assessment reports for new and existing chemical assessments are freely available from the web (**www.nicnas.gov.au**). Summary Reports are published in the *Commonwealth Chemical Gazette* (http://www.nicnas.gov.au/publications/#gazette).

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

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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

Diethylhexyl phthalate (DEHP) (CAS No 117-81-7) was declared as a Priority Existing Chemical (PEC) for public health risk assessment 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 DEHP as plasticizers in industrial and consumer products
- consumer products being significant sources of repeated and long term exposure of the public to DEHP through migration and leaching from the products
- the potential for adverse health effects, particularly reproductive effects from DEHP exposure, especially in certain subpopulations
- current restrictions overseas for the use of DEHP in certain consumer products.

The purpose and scope of this PEC assessment is to determine the risks to adults and children from DEHP in consumer applications with particular potential for repeated or prolonged exposure, such as adult cosmetics and children's toys and child care articles.

Manufacture and importation

Data collected through calls for information specific for the assessment of DEHP and for other purposes (e.g. compiling the High Volume Industrial Chemicals - HVIC list) suggest that most of the DEHP introduced in Australia (excess of 2500 tonnes in 2004; between 10 000 and 99 000 tonnes in 2006) is for industrial applications. DEHP is imported in finished products or mixtures and as a raw material for local manufacture. Manufacture of DEHP as a raw material was not reported.

The amount of DEHP used for applications with the potential for public exposure, such as toys, childcare articles and cosmetics is likely to be significantly lower. One applicant who imports DEHP as a raw material that may be used in these specific applications indicated importation volumes of approximately 67 tonnes in 2005 and 60 tonnes in 2006.

Uses

Information on worldwide use of DEHP indicates that while it has wide spread use as a plasticiser for PVC for a variety of applications, significant restrictions have been implemented on its use in toys, childcare articles and cosmetics in Europe and USA.

The information collected by NICNAS identified that in Australia DEHP is imported as a component of perfumery and cosmetic products of unidentified origin with typical concentrations of approximately 0.05%. Some businesses indicated phasing out of DEHP in cosmetic applications following the ban of DEHP for use in cosmetics in the European Union (EU). However, given the absence of regulatory measures limiting the use of DEHP in cosmetics in Australia its potential use in these applications cannot be excluded.

The information also suggests that currently the use of DEHP in children's toys and childcare articles in Australia is limited. However, given the absence of regulatory measures restricting DEHP use in these applications, the potential for introduction and use of DEHP in children's toys and childcare articles in Australia cannot be excluded.

DEHP is used ubiquitously in Australia in a range of industrial and consumer applications mainly as a plasticiser (plastic softener) for polyvinyl chloride (PVC) products but also in other polymers for coatings, adhesives and resins. It is one of a closely related group of phthalates, which, in many cases, can be mixed or substituted for each other in individual applications.

Health effects

DEHP is rapidly and almost completely absorbed following oral or inhalation exposure. A bioavailability of 100% is assumed for these routes. In contrast, bioavailability via dermal absorption is not likely to exceed 5%.

DEHP has low acute toxicity via all routes and low skin and eye irritation potential. There is no evidence of skin sensitization for DEHP in animals or humans.

Repeated exposure to DEHP in rodents is associated consistently with adverse effects on the liver (hepatomegaly, peroxisome proliferation and hepatocellular tumours), kidneys (increased weights, mineralisation of renal papilla, tubule cell pigments and chronic progressive nephropathy) and the reproductive system mainly in males (organ toxicity following pre and postnatal exposure resulting in fertility and developmental effects). Mononuclear cell leukaemia (MCL) and Leydig cell tumours were also observed inconsistently in rat studies.

The major molecular mechanism underlying hepatotoxicity of DEHP in rats and mice involves activation of peroxisome proliferator activated receptor alpha (PPARα), a mechanism that is not considered relevant for humans. MCL is not found in other mammalian species and has no comparable type in humans. Consequently, the liver effects and MCL observed following DEHP exposure in rodents are regarded to be species specific and not relevant to humans.

The mechanism underlaying renal toxicity of DEHP is not clear but does not appear to be related to peroxisome proliferation as kidney lesions were found in both PPARα-null and wild-type mice. Therefore, the relevance of this effect to humans cannot be excluded. The LOAEL for kidney toxicity (increases in absolute and relative kidney weights) in a well conducted 104-week rat dietary study was 146.6 mg/kg bw/d. The NOAEL was 28.9 mg/kg bw/d.

Testicular toxicity manifests as decreased testes weights, testicular atrophy, increased bilateral aspermatogenesis, immature or abnormal sperm forms, seminiferous tubular degeneration, Sertoli cell vacuolation or complete loss of spermatogenesis. The LOAEL for testicular effects is established at 37.6 mg/kg bw/d based on increased incidence of Sertoli cell vacuolation in a 13-week rat dietary study. The NOAEL is 3.7 g/kg bw/d.

Multigenerational studies with rodents reveal adverse reproductive effects of DEHP manifesting as decreased fertility and adverse developmental effects on progeny.

A LOAEL for effects on fertility is established at 140 mg/kg bw/d based on decreased number of litters and viable pups in the progeny of adult mice treated with DEHP for 14 weeks starting 7 days premating. The NOAEL is 14 mg/kg bw/d. Fertility of both sexes was affected as demonstrated by a cross-over mating trial at the highest dose of 425 mg/kg bw/d.

Interestingly, while testicular histomorphology was affected at high doses in this study, fertility effects in females were not correlated with any obvious organ toxicity.

Parental and early-postnatal exposure to DEHP in rodents also affects the reproductive development of progeny, particularly males. At high doses, overt structural malformations of the tail, brain, urinary tract, vertebral column and sternum are observed. A LOAEL for developmental toxicity in male progeny is established at 5 mg/kg bw/d, based on increased testes weight in a study of prepuberal rats exposed during gestation and lactation. The NOAEL for this effect is 1.2 mg/kg bw/d. In female progeny in the same study, a LOAEL for developmental toxicity is established at 15 mg/kg bw/d, based on a significant delay in vaginal opening. The NOAEL was 5 mg/kg bw/d.

In a three-generational dietary study in rats, a LOAEL of 14 mg/kg bw/d is established for male developmental toxicity based on decreased testes weight and seminiferous tubule atrophy in F1 and F2 generations. The NOAEL in this study is 4.8 mg/kg bw/d. At higher doses, decreased in utero survival, reduced anogenital distance (AGD), undescended testes, retained nipples/areolae, incomplete preputial separation and disruption of spermatogenesis were also observed in F1 and F2 generations.

Biochemical studies in rodents reveal association of DEHP exposure with alterations in Leydig cell steroidogenesis, serum levels of testosterone and luteinizing hormone (LH), and expression of genes crucial for development of the male reproductive system. A LOAEL of 10 mg/kg bw/d is established based on increased serum LH and testosterone levels in rats exposed to DEHP for 28 days during postnatal day (PND) 21-48. The NOAEL for these biochemical alterations is 1 mg/kg bw/d.

Overall, rodent studies suggest that the type and severity of reproductive effects from DEHP exposures depend on the time and duration of dosing, and also the age at which effects are monitored. Generally, younger animals are more sensitive than older animals.

Lifetime dietary exposures to DEHP were associated also with dose-dependent increases in the incidence of Leydig cell tumours in some rat studies. However, overall, data are insufficient to determine an association between DEHP exposures and testicular neoplasms.

In humans, studies of potential effects of DEHP on fertility and development are limited and generally based on examining correlations between urinary metabolite levels and reproductive parameters. Overall, available studies do not identify significant, consistent associations between DEHP exposures and reproductive parameters either in adults or children.

Consistent observations of reproductive effects of DEHP in rodents together with data on mode of action suggesting effects on steroidogenesis and expression of genes critical for reproductive system development common to both rodents and humans, suggest that the reproductive toxic effects of DEHP seen in rodents are relevant for humans. Overall, studies support a NOAEL for fertility and developmental effects of DEHP in the dose range of 1–10 mg/kg bw/d. These data are therefore considered for the risk assessment of DEHP in humans.

Public exposure and health risk

Biomonitoring data for assessment of DEHP exposure are not available for the Australian general population or specific subpopulations. In general biomonitoring data are not very useful in determining the particular contribution of a specific application of the chemical to the overall exposure of the population. However it may be useful for monitoring relative levels of exposure in different subpopulations (e.g. infants, children or adults) or, if they

have sufficient power, for monitoring general trends in exposure levels from all significant sources of the chemical. They are also useful in determining whether the exposures calculated through modelling are within the observed range, and their magnitude compared with the integrated exposure of the population.

In this assessment, public health risks from modelled DEHP exposure were assessed using a Margin of Exposure (MOE) approach for two exposure scenarios:

a) use of toys and childcare articles by children, and

b) use of cosmetic products by the general population.

For children, two routes of exposure to DEHP were considered - dermal exposure during normal handling of toys and childcare articles and oral exposure during intentional or inadvertent mouthing, sucking and chewing of these products, due to leaching of DEHP from the plastic. The rates of leaching of DEHP are based on overseas in vivo and in vitro studies conducted with PVC containing the similar phthalate DINP. The migration rates from plastic articles determined for DINP are considered applicable to toys and childcare articles containing DEHP.

Overseas mouthing studies indicated that children's mouthing behaviour, and therefore the potential for oral exposure, is maximal, reaching up to 3hr/day, in the period between 6 and 12 months of age. Based on these data, 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/day.

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

Health risks for children were estimated for both renal and reproductive effects potentially associated with repeated combined handling and mouthing of toys containing DEHP. Assessments of MOE comparing the DEHP dose at which no adverse reproductive effects were observed in experimental systems and estimated internal DEHP doses for children, derived a MOE for typical conditions of toy use of 157. The MOE for the worst case toy use was 20. Given that MOEs below 100 indicate a risk for a particular adverse effect, the MOE derived for children in this assessment indicates a concern, especially for those children for whom toy use pattern and total contact with toys may be higher than typical, given the sensitivity of developing reproductive organs during the first few months after birth.

Risk estimates for renal effects for the typical and worst case scenarios of toy use by children derive MOEs of 950 and 120, respectively. These MOEs above 100 indicate a low risk of renal effects in children.

The main route of exposure to DEHP from use of cosmetics 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 for combined cosmetics use was derived based on European use patterns of cosmetics.

Given the low acute toxicity of DEHP, the risk of acute adverse effects for consumers exposed to DEHP through cosmetics is low. However, similar to the risk assessment for children, the potential risks from DEHP from cosmetic use relate to renal and reproductive

effects. Estimation of margins of exposure (MOE) comparing the DEHP dose at which no adverse reproductive effects were observed in experimental systems and estimated internal DEHP doses in individuals using cosmetics containing DEHP, derived a MOE for worst case cosmetics use scenario of 26.6. For renal effects, the MOE for the worst case scenario was 441. The low MOE for reproductive effects indicates a concern for the general population and high concern for the subpopulations most at risk for reproductive developmental effects in their progeny i.e. pregnant and breastfeeding women.

Recommendations

This section provides the recommendations arising from the assessment of DEHP. Recommendations are directed at the appropriate regulatory bodies with responsibilities for regulating chemicals in products and articles. Implicit in these recommendations is that best practice is implemented to minimise public exposure.

Recommendation 1 - to the Australian Competition and Consumer Commission (ACCC)

It is recommended that the Australian Competition and Consumer Commission (ACCC) consider appropriate regulatory measures to limit exposure to DEHP resulting from the use of DEHP in toys and childcare articles where significant mouth contact may occur.

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

- Worst case estimates of the MOE for use of DEHP in children's toys and childcare articles indicate that the risk of reproductive toxicity in children from the use of these products containing DEHP is unacceptable.
- Oral exposure to DEHP through mouthing of toys and childcare articles is the major route of exposure to DEHP
- Reproductive developmental toxicity in children is a serious long term health effect
- Currently there are no restrictions in Australia on the use of DEHP in consumer products including children's toys and childcare articles and there is a potential for introduction and subsequent exposure of children to DEHP via these products.

Recommendation 2 - to the National Drugs and Poisons Schedule Committee[1] (NDPSC)

It is recommended that the National Drugs and Poisons Schedule Committee (NDPSC) consider scheduling the cosmetic use of DEHP in Appendix C of the SUSDP[2] to limit the potential exposure of the public to DEHP from use in cosmetics.

Recommendation 2 is based on the following findings of the PEC assessment:

- Estimates of the margin of exposure (MOE) for use of DEHP in cosmetics indicate that the risk of reproductive toxicity for the general population from the use of cosmetics containing DEHP is unacceptable.
- Reproductive toxicity is a serious long term health effect
- Currently there are no restrictions in Australia on the use of DEHP in cosmetics and there is a potential for introduction and widespread use of cosmetic products containing DEHP.

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[1] The National Drugs and Poisons Schedule Committee (NDPSC) has been responsible for determining the scheduling of drugs and poisons under the Standard for the Uniform Scheduling of Drugs and Poisons. From July 1 2010 the NDPSC has been replaced by two new expert advisory committees, the Advisory Committee on Medicines Scheduling and the Advisory Committee on Chemicals Scheduling. These committees have been established to provide advice and make recommendations to the Secretary of the Department of Health and Ageing (or delegate) on medicines and chemicals scheduling decisions, with the decision maker now being the Secretary of the Department of Healht and Ageing (or delegate).

[2] The Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP) will be renamed the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP), which will be available from 1 September 2010.

Secondary Notification

Under Section 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 DEHP, specific circumstances include the following:

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

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

Acronyms & Abbreviations

2-EH	2-ethylhexanol
AGD	anogenital distance
AICS	Australian Inventory of Chemical Substances
BBP	butylbenzyl phthalate
bw	bodyweight
CAS	Chemical Abstracts Service
CERHR	Center for the Evaluation of Risks to Human Reproduction (US)
CHO	Chinese hamster ovary
d	day
DBP	di-n-butyl phthalate
DCHP	dicyclohexyl phthalate
DEHP	diethylhexyl phthalate
DEP	diethyl phthalate
DIBP	diisobutyl phthalate
DINP	diisononyl phthalate
DIOP	diisooctyl phthalate
DMP	dimethyl phthalate
DNA	Deoxyribonucleic acid
DnNP	di-n-nonyl phthalate
DnOP	di-*n*-octyl phthalate
E2	17β-oestradiol
EC	European Commission
ECB	European Chemicals Bureau
ECHA	European Chemicals Agency
EU	European Union
EU RAR	European Union Risk Assessment Report
FDA	Food and Drug Administration (US)
g	gram
GD	gestation day
GHS	Globally harmonized system of classification and labelling of chemicals

h	hour
HPT	hypothalamic-pituitary-thyroid
HPVC	High production volume chemical
HVICL	High Volume Industrial Chemicals List
insl3	insulin-like 3 peptide
iv	intravenous
kg	kilogram
L	litre
LC50	median lethal concentration
LD50	median lethal dose
LH	luteinising hormone
LOAEL	lowest-observed-adverse-effect level
m^3	cubic metre
MCL	mononuclear cell leukaemia
mg	milligram
mg/cm^3	milligrams per cubic centimetre
mg/kg bw	milligrams per kilogram bodyweight
mg/kg bw/d	mg/kg bodyweight/day
mL	millilitre
µg	microgram
MEHHP	mono (2-ethyl-5-hydroxyhexyl) phthalate
MEHP	monoethylhexyl phthalate
MEOHP	mono-(2-ethyl-5-oxohexyl) phthalate
mPa	milliPascal
MRNA	messenger ribonucleic acid
ND	new data
NDPSC	National Drugs and Poisons Scheduling Committee. Note that the National Drugs and Poisons Schedule Committee (NDPSC) has been responsible for determining the scheduling of drugs and poisons under the Standard for the Uniform Scheduling of Drugs and Poisons. From July 1 2010 the NDPSC has been replaced by two new expert advisory committees, the Advisory Committee on Medicines Scheduling and the Advisory Committee on Chemicals Scheduling. These committees have been established to provide advice and make recommendations to the Secretary of the Department of Health and Ageing (or delegate) on medicines and chemicals scheduling decisions, with the decision maker now being the Secretary of the Department of Healht and Ageing (or delegate).

NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Cooperation and Development
PEC	priority existing chemical
PND	postnatal day
ppm	parts per million
PVC	polyvinyl chloride
SCCP	Scientific Committee on Cosmetic Products (EU)
SD	Standard deviation or Sprague-Dawley (rats), as indicated in the text
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons. Note that the Standard for the Uniform Scheduling of Drugs and Poisons will be renamed the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP), which will be available from 1 September 2010.
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons (see note above)
US	United States
US EPA	United States Environmental Protection Agency
wt	weight

1. Introduction

1.1 Declaration

Diethylhexyl phthalate (DEHP) (CAS No 117-81-7) 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. The basis for the declaration was the actual and potential use of DEHP 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 DEHP;
- determine the use and functions of DEHP in Australia in the specific consumer applications of children's toys, childcare articles and cosmetics;
- determine any adverse health effects associated with exposure to DEHP;
- determine the extent of exposure of children and adults to DEHP from these applications;
- characterise the risks to humans posed by exposure to DEHP 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;
- Childcare 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 DEHP as PECs, importers and manufacturers of DEHP as a raw material for use in children's toys, childcare articles and cosmetics, and importers of cosmetics containing DEHP, were requested to apply for assessment and supply information on the use of DEHP. Unpublished information on health effects of phthalates including DEHP 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 childcare articles for similar information on phthalates, including DEHP, 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, key reviews on DEHP prepared by the European Chemicals Bureau (ECB, 2006), the Center for the Evaluation of Risks to Human Reproduction (CERHR, 2005) and the Agency for Toxic Substances and Disease Registry (ATSDR, 2002) were consulted. The European Chemicals Bureau's final DEHP report was published in 2008 (ECB, 2008). The ECB 2008 report was used to cross check references to the ECB 2006 report in the NICNAS report. Where differences were noted the text was modified and ECB 2008 was also referenced. Where ECB 2006 was consulted as a source, the secondary references have been indicated as described below.

Information from these reviews was supplemented with relevant studies from more recent literature surveys conducted up to July 2008. After this date, new data were identified by regular search alerts on phthalates through PubMed and ScienceDirect database systems. Reports with significant new information were included in this assessment.

In this report, references not marked with an asterisk were reviewed for the purposes of this assessment. References not examined but quoted from the key reviews as secondary citations are also noted in this assessment and marked with an asterisk.

Hazard information containing reports published prior to September 2007 is also in the *Phthalates Hazard Compendium* providing a comparative analysis of key toxicity endpoints for 24 *ortho*-phthalates (NICNAS, 2008).

1.4 Peer review

The report has been subjected to internal peer review by NICNAS during all stages of preparation. Human health hazard sections were also reviewed by an external expert, Dr. Peter Abbott currently at Bioscience Consulting.

1.5 Applicants

Following the declaration of DEHP as a Priority Existing Chemical, five 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

6/574 St Kilda Rd

Melbourne VIC 3004

Department of Environment and Conservation (NSW)

59-61 Goulburn St

Sydney NSW 2000

International Sales and Marketing

260-262 Highett Road, VIC

Highett VIC 3190

Sigma Aldrich Pty Ltd

12 Anella Ave

Castle Hill NSW 2154

Toyo Tyre & Rubber

137-149 Airds Rd

Minto NSW 2566

2. Background

2.1. International perspective

Diethylhexyl phthalate (DEHP) is a member of the group of esters of phthalic acid known as phthalates, used ubiquitously as plasticisers worldwide. In addition, they are also used commonly as solvents.

The US Phthalate Esters Panel High Production Volume (HPV) Testing Group, 2001 and OECD, 2004 derived three categories of phthalates based on use, physicochemical and toxicological properties. Low molecular weight 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, DEHP belongs to this mid-molecular weight phthalate group known as transitional phthalates.

DEHP is used as a plasticiser in a diverse range of industrial and consumer products and applications such as automotive components, building and construction materials, but also in food contact materials and medical devices such as flexible tubing, intravenous bags and catheters. DEHP is also used in soft plastic toys and childcare articles.

In addition, DEHP is used in non-PVC polymers and non-polymer uses such as adhesives and sealants, lacquers and paints, printing inks for paper, plastics and textiles and also in rubber and ceramics for electronics. DEHP is also used in cosmetic, mainly perfumery, products.

The physicochemical properties of phthalates such as DEHP that impart usefulness as plasticisers also permit their migration and leaching from polymer matrices. As a plasticiser, DEHP can be present in high concentration (up to approximately 40%-50% w/w) in polymer materials. The potential for leaching from plastics and the use in consumer products such as cosmetics together with the reproductive toxicity profile for DEHP and some other related phthalates, have led to concerns over the potential for health impacts from exposure to DEHP. Particular concerns exist for consumer uses with potential for exposure of the young from toys and childcare articles or prolonged deliberate exposure though the use of cosmetics for the general population.

Historically, studies of the health effects of certain phthalates have identified reproductive and developmental toxicity as of particular concern. Accordingly, several overseas jurisdictions such as the European Community (EC), USA and Canada have taken regulatory action on a number of phthalates, including DEHP, for particular uses.

In the EU, permanent restrictions on the use of six phthalate plasticisers in toys came into effect on 17 January 2007. The legislation was previously agreed to by the European Union in 2005 (Directive 2005/84/EC) and sets a limit of 0.1 wt % of the plasticised material for DEHP (and similarly for di-n-butyl phthalate (DBP) and butylbenzyl phthalate (BBP)) in toys and childcare articles. In addition, the

Cosmetic Directive bans DEHP (and DBP and BBP) from use as an ingredient in cosmetic products (Article 4b of the Cosmetic Directive 76/768/EEC, introduced in 2004) based on the restrictions for cosmetic use of chemicals with known carcinogenic, mutagenic or reproductive (CMR) toxicity potential (SCCNFP, 2001).

The following additional regulatory information on DEHP was obtained from the European Chemical Substances Information System's Data Sheet (EC, 2009):

- DEHP is included in Annex 1 of EC Directive 67/548/EEC, relating to the classification, packaging and labelling of dangerous substances;
- Under Directive 67/548/EEC, DEHP is classified as a Reproductive Toxicant Category 2 requiring the Risk phrases R60: May impair fertility and R61: May cause harm to the unborn child;
- DEHP has been reported by the EU as a High Production Volume Chemical (HPVC); and
- DEHP is included in a priority list under Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances.
- In January 2009 DEHP together with Dibutyl phthalate (DBP) and Benzyl butyl phthalate (BBP) was proposed for inclusion on the REACH List of Substances of Very High Concern (SVHC) which would be subject to authorization (Annex XIV) by the European Chemicals Agency (ECHA). The proposal was accepted by the member state committee in May 2009 and it is expected that the list will be finalised and adopted by the EC by the end of 2009.

In September 2007, Health Canada completed a public comment consultation on a proposal for legislative action on DEHP under the *Hazardous Products Act* which in June 2009 resulted in a proposal for inclusion of Phthalates Regulations, that prohibit the presence of DEHP at concentrations of greater than 1000 mg/kg (equivalent to 0.1% by mass) in the plasticised material of toys and childcare articles, when tested in accordance with a method that conforms to good laboratory practices (Health Canada, 2007).

As of September 2009, DEHP has been added to the Health Canada List of Prohibited and Restricted Cosmetic Ingredients (The Cosmetic Ingredient Hotlist), to reflect a declaration of 'toxic' under the *Canadian Environmental Protection Act* due to health concerns (Health Canada, 2009).

In July 2008, the US Congress passed a Consumer Safety Bill that will permanently prohibit the sale of children's toys or child care articles that contain more than 0.1% of DEHP, DBP or BBP.

In the USA, use of DEHP in personal care products was prohibited by legislation in the State of California, effective 1 January 2007.

Beyond the recent actions in USA and EU, and the proposed action in Canada, there are no regulatory restrictions on the use of DEHP in consumer applications such as children's toys, childcare articles and cosmetics in Australia, Asia and other non-EU countries. This raises the possibility of import into Australia of DEHP containing cosmetics and children's products manufactured in countries with no restrictions.

2.2 Australian perspective

In 1999, concern over health effects led to the 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, 25 phthalate chemicals, including DEHP, were identified as currently or potentially in industrial use in Australia. DEHP together with eight other phthalates, was also identified to be in actual or potential use in children's toys, childcare articles and/or cosmetics in Australia.

DEHP is currently listed in the Safe Work Australia Hazardous Substances Information System - HSIS (http://hsis.ascc.gov.au/Default.aspx) where it is classified as a Reproductive Toxicant category 2 based on an adopted classification from the European Chemicals Bureau (ECB). DEHP is not listed in the *Standard for Uniform Scheduling of Drugs and Poisons* (SUSDP, 2008).

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

DEHP is listed on the 2002 and 2006 Australian High Volume Industrial Chemicals List (HVICL). The HVICL contains chemicals with an annual introduction volume (importation and manufacture) of 1000 tonnes or more for the periods 2001-2002 and 2006.

2.3 Assessments by international bodies

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

- A Draft European Union Risk Assessment (RAR) report on DEHP (ECB, 2006) and its final verison (ECB, 2008)
- Opinion of the EU Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) on the safety of medical devices containing DEHP plasticized PVC or other plasticizers on neonates and other groups possibly at risk (SCENIHR, 2008)
- Opinion of the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) on the results of a second Risk Assessment of bis(2-ethylhexyl) phthalate (DEHP) - Human health part (CSTEE, 2004)
- Opinion of the EU Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) on medical devices containing DEHP Plasticised PVC; Neonates and other groups possibly at risk from DEHP toxicity. (SCMPMD, 2002)
- Opinion of the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) on Phthalate migration from soft PVC toys and child-care articles (CSTEE, 1998)
- SIDS Initial Assessment Profile (SIAP) of the OECD Screening Information Data Set (SIDS) on Bis(2-ethylhexyl)phthalate within the OECD High Production Volume (HPV) Chemicals Program (OECD, 2005)

- US Agency for Toxic Substances and Disease Registry Toxicological Profile for Di(2-Ethylhexyl) Phthalate (ATSDR, 2002)
- USA Food and Drug Administration (FDA) Safety assessment of di(2-ethylhexyl)phthalate (DEHP) released from medical devices. Center for Devices and Radiological Health (FDA, 2002)
- US Center for the Evaluation of Risks to Human Reproduction, Update on the Reproductive and Developmental Toxicity of Di (2-Ethylhexyl) Phthalate (CERHR, 2005).
- Health Canada Assessment of Exposure and Toxicity of DEHP in Medical Devices (Health Canada, 2002)
- Health Canada Assessment of DEHP in 1994 when it was declared toxic under the *Canadian Environmental Protection Act, 1999* (Government of Canada, Environment Canada, Health Canada, 1994)

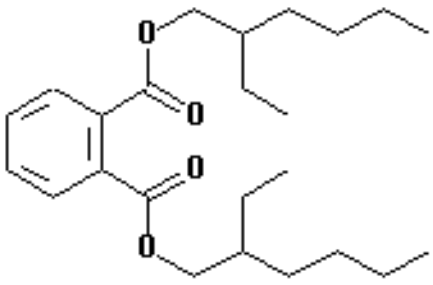
3. Identity, Properties and Analysis

Diethylhexyl phthalate (DEHP) is listed on the Australian Inventory of Chemical Substances (AICS) as 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester.

3.1 Chemical identity

Chemical Name:	Di(2-ethylhexyl)phthalate
CAS Nos.	117-81-7
EINECS No.	204-211-0
Synonyms:	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
	Phthalic acid, bis(2-ethylhexyl) ester
	Bis(2-ethylhexyl) 1,2-benzenedicarboxylate
	Bis(2-ethylhexyl) o-phthalate
	Bis(2-ethylhexyl) phthalate
	Di-2-ethylhexyl-phthalate
	Ethylhexyl phthalate
	Dioctyl phthalate
	Di(isooctyl) phthalate
	Octyl phthalate
Molecular Formula:	$C_{24}H_{38}O_4$
Molecular Weight:	390.56

Structural Formula:



Purity:	≥ 99.7% w/w
Impurities:	other phthalates

3.2 Physical and chemical properties

At room temperature, DEHP is an oily colourless liquid with slight odour.

Table 3.1 summarizes the physico-chemical properties of DEHP (values are adopted from ECB 2008).

Table 3.1 Summary of physico-chemical properties

Property	Value
Melting point	-55˚C to -50˚C
Boiling point	230˚C at 5mmHg 385˚C at 1013 hPa
Density	980 kg/m^3 (20˚C)
Vapour pressure	3.4 x10^{-8} kPa (20˚C)
Water solubility	3 x 10^{-5} g/L (20˚C)
Partition coefficient n-octanol/water (log Kow)	7.50
Henry's constant	4.43 Pa m^3/mol
Flash point	200˚C

DEHP is readily soluble in most organic solvents and miscible with alcohol, ether and most oils (Phthalate Esters Panel HPV Testing Group, 2001).

Conversion factors at 25°C and 1013 hPa: 1 ppm =15.94 mg/m^3

1 mg/m^3 = 0.063 ppm

4. Manufacture, Importation and Use

4.1 Manufacture and Importation

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

The total volume of DEHP imported to Australia for industrial uses in 2004 according to responses to a call for information on phthalates, was in excess of 2500 tonnes. In 2006, according to HVIC data, the importation volume was in the range of 10 000 and 99 000 tonnes. The amount of DEHP used in applications with the potential for public exposure through use in toys, childcare articles and cosmetics is likely to be significantly lower. One applicant who imports DEHP as a raw material that may be used in these applications indicated importation volumes of approximately 67 tonnes in 2005 and 60 tonnes in 2006. No further DEHP specific information is available on the introduction volume as either a raw material or through import of finished products available to the public.

4.2 Uses of DEHP

4.2.1 Use in Australia

According to information collected by NICNAS through calls for information from introducers of DEHP in 2004 and 2006, this chemical is used industrially in Australia as a plasticiser in PVC and in other polymers for coatings, adhesives and resins. Applications include flooring, waterproofing, PVC labels, surface repair resin moulds, epoxy and polyurethane products, rubber components in brake assemblies and hot melt adhesives for automotive assembly and repair. A small amount is also used for cable sheathing/insulation.

DEHP was identified by one company as imported as a raw material that could be used in toys, childcare articles and/or cosmetics. Another company indicated importation of perfumery and cosmetic products containing DEHP. A typical concentration of DEHP in these products was identified as approximately 0.05%. No additional data on product types or origin, or on respective DEHP contents were available. One company noted phasing out of the chemical in cosmetic applications in 2004 following the ban on use in cosmetics in the EU.

DEHP was also identified as being in use or with the potential for use in children's toys.

4.2.2 Uses overseas

The worldwide consumption of plasticisers was estimated at 3.5 million tonnes/year (Cears* & Poppe, 1993). The global production of DEHP was estimated to be between 1 and 4 million tonnes in 1994 (Klöpffer 1994 cited in

Huber* et al., 1996). The production of DEHP in Japan was 348 600 tonnes in 1993 with imports during the same period of 17 400 tonnes (MITI*, 1992). The production volume of DEHP in Western Europe for 1997 was 595 000 tonnes (ECPI*, 1998).

Specific information on production, import and export volumes of DEHP was not available in the USA. Estimates were based on the information available for a group of dioctyl phthalates (DOP) which includes DEHP, diisooctyl phthalate (DIOP), and di-n-octyl phthalate (DnOP) (ATSDR, 2002). Domestic production of DOP in 1998 was 285 million pounds and the projected volume for 1999 was 241 million pounds. Import quantities of DOPs were about 4 million pounds in 1998 and exports about 14–27 million pounds per year from 1994 to 1998 (ATSDR, 2002). The information on the use pattern indicates that approximately 95% of DEHP is used as a plasticiser in PVC for a variety of applications (ATSDR, 2002). A number of companies indicated that they have discontinued the use of DEHP in toys and childcare articles produced domestically in the USA (ATSDR, 2002).

More recent information from the Toxics Use Reduction Institute (TURI) indicates that in 2002 US manufacturers produced approximately 240 million pounds of DEHP. The uses of DEHP fall into two major categories: polymer uses (e.g., consumer products such as footwear, shower curtains and toys, medical devices and commercial/industrial uses) and non-polymer uses (e.g., dielectric fluids, paints, adhesives and inks) (TURI, 2009) Similar to the ASTDR (2002) analysis the TURI data show that non-polymer uses represent less than 5% of the total DEHP used in USA (TURI, 2009).

TURI analysis of data for different industry sectors using DEHP in Massachusetts from 1994 to 2004 showed that total use of DEHP decreased to 3.7 million pounds in 2004 from 9.7 million pounds in 1990 (TURI, 2009)

Significant changes in the use per different sectors were identified. Five sectors (paint and pigments, electrical capacitors, footwear, specialty paper products and rubber products) experienced 100% reduction in their reportable use of DEHP. While the companies in the Rubber Products sector no longer manufacture in Massachusetts, both the Footwear and Electrical Capacitors industry sectors have largely moved away from the use of DEHP towards other chemicals. The Paints and Pigments sector has also reduced all use of DEHP below reporting thresholds (10 000 pounds). The Specialty Paper Products sector eliminated its use of DEHP as their processes no longer require the plasticized polymer coating previously used (TURI, 2009).

Industry sectors that experienced significant reductions (95%-20%) in the use of DEHP from 1990 to 2004 include the Plastics Products, Textiles and Resins sectors (TURI, 2009).

In contrast, increases in the use of DEHP was identified in the Medical Device sector (over 300%) and chemical packaging (less than 50%) (TURI, 2009).

It is not clear how representative the Massachusetts use data are for USA overall. No such analysis was presented for all of the USA.

In the EU, DEHP use represents around half of the total volume of phthalates used as plasticisers (based on ECB, 2006 and ECB 2008). In the ECB reports, DEHP-containing PVC was identified as being used in a variety of consumer products e.g.

toys, automotive components, furniture, shoes and boots, outdoor and rainwear, building material such as flooring, cables, profiles and roofs. DEHP is also used in medical products like blood bags, dialysis equipment. The use profile in the EU is likely to change significantly due to the current EU restriction on use in toys, childcare articles and cosmetics.

The most recent technical report to ECHA on manufacture, import, export, uses and releases of DEHP in EU (ECHA, 2009) found that the manufacture of DEHP has decreased significantly over the last 10 years from 595 000 tonnes/year in the 15 EU countries in 1997 to 340 000 tonnes/year in 2007, a number which now includes data from 12 new member states (ECHA, 2009).

A net export of raw DEHP was estimated for 2007 at approximately 50 000 tonnes tonnes/year which also represents a decrease since 2005 (ECHA, 2009). Export of DEHP in preparations was estimated at approximately 10 000 tonnes/year in 2007 (ECHA, 2009). Thus, the net use in the EU was estimated to be approximately 280 000 tonnes/year in 2007.

The report identified a very large number of diverse articles and preparations, which are used ubiquitously in the EU (ECHA, 2009). Cosmetics and toys were not specifically identified.

4.2.3 Uses of phthalates and possibilities for substitution

Information on the use patterns of phthalates indicate generally that the lower molecular weight phthalates are used as solvents whilst the higher molecular weight phthalates are used as plasticisers (NICNAS, 2008a). The 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. The phthalates exhibit many orders of magnitude increase in the octanol-water partition coefficient (Kow) and an order of magnitude of ten for decrease in vapour pressure as side chain length increases from 1 to 13 carbons. Water solubility is also inversely related to molecular weight and side chain length (NICNAS, 2008a). 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).

Based on these physicochemical properties, a high molecular weight phthalate ester (for example, DIDP) will be quite different to a low molecular weight phthalate ester such as DMP. However the difference in properties between two phthalates of similar molecular weight, such as 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 DEHP with DINP as a plasticiser, is more probable than substitution with a very different phthalate 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 Ingredients Dictionary and Handbook (Gottschalck & McEwen, 2006), 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, with DCHP, DBP, DINP, DIDP, DIBP, BBP and DEHP

(maximum concentration 167 ppm) 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 & 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. However, in the absence of information to characterise these limits on substitutability, it is necessary to assume complete substitutability. Known use concentrations from well characterised phthalates are used in this assessment to undertake an exposure assessment for DEHP for scenarios where use data are lacking.

5. Public Exposure

Scope

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

- Use in children's toys and childcare articles; and
- Use in cosmetics

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

5.1 Methodology for assessing exposure

It is acknowledged that there are uncertainties in deriving exposure estimates. Actual measured data are always preferred in exposure assessments. Modelled data may be used if measured data are not available. 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.

In particular, exposure to DEHP in children's toys and childcare articles was estimated for children via both the oral and dermal routes.

Oral exposure was modelled by:

- Estimation of highest plausable concentrations of DEHP in toys and childcare articles in Australia; and
- Estimation of the available fraction of DEHP based on the results of international experimental studies of childrens' mouthing behaviour and of extractability of phthalate plasticisers under mouthing conditions (Appendix).

Dermal exposure was modelled by:

- Estimation of highest plausable concentrations of DEHP in toys and childcare articles in Australia; and
- Use of default values for exposed surface area and estimates of dermal contact time with toys; and
- Use of the estimate of the migration rate of DEHP from PVC matrix through the skin based on experimental studies (Appendix).

Exposure of the general population to DEHP from cosmetics was estimated for both the dermal and inhalation routes. Insufficient information on the usage levels of DEHP in cosmetic products is available, and therefore the estimate is based on usage levels of an alternative phthalate, in this case diethyl phthalate (DEP) reported in cosmetic products in Australia. These estimates are considered valid for DEHP because of the possibility of substitution of one phthalate for another (see Section 5.3.1), but are subject to the uncertainties described in Section 4.2.3.

Dermal exposure was modelled by:

- Estimation of highest plausible concentrations of DEHP in cosmetic products for dermal application in Australia; and
- Use of default values for usage volumes and frequency for cosmetic products, and
- Use of an estimate for dermal bioavailability of DEHP (see Section 6.1).

Inhalation exposure was modelled by:

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

International biomonitoring data provide estimation of overall exposure of the general or specific subpopulations of the public to DEHP. 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 for these exposure 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 Sections 8.3.1 and 8.3.2.

5.2 Children's toys and childcare articles

5.2.1 Sources of exposure

According to data provided by local suppliers, several phthalates including DEHP 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 is necessary to use overseas data to quantify the presence of phthalates in soft toys and establish possible levels of exposure to children.

It should be noted that the overseas data on levels of phthalates in toys pre-date EU Directive 2005/84/EC prohibiting the use of DEHP in all children's toys at levels above 0.1%, effective January 2007 (Directive 2005/84/EC) and which is likely to

have affected the use of DEHP internationally. The limited Australian information obtained through a voluntary call for information in 2006 indicates that the concentration of DEHP in toys available in Australia does not exceed 0.1%. However, considering that the information collected covers only a small proportion of available toys, and the current absence of restrictions on DEHP content in toys in Australia and many other countries, the available pre-2005 overseas data are used to establish a reasonable worst-case scenario of DEHP exposure to children through the use of toys.

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, diisooctyl phthalate (DIOP) and di-n-nonyl phthalate (DnNP), were also found. DEHP was found in only one (a handbag) of the 35 samples at a concentration of 19.05% w/w.

DINP was also the predominant phthalate in soft PVC toys, evaluated by Health Canada Safety Laboratory (Health Canada, 1998). The content of DINP was found to range from 3.9% to 44% by weight.

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. However, few of the sampled toys contained DEHP as the dominant phthalate plasticiser (8%, with a variety of countries of origin), with the majority of the remainder having <1% DEHP in conjunction with higher levels of DINP. Other phthalates found included diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), diisobutyl phthalate (DIBP), butylbenzyl phthalate (BBP), di-n-octyl phthalate (DnOP), DIOP, DnNP and di-isodecyl phthalate (DIDP). 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) was dominant.

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; Rastogi and Worsoe, 2001). The content of DEHP in the tested toys was found to range from 0.06% to 31%.

In 2006, the Intergovernmental Forum for Chemical Safety (IFCS) published a paper on Toys and Chemical Safety (IFCS, 2006) containing recent information on selected chemicals, including phthalates, in toys available in industrialized countries. This review indicated that DEHP may be present in certain children toys at weight concentrations exceeding 40%.

Health Canada (Canada Gazette, 2009) analysed 100 toys for phthalate content during 2007. Of these, 72 had PVC parts. 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 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 phthalates were typically present in toys at weight concentrations of approximately 5%-50%, with the predominant phthalates being DINP and DEHP. The DEHP concentration in toys varied from 0.08% to 19.05%. Other phthalates such as DEP, DnBP, DIBP, BBP, DnOP, DIOP, DnNP and DIDP were also found in toys.

5.2.2 Routes of exposure

Two routes of exposure to DEHP are considered to be likely during use of plastic toys and childcare articles. Dermal exposure may occur during normal handling and oral exposure through intentional or inadvertent chewing, sucking and biting of these products. Inhalation exposure to DEHP from these products is considered negligible due to the low vapour pressure of DEHP.

When children mouth toys, phthalate plasticisers can migrate into the saliva and subsequently be swallowed as well as absorbed 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 children spend with the product in their 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 are mostly performed on PVC containing DINP and are summarised in the Appendix. The results demonstrate that migration rate of phthalates from articles appears largely determined by the magnitude of the mechanical force applied to an article, and less affected by the physicochemical characteristics or concentration of a particular phthalate. Therefore, although migration data specific for DEHP or other phthalates are not as well characterised, the migration rates determined for DINP under chewing condition can be extrapolated to other phthalates such as DEHP assuming similar product uses and concentrations in products. Therefore, the estimates of typical and worst case migration rates of 26.03 $\mu g/cm^2/h$ and 57.93 $\mu g/cm^2/h$ respectively, based on PVC

articles containing 15%-54% DINP (consistent with the range of usage levels of DEHP as discussed in Section 5.2.1) are considered appropriate and applicable for estimates of oral exposure to DEHP.

5.2.3 Estimates of oral exposure for children from toys and childcare articles

Estimated oral exposures were calculated from the estimated migration rate for DEHP, typical body weight for children and estimated mouthing duration. The main estimate is for a 6 month old infant. This is based on the studies which demonstrate that 6 month old infants are within an age range showing maximum mouthing behaviour, and have the lowest body weight in this age range (Appendix A Table A.1). The following assumptions were also used:

- A child of 6 months weighs 7.5 kg. The mean body weight is based on the 50th percentile weight of 6-month old children (combined sexes) (USEPA, 2006).
- The surface area of a child's open mouth and the typical surface of an article available for mouthing at any one time is approximately 10 cm^2 (LGC, 1998).
- The maximum time the child spends mouthing toys is 3 h/day and a typical mouthing time is around 0.8 h/day (Appendix); and
- Phthalate bioavailability via the oral route is 100% (Section 6.1).

For a 6-month old child, the internal phthalate dose from oral exposure was calculated from the equation shown below:

$$D_{int,oral} = \frac{M \bullet S_{mouth} \bullet t \bullet n \bullet \frac{B_{oral}}{100}}{BW} \qquad \text{Equation 1}$$

Where:

$D_{int,oral}$	=	Internal dose via the oral route, μg/kg bw/day
M	=	Migration rate of DEHP from toys, μg/cm^2/h
S_{mouth}	=	Surface area of a child's open mouth, cm^2
t	=	Mouthing time, h
n	=	Frequency/day
B_{oral}	=	Bioavailability via the oral route, %
BW	=	Child bodyweight, kg

The parameter values and estimations of DEHP internal doses for both the typical and the worst-case scenario are shown in Table 5.1.

Table 5.1: Exposure parameters and estimated daily internal dose from oral exposure to children mouthing toys and childcare articles

	M (μg/cm^2/h)	**BW** (kg)	**S_{mouth}** (cm^2)	**t n*** (h/day)	**$D_{int.oral}$** (μg/kg bw/day)
Typical Exposure Scenario	26.03	7.5	10	0.8	27.8
Worst-case Exposure Scenario	57.93	7.5	10	3	231.7

* the aggregate mouthing time per day (product of mouthing time (t) and frequency (n)) is reported since the individual values of t and n are not available.

The estimate of daily exposure using the worst-case scenario is comparable with the estimate in the EU RAR (2006) of 200 µg/kg bw/day for oral exposure to DEHP from the use of children's toys and child-care articles**.**

5.2.4 Estimates of dermal exposure for children from toys and childcare articles

Dermal exposure can occur from absorption of phthalates via the hands and lips of the child. DEHP is partially dissolved in saliva, which can increase the amount of phthalate available for dermal absorption.

Limited quantitative absorption data are available for DEHP. Deisinger et al. (1998) investigated the skin absorption of DEHP from PVC film in rats. Sheets of PVC film (15cm^2) with [14C]DEHP (total of 40.4% DEHP w/w) were applied to shaved backs of 8 male rats in two separate experiments. The mean dermal absorption of DEHP in rats was determined to be 0.24 µg/cm^2/h (Section 6.1).

In in vitro tests, rat skin was determined to be 4 times more permeable to DEHP than human skin (Barber et al., 1992* and Scott et al., 1987). Equivalent comparative in vivo data are not available. The rate of dermal absorption of 0.24 µg DEHP/cm^2/h, determined in the in vivo test in rats, is used for the exposure estimates. No information on relative permeability of adult and infant skin to DEHP under these conditions was available.

In this scenario, exposure is proportional to the amount of time spent handling the toys, the internal dose is dependent on the time handling the toys and the rate of dermal absorption. Dermal exposure to DEHP was calculated based on the area of skin in contact with the toy, the duration of contact, and the rate of dermal absorption of DEHP through the skin.

The following additional assumptions were also used in calculating dermal exposure:

- A child of 6 months weighs 7.5 kg (USEPA, 2006);
- The maximum time the child spends handling toys is 3 h/day (ECB 2006 and ECB, 2008) and a typical contact time is around 0.8 hour per day (Groot et al., 1998; Juberg et al., 2001. see Appendix); and
- The contact surface area is 100 cm^2 based on exposure to lips and hands (Exponent, Inc., 2007).

For a 6-month old child, the internal dose from dermal exposure was calculated using the equation shown below:

$$D_{int,derm} = \frac{R_{derm} \bullet S_{derm} \bullet t \bullet n}{BW} \qquad \text{Equation 2}$$

Where:

$D_{int,derm}$	=	Internal dose via the dermal route, µg/kg bw/day
R_{derm}	=	Dermal absorption rate of DEHP in skin, µg/cm^2/h
S_{derm}	=	Surface area of a child's lips and hands, cm^2
t	=	Time of contact, h
n	=	Frequency/day
BW	=	Child bodyweight, kg

The exposure factors and calculations of DEHP internal doses from dermal exposure for both the typical exposure scenario and the worst-case scenario are shown in Table 5.2.

Table 5.2: Exposure parameters and calculated daily internal doses from dermal exposure to children mouthing toys and childcare articles

	R_{derm} (μg/cm²/h)	BW (kg)	S_{derm} (cm²)	t · n* (h/day)	$D_{int,derm}$ (μg/kg bw/day)
Typical Exposure Scenario	0.24	7.5	100	0.8	2.6
Worst-case Exposure Scenario	0.24	7.5	100	3	9.6

* the aggregate contact time per day (product of contact time (t) and frequency (n)) is reported since the individual values of t and n are not available.

5.2.5 Combined exposure estimates for children from contact with toys and childcare articles

The combined exposure arising from both dermal and oral contact with children's toys and childcare 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/day)	Worst-case D_{int} (μg/kg bw/day)
Oral	27.8	231.7
Dermal	2.6	9.6
Combined	30.4	241.3

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 and Havery; 2006*; US FDA, 2008).

As discussed in Section 4.2.1, information available to NICNAS indicates that the use of DEHP in cosmetic and personal care products in Australia is limited. In 2006, only one company provided information that DEHP is imported as a component of finished cosmetics and fragrances at a typical concentration of 0.05%. Another company reported that import of personal care products containing DEHP was discontinued after 2004. DMP, DEP, DBP and DnOP are currently used, or have the potential for use, in these applications. DEP is by far the predominant phthalate used in cosmetics with current data showing the presence of DEP in all cosmetic product types.

Worldwide, the phthalates predominantly found in personal care and cosmetics products are diethyl phthalate (DEP) and dibutyl phthalate (DBP) (Hubinger and Havery, 2006*; US FDA, 2008). DEHP has also been found in a very small number of products available in Korea at concentrations up to 18.3 mg/kg in perfumes and up to 25.1 mg/kg in nail polish (Koo et al., 2004). Trace amounts of DEHP (up to 167 mg/kg or 0.0167%) were found in 14 of 36 perfumery products tested in EU (Peters, 2005). As DEHP has been prohibited for use in cosmetics since 2004 in the EU (Article 4b of the Cosmetic Directive), it was suggested that the trace amount of DEHP in these products could be due to leaching during early stages of formulation from plastic manufacturing equipment (containers, pipes, pumps) or from plastic tubing as part of the packaged product (SCCP, 2007). However, only very low levels of DEHP were found in one sample of plastic tubing in one product (Peters, 2005).

In theory, it could be possible to use biomonitoring data to further characterise exposure through use of cosmetic and personal care products. However, DEHP is ubiquitous and it is very difficult to specifically assess the contribution of DEHP exposure through these products, unless there is available information on the phthalate content and use rates of the products. One recent US study (Sathyanarayana et al., 2008) monitored the presence of metabolites of 9 phthalates, including DEHP, in the urine of 163 infants in relation to mother's reported use of 5 types of baby care products within the 24 hours 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 the baby care products. 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 results from the perspective of comparison with the doses estimated in this assessment are discussed further in Section 5.4.

Containers

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

Data are available relating to DEHP leaching from PVC storage bags and tubing for medical use. PVC used in medical devices contains a relatively high proportion (20%-40%) of plasticiser (US FDA, 2002; Health Canada, 2002). The mean levels of DEHP reported in blood or blood products stored in DEHP-containing PVC bags ranged from 0 to 650 μg/mL, depending on storage conditions, duration of storage and blood product stored. The highest content of 650 μg/mL was detected in platelet concentrate supernatant stored in PVC bag for 42 days at 4^{o}C (Labow et al., 1986*). The DEHP content extracted from drug formulations stored in plasticised PVC ranged from 0.2 to 54.64 μg/mL, varying significantly depending on the contact area, temperature and storage conditions. The highest DEHP concentrations were reached when multiple lipophilic drugs were pre-mixed in intravenous fluid bags and agitated for 1 h (Loff et al., 2000; Ito et al., 2005b).

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. DEHP was detected in only one of the four eye drops samples at 112.6 ± 26.9 ng/mL.

From the above studies, it is difficult to determine the likelihood or extent to which DEHP used as a plasticiser in cosmetic product containers may leach into the product. Data are available for DEHP in PVC medical devices, but medical device use requires additional properties (e.g. extreme flexibility, transparency, ability to be sterilized) that may not be directly applicable to material required for cosmetic containers. Therefore, extrapolation of levels of plasticisers from medical device to cosmetic container use and the likely rate of contamination of cosmetic products based on leaching from medical plastics is not possible. Available limited data suggest that contamination of cosmetic products from DEHP leaching from packaging or during manufacture is likely to be at very low levels.

Concentration estimates for use in exposure assessment

Australian information on the concentrations of DEHP in cosmetic products includes only one company providing information that DEHP is imported as a component of finished cosmetics and fragrances at a typical concentration of 0.05%. The typical concentration cannot be used to determine the likely concentration of DEHP across a range of types of cosmetic product, for use in the exposure assessment. The limited information from overseas sources may reflect the effect of the EU prohibition of DEHP in cosmetics. However, in light of the absence of restrictions on use of DEHP in cosmetics in Australia and many other countries, it is not possible to assume that all products marketed in Australia meet the EU standards.

In the absence of sufficient information on the actual concentrations of DEHP in cosmetics in Australia, the assumption of complete substitutability of phthalates, discussed in Section 4.2.3, is used to give a plausible worst case estimate of exposure. The exposure assessment scenario described here is aimed at determining exposure to DEHP based on the assumption that it could replace all DEP currently used in cosmetics. Therefore the content of DEHP in cosmetic products for the purposes of exposure assessment was assumed to be similar to concentrations of DEP currently reported in different cosmetic product types in Australia as this provides a basis to estimate a potential level of exposure to DEHP from cosmetic use. The values obtained by this method are given for a range of product types in Table 5.4.

5.3.2 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 application of creams or liquid products. Inhalation exposure may occur through breathing overspray from products applied as aerosols. Due to the low vapour pressure of DEHP, inhalation exposure to DEHP 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 likely to occur only infrequently and will involve very small amounts of phthalates.

Current information does not indicate use of phthalates in oral cosmetics such as toothpastes, mouthwashes, lipsticks and lip-glosses, products that are mostly likely to be subject to inadvertent ingestion. Therefore, the potential for public exposure via this route is expected to be negligible and, hence, is not characterised further.

5.3.3 Estimates of dermal exposure

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 a few minutes, although some residual product may remain. In contrast, for leave-on products, exposure may last for several hours.

Dermal exposure to DEHP 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 DEHP. 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 (TGD) on Risk Assessment of the European Chemicals Bureau (EC, 2003) and The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP) Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation (SCCNFP, 2003 and 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 DEHP via the dermal route was assessed to be 5% (based on a number of studies discussed in Section 6.1.1). The internal dose arising from dermal exposure to cosmetic and personal care products were estimated using Equation 3 below:

$$D_{int,derm} = \frac{A_{prod} \bullet n \bullet \frac{C}{100} \bullet \frac{B_{derm}}{100} \bullet RF \bullet CF}{BW} \qquad \text{Equation 3}$$

Where:

$D_{int,derm}$	=	Internal dose via the dermal route, µg/kg bw/day
A_{prod}	=	Amount of cosmetic and personal care product applied to skin, mg/event
n	=	Frequency of product application, event/day
C	=	Concentration of DEHP in product, %
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 DEHP 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

Product Type	A_{prod}[a] (mg/event)	n[a] (events/day)	RF[a]	C[b] (%)	$D_{int,derm}$ (μg/kg bw/day)
Leave-on products					
Body antiperspirant roll-on / liquid	500	1	1	0.002	0.0071
Cologne / aftershave / Splash	1200	2	1	0.97	16.63
Nail polish	250	3/7	1	25	19.13
Face cream / Moisturizer	800	1	1	0.42	2.40
Body lotion	7500	2	1	0.25	26.79
Perfume spray	637.5[c]	5	1	2.5	56.92
Rinse-off products					
Soap bars	800	6	0.01	0.15	0.051
Shower products	5000	2	0.01	0.48	0.34
Shampoo / conditioner	12000	1	0.01	0.05	0.043
Shaving products (cream, gel, stick, lather)	2000	1	0.01	0.005	0.00071

[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]Concentration of DEHP, derived from the maximum concentration of phthalate (DEP) reported in these products in Australia.
[c] Assuming 85% of the spray product amount ends up on the skin (RIVM, 2006).

The internal dermal exposures calculated using Equation 3 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 DEHP, 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 amount (95th percentile) of cosmetic products used per day in the Hall et al. (2007) study were: 8.651 g/day for body lotion, 1.806 g/day for liquid deodorant, 1.801 g/day for facial moisturiser and 12.181 g/day for shampoo. These probabilistic estimates are comparable to the amount of product applied as reported in the EU TGD (EC, 2003) and SCCP (2006). Taking into account probabilistic estimates (95th percentiles) for the externally applied doses of 4 types of cosmetic products calculated by Hall et al. and considering the bioavailability and assumed concentration of DEHP in these cosmetic product types from Table 5.4, the derived internal DEHP dose from liquid deodorant is estimated as 0.027 μg/kg bw/day, from face moisturiser as 5.4 μg/kg bw/day, from body lotion as 15.4 μg/kg bw/day and from shampoo as 0.044 μg/kg bw/day. These data for DEHP exposure derived from probabilistic estimates of product exposures are comparable to the data derived from point estimates above.

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 122.31 μg/kg bw/day.

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 in the opinion 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 is 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 DEHP 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 DEHP 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/day)**	**SA/BW ratio**	$D_{int,derm}$ **(μg/kg bw/day)**
Newborn	26.79	2.3	61.7
6 months	26.79	1.8	48.2
12 months	26.79	1.6	42.9

These estimates are subject to a high degree of uncertainty, as it is not known whether DEHP has been used in products of this type.

5.3.4 Estimates of inhalation exposure

Inhalation exposure to DEHP 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 were used in the calculations:

- Adult inhalation rate is 22 m^3/day (enHealth, 2003);
- Phthalate bioavailability via the inhalation route is 100%;
- The average body weight is 70 kg;
- 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 - 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:

$$D_{int,inh} = \frac{A_{prod} \bullet n \bullet \frac{C}{100} \bullet \frac{B_{inh}}{100} \bullet t \bullet IR_{air} \bullet CF_1 \bullet CF_2}{BW \bullet V_{room}} \qquad \text{Equation 4}$$

Where:

$D_{int,inh}$	=	Internal dose via the inhalation route, μg/kg bw/day
A_{prod}	=	Amount of deodorant or perfume spray, mg/event
n	=	Frequency of spray application, event/day
C	=	Concentration of DEHP 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/day
CF_1	=	Conversion factor (time), 1 day/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 (TGD) on Risk Assessment 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 DEHP are extrapolated from the maximum concentration of phthalates (DEP) reported in

these products in Australia. The typical use pattern and calculations of DEHP 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}[a] (mg/event)	n[a] (events/day)	C[b] (%)	$D_{int,inh}$ (µg/kg bw/day)
Perfume spray	750	1-5	2.5	32.4
Antiperspirant / deodorant spray	3000	1-3	0.37	11.5

[a]Typical values for use parameters derived from EU TGD (EC, 2003).
[b]Concentration of DEHP, derived from the maximum concentration of phthalate (DEP) reported in these products in Australia

For a worst-case scenario estimation, the internal dose from inhalation exposure is determined to be 32.4 µg/kg bw/day, as it is considered more likely that only one of these two types of products would be used by an individual on a single day.

5.3.5 Combined exposure from contact with cosmetic products

The systemic exposure to DEHP, internal dose- D_{int}, arising from the combined use of cosmetic products containing DEHP at the assumed maximum levels is summarised in Table 5.6.

Table 5.7: Total estimated exposure to DEHP from cosmetic use

Route of Exposure	D_{int} (µg/kg bw/day)
Dermal	122.3
Inhalation	32.4
Combined	154.7

5.4 Biomonitoring data

Biomonitoring data for a particular chemical or its metabolites represent exposure to the chemical from all sources and pathways. The toxicokinetics of DEHP demonstrates that DEHP 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 limits 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. For this purpose modelling is most suitable. 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 DEHP as determined from the concentrations of the urinary metabolites of DEHP, namely,

MEHP (monoethylhexyl phthalate) and the oxidative metabolites of MEHP, MEOHP and MEHHP. In most studies, MEHP was the only biomarker used. All these studies were conducted prior to the EU restrictions on use of DEHP in toys, introduced in 2007, and some predate the change to the Cosmetics Directive in the EU in 2004. These studies are summarised in Table 5.8.

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 DEHP doses than the population average. For example, for female adults, the maximum calculated exposure from biomonitoring data was 43.2 μg/kg bw/day (Wormuth et al., 2006). This indicates that there are likely to be high exposure scenarios applicable to a subset of the population.

Table 5.8: Summary of biomonitoring data estimating exposure to DEHP (in μg/kg bw/day)

Study	Population Group	Mean	Median	95th Percentile
Calafat & McKee (2006a)	2772 people from the American population 6 to above 20 years old	0.9 – 2.2*	-	7.1 – 16.8*
Marsee et al. (2006)	214 mother-infant pairs observed for MEHP levels	-	1.32	9.32
Wormuth et al. (2006)	Compilation of several German studies for the general population	-	4.2 (children) 2.6 (females) 2.3 (males)	8.4 (children) 12.0 (females) 10.3 (males)
CERHR (2005)	Estimated doses for US population groups	-	-	30 (ages 20+) 25 (ages 12-19) 30 (ages 6-11)

* Reported range of values depending on the metabolite used as biomarker.

The calculated worst case DEHP exposure to cosmetics and personal care products are greater than the biomonitoring data of the DEHP metabolite, due to the worst case assumptions used. However the typical mouthing scenario and 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 relevant for highly exposed individuals. The results seen in the biomonitoring studies are also consistent with the basis of the exposure assessment of DEHP, as they indicate that the general population exposure is much lower than the individual exposure which can arise from these specific high exposure scenarios.

6. Human Health Hazard Assessment

The Existing Chemical Hazard Assessment Report on DEHP was completed and published by NICNAS in June 2008 (NICNAS, 2008b) using as data sources the key international reviews prepared by the (i) the European Chemicals Bureau (ECB, 2006); (ii) the Center for the Evaluation of Risks to Human Reproduction (CERHR, 2005); and (iii) the Agency for Toxic Substances and Disease Registry (ATSDR, 2002). This chapter of the PEC assessment report is largely based on the Existing Chemical Hazard Assessment Report (NICNAS, 2008b), but has been supplemented with an evaluation of new data from comprehensive searches of DEHP related literature up to July 2008 and relevant studies identified up to October 2009 .

In order to identify the more recently evaluated studies, the references in the text to these studies are marked with 'ND', for 'new data' (e.g. 2007 **ND**).

6.1 Kinetics and metabolism

The toxicokinetics of DEHP has been reviewed extensively. Toxicokinetic studies in experimental animals have been performed for the oral, inhalation, dermal and parenteral routes of exposure. The majority of studies are performed in rats via the oral route. A limited number of studies examine the toxicokinetics of DEHP in humans.

6.1.1 Absorption

Absorption via the oral route

The rate and extent of intestinal absorption of DEHP have been estimated mostly indirectly by measuring urinary excretion of 14C-DEHP-derived radioactivity after oral administration. Only a few studies report on DEHP-derived 14C levels in blood. Taken together, reports indicate that DEHP, probably as its first hydrolytic metabolite (see section 6.1.3) monoethylhexyl phthalate (MEHP), is rapidly absorbed from the gastrointestinal (GI) tract following oral administration.

The extent of oral absorption in rats, non-human primates and humans has been estimated as 50% for doses up to 200 mg/kg bw. At higher doses, absorption in non-human primates is dose-limited, in contrast to rodents (Albro et al., 1982*; Rhodes et al., 1983*). However, more recent studies with human volunteers indicate that with low microgram doses similar to those to which humans are likely to be exposed normally, absorption of DEHP after oral exposure may be higher than 50%. In a study by Kotch et al. (2005) in which a male volunteer was orally exposed to microgram doses of radiolabelled DEHP (4.7 µg/kg bw, 28.7 µg /kg bw and 650 µg /kg bw) 67% of the DEHP dose was found eliminated in urine after 24 hours and about 75% was eliminated after two days. No dose dependency in metabolism and excretion was observed. This study suggested that in humans most of the orally administered DEHP is systemically absorbed and excreted in urine.

The bioavailability of orally administered DEHP appears to be higher in young than in old rats (Sjöberg et al., 1985c). The higher proportion of intestinal tissue in relation to body weight (Younoszai & Ranshaw, 1973), and the relatively higher blood flow through the gastro-intestinal tract (Varga and Csaky, 1976) have been suggested as the likely factors causing an increased absorption in young animals. No information is available concerning differences in absorption and bioavailability of orally administered DEHP between human adults or children. Based on human adult data and age differences observed in rats, bioavailability of DEHP via the oral route in both children and adults is estimated to be 100%.

Absorption via the dermal route

The rate of dermal absorption of DEHP appears to be relatively low.

In two studies with rats (Melnick et al., 1987*; Elsisi et al., 1989), 95% and 86% of the applied dose of radio labelled DEHP remained at the site of application after 5 or 7 days, respectively. Dermal absorption, considered as the cumulative amount detected over time in excreta and tissues excluding the dosed skin, was calculated in these two studies to be 9% and 6.5%, respectively.

Another study with rats determined the percutaneous absorption rate for DEHP from PVC plastic film. Sheets of PVC film (15cm^2) with a total of 40.4% w/w 14C-DEHP were applied to shaved backs of 8 male rats in two separate experiments. The mean dermal absorption of DEHP in rats was determined to be 0.24 μg/cm^2/h (Deisinger et al., 1998).

Dermal absorption of DEHP has also been examined in guinea pigs. Ng et al. (1992*) found that 3% (7% when corrected for incomplete excretion) of applied radiolabelled DEHP was absorbed and excreted in the first 24 hours while 21% (53% cumulative and corrected) was excreted after 7 days. Dermal absorption, considered as the cumulative amount detected in excreta and tissues, excluding the dosed skin, was calculated to be 26%.

In another study with female Hartley hairless guinea pigs (Chu et al., 1996*) the bioavailability of dermally administered 14C-DEHP was determined in four different experiments for different application times (24 hours to 14 days) and doses (between 107 and 529 μg/cm^2). Dermal absorption (considered as the cumulative amount detected in excreta and tissues, including the amount remaining in the skin after washing, was calculated to be 9.7%-18.9% from the four different experiments.

No human in vivo dermal absorption studies were available.

Percutaneous absorption of DEHP has also been examined in vitro using rat, guinea pig, and human skin and/or epidermal preparations (Scott et al., 1987, Barber et al., 1992*, Pelling et al., 1998*). Permeability to DEHP was 4-fold higher in rat compared to human skin preparations (Scott et al., 1987; Barber et al., 1992*). In 50% v/v aqueous ethanol vehicle, the in vitro percutaneous steady state absorption rate for DEHP was determined to be 5.6 and 22.4 μg/cm^2/h for human and rat epidermis respectively (Scott et al., 1987).

Considering the in vivo data results demonstrating 9% and 26% dermal absorption of DEHP in rats and guinea pigs, respectively, together with the comparative in vitro studies demonstrating that human skin is significantly less permeable (4-fold)

to DEHP than rat skin, the dermal bioavailability of DEHP in humans is not likely to exceed 5%.

Absorption via the inhalation route

Absorption of DEHP occurs via the respiratory tract in animals and humans but quantitative absorption data for inhalation exposure are not available.

In rats exposed to an aerosol containing radiolabelled DEHP, about 90% of the radioactivity was almost equally distributed in urine and faeces within 72 hours (General Motors, 1982a*b*).

Case studies of patients and workers indicate absorption of DEHP through the lungs. The primary metabolite MEHP and three other metabolites were identified in workers exposed to DEHP by inhalation (Liss et al. 1985*; Dirven et al., 1993a*, b*). There was a large human inter-individual variation in percentages of MEHP detected, ranging from 20% to 100%.

DEHP, but not MEHP, has been detected in the urine of infants undergoing respiratory therapy with plasticised medical devices, suggesting possible leaching of DEHP from the PVC tubes and direct absorption via the inhalation route (Roth et al. 1988*).

Absorption via the parenteral route

Systemic exposure to DEHP via parenteral routes bypass intestinal lipases, so the amounts of DEHP in organs and tissues would be expected to be higher. This is evident in data from studies of exchange transfusions and haemodialysis in humans where initially there is more DEHP than MEHP in the blood (Pollack et al., 1985a*, b*; Sjoberg et al., 1985a*). DEHP levels then decline rapidly with a half-life of 10 hours (Sjoberg et al., 1985a*) and MEHP levels increase until the time-averaged concentrations are roughly equal (Pollack et al., 1985b*).

Similar results have been seen in animal studies. Following arterial injection in rats, DEHP was rapidly cleared from the blood (half-life of 15 hours) (Pollack et al., 1985b*).

6.1.2 Distribution

Studies using radiolabel isotopes show the liver, kidney, testes and blood as the main sites of distribution following orally administered DEHP in rats and monkeys (Rhodes et al., 1986*). In mice intravenously injected with labelled DEHP, radioactivity was rapidly distributed in the gall bladder, intestine, urinary bladder, liver, kidney and brown fat (Lindgren et al., 1982*). There was no evidence of accumulation of DEHP or metabolites in animal tissues.

Limited human data from autopsies have indicated the presence of DEHP in adipose tissues (EPA, 1989). However, it was suggested that this may be an artefact from contamination of biological samples during tissue processing (EPA, 1989).

Orally administered DEHP, most likely via some of its metabolites, can cross the placental barrier in pregnant rats (Singh et al., 1975; Srivastava et al., 1989), and mice (Lindgren et al., 1982*) as shown by the detection of DEHP-derived radioactivity in foetal tissues. In mice treated orally with high doses of DEHP,

DEHP-derived radioactivity was also seen in the uterine fluid (Lindgren et al., 1982). In a most recent study with pregnant Sprague–Dawley rats orally treated with 0, 11, 33, 100, or 300 mg DEHP/kg bw/d on GD 8-18 (Calafat et al., 2006b), concentrations of MEHP in amniotic fluid were strongly correlated with corresponding maternal DEHP dose levels, consistent with transport of DEHP/MEHP across the placenta.

Stroheker et al. (2006) examined the distribution of DEHP-derived radioactivity in foetuses and offspring of Wistar rats following gavage administration of DEHP. DEHP-derived radioactivity passed through the placenta during the gestation period, and the majority of the radioactivity was detected in the liver of the foetuses, but not in the offspring. Relatively lower levels of radioactivity were detected in the gonads of the foetuses and offspring and no sex related differences were observed (Stroheker et al., 2006 **ND**).

6.1.3 Metabolism

Orally administered DEHP is rapidly hydrolysed by lipases to monoethylhexyl phthalate (MEHP) and 2-ethylhexanol (2-EH). Lipases are found in all tissues (intestinal mucosa, liver, kidney, lungs, skin, pancreas and adipose tissues) but especially in the pancreas (Albro, 1986*), correlating with the particularly rapid metabolism of DEHP in the intestine. Whereas unhydrolysed DEHP can be absorbed in the intestine, absorption is increased following hydrolysis to MEHP.

The rate of formation of MEHP from DEHP differs by several hundred-fold among species, with the highest in CD-1 mice, the next highest in Sprague–Dawley rats and the lowest in marmosets in all organs measured (liver, lungs, kidneys, and small intestine) (Ito et al., 2005).

Following hydrolytic cleavage of DEHP resulting in the formation of MEHP and 2-EH, MEHP is further metabolised via oxidative reactions resulting in the formation of numerous metabolites and a small amount of phthalic acid. Some of these oxidized derivatives are then conjugated with glucuronic acid prior to urinary excretion (Albro, 1986*, Astill, 1989*). The phthalic acid remains undegraded. Oxidation of 2-EH primarily yields 2-ethylhexanoic acid and several keto acid derivatives, which are also excreted in the urine.

In workers exposed to DEHP via inhalation, MEHP and three other main metabolites were identified in several studies (Liss et al., 1985*; Dirven et al., 1993a*, b*). Considerable human inter-individual variations in percentages of detected unmetabolised MEHP were reported.

6.1.4 Elimination and excretion

Orally administered DEHP is excreted mainly as metabolites, with a small amount of the parent compound, via urine and faeces. In rats, excretion of low oral doses of DEHP occurs mostly via the urine, whereas in monkeys, excretion occurs mostly via faeces. In rats and mice, elimination is rapid with 85%-90% of the dose of radiolabelled DEHP being excreted via urine and faeces in the first 24 hours. In monkeys, the 24 hour excretion rate was lower at 50%-80% (Astill, 1989*). A recent human study noted that most (75%) of the orally administered DEHP was eliminated as metabolites via urine within 2 days (Koch et al., 2005).

Excretion via the urine also appears to be the major route of elimination of DEHP after inhalation exposure in rats (General Motors, 1982a*, b*). DEHP derived

radioactivity is also detected in the excreta of animals dosed via the dermal route (Elsisi et al., 1989; Melnick et al., 1987*; Ng et al., 1992* ;Chu et al., 1996*).

In mice intravenously injected with radiolabelled DEHP, radioactivity was rapidly distributed to the gall bladder, intestine, urinary bladder, liver, kidney and brown fat (Lindgren et al., 1982*). Prolonged high levels in gall bladder and intestine after 24 hours suggest secretion via the bile is a major elimination route in mice. Following intravenous administration to marmosets, approximately 40% of the dose was excreted in urine and approximately 20% in the faeces (cumulative excretion). Around 28% remained in the lungs 7 days after administration with minimal levels in other tissues. Residual lung activity was postulated by the authors as reflecting insoluble emulsion entrapped within alveolar capillaries (Rhodes et al., 1983*;1986*).

6.2 Effects on laboratory animals and other test systems

6.2.1 Acute toxicity

Acute toxicity of a single dose of DEHP has been evaluated in a number of species after oral, dermal, inhalation and intravenous routes of administration.

DEHP has low acute oral, dermal and inhalation toxicity. Intravenous and intraperitoneal administration of DEHP results in higher acute toxicity than oral or dermal administration.

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

Table 6.1 – Acute animal toxicity studies (adapted from ECB (2006))

Study Type	Species	Results (LD50/LC50)	References
Oral	Rat	30600 mg/kg bw	Shibko & Blumenthal, 1973
	Rat	>20000 mg/kg bw	NTP, 1982*
	Rat	>40000 mg/kg bw	Nuodex, 1981a*
	Mouse	>20000 mg/kg bw	NTP, 1982*
	Mouse	>9860 mg/kg bw	Nuodex, 1981b*
	Guinea pig	26000 mg/kg bw	Krauskop, 1973*
	Rabbit	34000 mg/kg bw	Shaffer et al., 1945*
Dermal	Rabbit	24750 mg/kg bw	ATSDR, 2002
Inhalation (4 h)	Rat	>10.62 mg/L	Hüls, 1981*
Intravenous	Rat	200 mg/kg bw	Schulz et al., 1975*; Rubin & Chang, 1978*
	Mouse	1060 mg/kg bw	Health Canada, 2002
Intraperitoneal	Rat	5675 mg/kg bw	Shaffer et al., 1945*
	Mouse	2800 mg/kg bw	Lawrence et al., 1975*; Woodward et al., 1986*

No significant clinical or necropsy findings were reported for the acute oral, dermal and inhalation exposures to DEHP. Following a single iv administration of DEHP in rats, effects were observed on the lungs including oedema of the alveolar wall together with leukocyte infiltration and haemorrhage (LD50: 200 mg DEHP /kg; Schulz et al., 1975*; Rubin and Chang, 1978*).

6.2.2 Skin, eye and respiratory irritation

Skin irritation

Two skin irritation studies in rabbits were performed according to OECD guideline 404 (BASF, 1986*; Hüls, 1987*) and another irritation study in rabbits was performed according to FDA recommended methods (Hüls, 1981*). In the first study, no erythema or oedema was observed (BASF, 1986*). In the second, very slight erythema was observed in all rabbits that persisted for 48 hours (Hüls, 1987*). In one rabbit, this progressed to a well-defined erythema. All reactions were reversible. The report concluded that DEHP was a slight skin irritant. The third earlier study reported that DEHP caused mild to moderate skin irritation at 24 hours after application in an unknown number of animals. Reactions were reversible (Hüls, 1981*).

These studies show that DEHP causes minimal skin irritation in rabbits.

Eye irritation

Two eye irritation studies in rabbits were performed according to OECD guideline 404 (BASF, 1986*; Hüls, 1987*) and another eye irritation study in rabbits was performed according to FDA recommended methods (Hüls, 1981*).

No reaction was observed in the cornea or iris in any of the studies. All three studies reported mild conjuctival redness after one hour. In the earlier study, mild conjunctival redness was observed in five eyes, one hour after dosing and in three eyes, 24 hours after application (Huls, 1981*). Where reported, all reactions resolved at later timepoints.

These studies show that DEHP causes minimal eye irritation in rabbits

Respiratory irritation

Data are insufficient to determine the respiratory irritation potential of DEHP. No studies specifically addressing this issue have been found. One acute toxicity study included examination of the lungs. Exposure to 10 mg/L DEHP for 4 hours induced dark red foci and patches in the lungs of 19/31 rats (Hüls, 1981*). It is unknown if these effects were reversible.

6.2.3 Sensitisation and allergen potentiation

Skin sensitisation

Two skin sensitisation studies in guinea pigs, one using the Magnusson-Kligman maximization test protocol (Hüls, 1981*) and another, the Buehler test protocol (Exxon, 1994*), reported no positive reactions.

These studies show that DEHP is not a skin sensitising in guinea pigs.

Respiratory sensitisation

An in vitro study provides limited support linking DEHP with respiratory hyperresponsiveness. MEHP (but not DEHP) provoked reversible hyperresponsiveness to methacholine in rat tracheal tissue (Doelman et al., 1990*). The authors concluded that only continuous exposure to DEHP might cause bronchial hyperresponsiveness.

Data are insufficient to determine the respiratory sensitization potential of DEHP.

Potentiation of immune response

DEHP has recently been implicated as an adjuvant that could potentiate the inflammatory effects of two known allergens in mice (Larsen et al. 2001, Takano et al., 2006 **ND**). However, the experimental design of some of these studies with regard to the route of administration of DEHP (subcutaneous and intraperitoneal) has been criticised as inadequate for determination of the overall adjuvant potential of DEHP. In addition, other studies have failed to demonstrate adjuvant activity of topically administered DEHP for the same allergens in mice (Dearman et al. 2008 **ND**). Inhalation exposure to DEHP did not affect immunologic response to airborne adjuvant except in extremely high doses (13 mg/m^3) (Larsen et al. 2007).

Based on the current limited and contradictory data, a potential role for DEHP as an adjuvant for allergic responses is unclear.

6.2.4 Repeat dose toxicity

Oral route

DEHP has been tested for repeated dose effects via the oral route in many studies particularly in the rat but also in the mouse and marmoset monkey. Adverse effects are reported in the liver, testes and kidney.

Liver hypertrophy, increased liver weights and peroxisome proliferation were noted in most of the repeated dose studies. In a 104-week dietary study F-344 rats (70/sex/group) were fed with DEHP at dose levels up to 12 500 ppm (789/938.5 mg/kg bw/d m/f respectively), hepatotoxicity (significant increases in serum albumin, absolute and/or relative liver weights and peroxisome proliferation) was observed in both sexes at 2500 ppm (146.6/181.7 mg/kg bw/d calculated for male/female body weight) and above (Moore, 1996*). NOAEL was 28.9/36.1 mg/kg bw/d for males/females.

Testicular effects such as decreased weights, testicular atrophy, increased bilateral aspermatogenesis, immature or abnormal sperm forms, seminiferous tubular degeneration, Sertoli cell vacuolation or complete loss of spermatogenesis were also evident in most of the repeated dose studies. In a 13-week dietary study with Sprague-Dawley rats (10/sex/dose) fed with DEHP at dose levels up to 5000 ppm (375.2/419.3 mg/kg bw/d, m/f, respectively), a LOAEL of 500 ppm (37.6 mg/kg bw/d) was established based on an increased incidence of Sertoli cell vacuolation. Significantly decreased absolute and relative testicular weights, mild to moderate seminiferous tubule atrophy and Sertoli cell vacuolation were observed at higher doses. A NOAEL of 50 ppm (3.7 mg/kg bw/d) was identified (Poon et al., 1997).

Effects of repeated DEHP treatment on the kidneys included increases in kidney weights, mineralisation of renal papilla, tubule cell pigments and chronic

progressive nephropathy. The majority of these changes were seen in both sexes in different species in studies of varying durations. In chronic studies in rats and mice (Moore, 1996*; Moore, 1997*), there was no indication that these DEHP-related changes in the kidneys were reversible upon cessation of exposure. From the 104-week rat dietary study of Moore (1996*), a NOAEL for kidney effects was established at 500 ppm (28.9/36.1 mg/kg bw/d for m/f respectively). The LOAEL in this study was 146.6 mg/kg bw/d (m) and 181.7 mg/kg bw/d (f), based on increased absolute and relative kidney weights.

McKee et al. (2004) describes a study by Tomonari et al. (2003*), reported as an abstract only, in which marmoset monkeys were given gavage doses of 0, 100, 500 or 2500 mg DEHP /kg bw/d for 65 weeks. At all doses tested, DEHP had no effects on liver and testicular weights. Weights of the other accessory male reproductive organs in the monkey were similarly unaffected by treatment. Microscopic examinations did not reveal any testicular lesions and there were no differences in sperm counts. No differences were observed in testicular 3-beta hydroxysteroid dehydrogenase levels or peroxisome proliferator-activated receptor mRNA expression between control and DEHP-treated animals. The NOAEL from this study was 2500 mg/kg bw/d.

A subsequent report by Tomonari et al. (2006) describes 90-115 day old marmoset monkeys (5-6/sex/group) given DEHP doses of 0, 100, 500 or 2500 mg/kg bw by gavage for 65-weeks. Blood samples were taken throughout the study and analysed for DEHP, MEHP, zinc and testicular enzyme activity. At the end of the study, the liver and primary and secondary sex organs were weighed and examined histologically. Peroxisomal enzyme activities were measured in liver samples. There were no treatment-related changes in body weights, liver weights or male reproductive organ weights. Absolute and relative uterine weights were increased significantly at 500 mg/kg bw/d and absolute and relative ovarian weights were increased significantly at 500 and 2500 mg/kg bw/d. These increases were not dose related. There were no microscopic changes in male gonads, secondary organs, Leydig, Sertoli or spermatogenic cells. No increases in hepatic peroxisomal enzyme activities were noted. The NOAEL was 2500 mg/kg bw/d.

Dermal route

In the only available dermal study, 0.2 mL of 10, 30, 50, or 100% DEHP in olive oil was administered percutaneously to mice for one month (Watari et al., 1978*). Macroscopically, the liver was greatly enlarged. Inflammatory signs were observed in the peritoneum in the two highest dose groups. Hepatic cells showed atrophied nuclei and frequently contained fat droplets. The authors concluded that DEHP is absorbed and accumulates in the liver. This study was considered to have several limitations in ECB, 2006 and 2008.

Inhalation route

Four inhalation studies in experimental animals were identified. In the first study, rats were exposed to up to 1000 mg/m^3 for 6 hours per day, 5 days per week for 4 weeks (BASF, 1990*; Klimisch et al., 1992*). In the highest dose group, there was a significant increase in relative lung weights in male rats accompanied by foam cell (macrophage) proliferation and thickening of the alveolar septa. Liver weights were slightly increased but unusually, this was not accompanied by peroxisome proliferation that had been reported in a similar range-finding study conducted

earlier by BASF (Merkle et al., 1988*). No testicular toxicity was detected histologically.

A poorly described study of male mice (20 animals) exposed to air saturated with vapours of DEHP (purity not specified) for 2 hours per day, 3 days per week for 4-16 weeks, failed to reveal consistent abnormalities which could be attributed to inhalation of DEHP (Lawrence et al., 1975*). No further data were available.

The only long-term inhalation study available was on hamsters (Schmezer et al., 1988*). However, only a single, very low dose (continuous inhalation of 15 $\mu g/m^3$ for 23 months) was used. No signs of any toxicological effects were reported.

Kurahashi et al. (2005) exposed 4-week old male Wistar rats (4/group) to doses of DEHP at 0, 5 or 25 mg/m^3, 6h per day, for 4 or 8 weeks. There were no differences in body or testes weights. Seminal vesicle weight was reduced after 8 weeks but not 4 weeks exposure to both doses. Histological examination showed no significant pathological changes in the testes after 4 or 8 weeks exposure to either dose. The study did not show a dose-response effect.

Using peroxisome proliferator-activated nuclear receptor alpha (PPARα)-null mice, Lapinskas et al. (2005) recently showed that expression of PPARα is necessary for DEHP- and dibutyl phthalate (DBP)-induced liver effects (hepatomegaly and induction of fatty acid metabolising enzymes).

Parenteral route

Several published reports were found on the effects of DEHP administered intravenously (IV) to animals (Jacobson et al., 1977*; Sjoberg et al., 1985b*; Greener et al., 1987*; Baxter Healthcare Corporation, 2000*; Cammack et al., 2003).

Jacobson et al. (1977*) studied hepatic effects in 6-month-old rhesus monkeys receiving transfusions of plasma from DEHP-plasticised polyvinyl chloride (PVC) bags over a 6-month or 1 year period. The average total exposures to DEHP for the groups of monkeys transfused weekly for one year were: Group 1 (plasma stored at 20°C): 3 monkeys at a mean dose of 27 mg/kg bw; Group 2 (plasma stored at 4°C): 2 monkeys at a mean dose of 8 mg/kg bw; Group 3 (transfused biweekly for 6 months, platelet poor plasma stored at 22°C): 2 monkeys at a mean dose of 32 mg/kg bw; Group 4 (untransfused control group): 3 monkeys and Group 5 (platelet-rich plasma stored for 48 hours at 22 °C in polyethylene blood bags): 2 monkeys. Three of the seven monkeys transfused from DEHP-plasticised PVC bags showed some impairment of hepatic perfusion and four out of seven monkeys demonstrated abnormal sulfobromo-phthalein clearance indicative of subclinical liver disease. Six out of the seven had abnormal liver histology (aggregates of inflammatory cells, hepatocyte degeneration, and multi- and bi-nucleated giant cells) upon completion of transfusion period that persisted in three of the five surviving monkeys throughout the follow-up period of 26 months. None of the five control animals had abnormal liver histology. The results of this study are confounded by the small sample size, inconsistent responses in the two groups that received the largest (and similar) doses, use of pooled plasma to re-transfuse into the monkeys and appearance of a tuberculosis outbreak in the monkey colony that might have contributed to the hepatic effects.

Sprague Dawley rats (5-6/group) were cannulated and 3 hour infusions of 0, 5, 50, or 500 mg/kg DEHP were performed every other day for a total of six infusions over 12 days (Sjoberg et al., 1985b*). This was equivalent to time-weighted average doses of 2.5, 25, and 250 mg/kg bw/d. The DEHP was emulsified with egg yolk phosphatides and administered in a glycerol solution. Animals were sacrificed 2-3 hours after the last infusion. The results showed a dose-related decrease in body weight gain, an increase in relative liver weight at the middle and highest doses but no change in clinical chemistry parameters. Liver and kidney histology appeared unchanged except for an increase in hepatic peroxisomes. There was no change in the relative weight of the reproductive organs but transmission electron microscopic examination revealed slight enlargements of the smooth endoplasmic reticulum in Sertoli cells at the highest dose in three of five rats. The NOAEL was 25 mg/kg bw/d, with hepatic changes at 250 mg/kg bw/d.

Rhodes et al. (1986*) reported an intraperitoneal marmoset study. Five marmosets were dosed with 1 g/kg bw/d of DEHP in corn oil for 14 days. There was no indication of the length of time reported between the last dose and necroscopy. At necropsy, blood was taken for toxicokinetic studies, a gross examination was made and selected tissues were subject to microscopic examination. The marmoset data were considered by CERHR (2005) to be confusing and poorly reported: a single set of bar graphs was presented, while two studies were performed. The authors state that organ weights were not changed in marmosets at 1 mg/kg bw/d but provided no data. Based on histology and biochemical measures, peroxisomal proliferation was not induced in marmosets. The authors presented no histological findings of testes. This limits the study as testicular pathology is the most sensitive endpoint at this exposure level, and poor histology could well mean that lesions could go undetected.

Cammack et al. (2003) conducted a 21-day repeat dose study of DEHP in neonatal (3- to 5-day old) rats. Rats were injected intravenously with 0, 60, 300 or 600 mg/kg bw/d. A second group of animals was dosed for 21 days then held for a recovery period until 90 days of age. Terminal body weight was significantly less in the high dose group only. At the end of the 21-day dosing period, mean liver weight was increased and mean testes weight was decreased in the two higher dose groups. Testicular atrophy was observed in all animals in the 300 and 600 mg/kg bw/d treatment groups. The NOAEL in the study was 60 mg/kg bw/d.

6.2.5 Genotoxicity

The genotoxicity of DEHP has been reviewed extensively (IARC, 2000; ECB, 2006 and 2008). DEHP has been tested in a variety of short-term genotoxicity assays with predominantly negative results. Overall, DEHP is regarded as non-genotoxic.

In vitro

In 15 published reverse mutation assays in bacteria, all results were negative (IARC, 2000; ECB, 2006 and 2008). The maximum concentration used was 14700 µg/plate. Two studies in fungi were negative, failing to show any evidence of mutation or recombination events. Primary DNA damage, mutation, sister chromatid exchange or chromosomal aberrations were not induced in most assays with cultured mammalian cells. Some of these in vitro systems are also sensitive to

non-genotoxic substances which are tumour promoters and/or peroxisome proliferators.

In vivo

Results were generally negative in in vivo studies (mouse, rat and Drosophila melanogaster) testing DEHP and its main metabolites MEHP and 2-EH. Low levels of mutation but not DNA damage were induced in somatic cells of Drosophila melanogaster. Gene mutations were not induced in vivo in the liver of dosed mice and there was no evidence of chromosomal aberrations in mice or rats in vivo.

6.2.6 Carcinogenicity

The carcinogenicity of DEHP has been investigated extensively in vivo and in vitro. Key studies are outlined below.

Oral route

In a carcinogenicity study, B6C3F1 mice (70/sex/group) received DEHP in the diet at concentrations of 0, 100, 500, 1500, or 6000 ppm (m/f: 0/0, 19.2/23.8, 98.5/116.8, 292.2/354.2, or 1266.1/1458.2 mg/kg bw/d) for 104 weeks (Moore, 1997*). In an additional recovery group, mice were dosed with 6000 ppm of DEHP for 78 weeks, followed by a 26-week recovery period. Significantly increased incidences of hepatocellular adenomas and carcinomas were observed at 1500 ppm and 6000 ppm in male mice. In these two high dose groups, induction of peroxisome proliferation but not hepatocellular proliferation was more pronounced in both sexes. In the 6000 ppm recovery group, the incidence of hepatocellular adenomas, but not carcinomas, was less than in the 6000 ppm group. The LOAEL for tumour induction (hepatocellular neoplasms in male mice) in this study was 1500 ppm (292 mg/kg bw/d). The NOAEL was 500 ppm (98 mg/kg bw/d).

In a chronic/carcinogenicity study, F344 rats (70/sex/group) received DEHP in the diet at doses of 0, 100, 500, 2500, or 12 500 ppm (m/f: 0/0, 5.8/7.3, 28.9/36.1, 146.6/181.7, or 789/938.5 mg/kg bw/d) for 104 weeks (Moore, 1996*). In an additional recovery group, rats (55/sex/group) were administered 12 500 ppm DEHP for 78 weeks, followed by a 26-week recovery period. Increases in hepatocellular adenomas and mononuclear cell leukaemia (MCL) in males at 2500 ppm and above and hepatocellular carcinomas in males and females at 12 500 ppm, were observed. However, the incidence of hepatocellular adenomas/carcinomas was decreased in recovery animals at 12 500 ppm (2-week recovery period), compared with the same dose group at the end of the dosing period. Peroxisome proliferation was induced from 2500 ppm. The LOAEL for tumour induction (hepatocellular neoplasms and MCL in male rats) was 2500 ppm (147 mg/kg bw/d for males). The NOAEL was 500 ppm (28.9 mg/kg bw/d, males).

The carcinogenicity of DEHP was tested in rats and mice in the US National Toxicology Program (NTP) in 1982-1983 (Kluwe et al., 1982*; NTP, 1982*; Kluwe et al., 1983*). F-344 rats and B6C3F1 mice were fed diets containing 0, 6000 or 12 000 ppm (rats), and 0, 3000 or 6000 ppm (mice) DEHP for 103 weeks. This corresponded to a daily DEHP intake of 0, 322, and 674 mg/kg bw/d for male rats; 0, 394, and 774 mg/kg bw/d for female rats; 0, 672 and 1325 mg/kg bw/d for male mice, and 0, 799 and 1821 mg/kg bw/d for female mice. There was a dose-dependent increased incidence of hepatocellular carcinomas in male and females

rats, with the increase statistically significant in females at the highest dose. The combined incidence of rats with hepatocellular carcinomas or neoplastic nodules was significantly greater than controls for females at both doses and for high dose males. In mice, a dose-related trend for hepatocellular carcinomas was observed for both sexes, with a significant increase in females at both doses and in high dose males. The incidence of hepatocellular carcinomas or adenomas when combined, was dose-related with a significant increase in both sexes at both doses. The LOAEL for tumour induction in rats and mice was 6000 ppm (320 mg/kg bw/d) and 3000 ppm (670 mg/kg bw/d), respectively. No NOAEL could be identified for either species.

In a lifelong exposure study, DEHP was administered in the diet at 0, 30, 95, and 300 mg/kg bw/d to male Sprague-Dawley rats beginning at an age of 90 - 110 days and continuing for the remaining lifetime of the animals (up to 159 weeks) (Voss et al., 2005). Significantly increased incidences of hepatocellular adenomas and carcinomas were observed at the highest dose. Similarly, the percentage of benign Leydig cell tumours in the highest dose group was almost twice as high as the percentage in the control group (28.3% versus 16.4%). Furthermore, there was a significant dose-related trend in the incidence of hepatic neoplasms and also Leydig cell tumours. The time-to-tumour analysis showed that the significantly higher incidence of Leydig cell tumours in this rat strain occurred even earlier than hepatocellular tumours. In addition, multiplicity of the DEHP-induced Leydig cell tumours increased with time in contrast to spontaneous Leydig cell tumours, which were mostly unilateral. In this study, the NOAEL for both liver and testicular carcinogenic effects was determined to be 95 mg/kg bw/d. However, the dose-related trend of increased Leydig cell tumours was observed commencing from the lowest dose, 30 mg/kg bw/d.

Inhalation route

The only inhalation study available is on Syrian golden hamsters continuously exposed to low levels (15 $\mu g/m^3$) of DEHP by inhalation for 23 months (Schmezer et al., 1988*). However, the study is considered inadequate to draw conclusions on the carcinogenicity of DEHP from inhalation exposure.

Studies on the mode of action

There is evidence suggesting that DEHP-induced peroxisome proliferation combined with suppression of hepatocellular apoptosis could be the major molecular mechanism for DEHP-induced hepatocarcinogenicity.

Using PPARα-null and wild-type male Sv/129 mice, Ward et al. (1998) demonstrated that PPARα is required for DEHP-induced liver lesions. Mice were fed ad libitum with either a control diet or one containing 12 000 ppm DEHP for up to 24 weeks. No signs of liver toxicity were detected in the PPARα-null mice while in wild type mice DEHP treatment induced typical lesions in the liver such as increase in the number of peroxisomes, induction of replicative DNA-synthesis, and hepatomegaly. However, evidence of lesions in kidneys and testes were found in both PPARα-null and wild-type mice, indicating a PPARα independent pathway for induction of toxicity in these organs.

More recently, using PPARα-null mice, Lapinskas et al. (2005) showed that expression of PPARα is required for DEHP-induced liver enlargement and

induction of fatty acid metabolising enzymes confirming earlier knockout mouse studies by Ward et al. (1998). An association between PPARα and liver cancer was demonstrated also through knock-out mice studies which showed the inability of the potent peroxisome proliferator and hepatocarcinogen Wy-14,643 to induce either increased cellular proliferation or hepatocarcinogenicity in PPARα-deficient animals (Lee et al., 1995; Peters et al., 1997).

The activation of PPARs by DEHP in species other than rodents appears to be less prominent. Syrian hamsters exhibit an intermediate response, whereas guinea pigs and monkeys appear to be relatively insensitive to DEHP associated PPARs activation (Lake et al., 1984; Rhodes et al., 1986; Short et al., 1987).

In a recent study, Ito et al. (2007 **ND**) examined the effect of low DEHP doses (doses that do not show significant hepatotaxiticity in rodents) in PPARα-null and wild-type male Sv/129 mice. Animals were fed ad libitum with a diet containing 0, 0.01% or 0.05% (100ppm and 500 ppm) DEHP from three weeks to 22 months of age (~ 90 weeks). Plasma levels of MEHP were similar between PPARα-null and wild-type groups indicating similar levels of DEHP exposure. No significant differences were observed in body weight or liver weight between the groups. However, statistically significant increases in total neoplastic changes (8 in 31 animals or 25.8%) were observed in the highest dose (0.05%) PPARα-null mice group, while only 2 hepatocellular adenomas were observed in 20 wild type animals (10%), as expected for these DEHP doses in wild type. Analysis of the basal level (in the absence of DEHP treatment) of 8-hydroxydeoxyguanosine (8-HdG) in the liver, an indicator of oxidative stress and DNA damage, showed that basal level of 8-HdG was significantly increased in PPARα-null mice compared to the wild type. DEHP treatment further increased 8-HdG levels in dose dependent manner in both genotypes. Also, DEHP appeared to differentially affect the expression of a number of genes involved in cell cycle regulation in liver of wild type compared to PPARα-null mice (Ito et al., 2007 **ND** and Takashima et al., 2008 **ND**). The results were interpreted to indicate that although the overall incidence of tumours was low, DEHP can induce hepatic carcinogenesis by PPARα-dependent and also by PPARα-independent pathway(s), the latter via proteins involved in response to oxidative damage and cell cycle regulation. The PPARα-independent pathway appears to be related to oxidative stress and is activated only in the PPARα-null mice and not in the wild type, where no increase in liver neoplasms was observed at these low DEHP doses.

6.2.7 Reproductive toxicity

Traditional hazard assessments consider effects on fertility separate from developmental toxicity. Fertility is tested by exposing sexually mature adults to a chemical and examining the effects on reproductive capacity. Developmental toxicity is studied by exposing pregnant dams or neonatal animals and looking for adverse effects on foetal development. Chemicals that affect the developing reproductive system following prenatal exposure may also affect sexual maturation or functional reproductive disorders that are only apparent at maturity. Developmental toxicity can therefore lead to adverse effects on fertility and the two endpoints cannot be clearly distinguished.

In this section, data are presented on the basis of test procedure. The effects on fertility (as adults) and development (as foetuses) are then discussed separately.

The effects of DEHP on reproductive endpoints have been tested in a variety of species including rats, mice, hamsters, ferrets and marmoset monkeys. Overall, rats were the most sensitive to reproductive effects followed by mice, hamsters and ferrets. Marmoset monkeys appear to be insensitive to DEHP-induced testicular toxicity, however the studies in this primate system are very limited in number and may not cover exposure at critical windows for toxicity. Key studies are described below.

Effects on fertility

Oral route

In a study by Poon et al. (1997) groups of 4-6 week old male and female Sprague-Dawley rats were exposed to 0, 5, 50, 500 and 5000 ppm DEHP in the diet for 13 weeks. Animals were reported to reach sexual maturity approximately 50 days into the study and thus were immature for only part of the study. These dietary concentrations corresponded to average DEHP doses of 0, 0.4, 3.7, 38, and 375 mg/kg bw/d, for the male rats. In the testes, Sertoli cell vacuolation, described as "mild," was seen in 7/10 males in the 500 ppm group, and 9/10 males in the 5000 ppm group. The highest group also showed decreased testes weights, bilateral, multifocal, or complete atrophy of the seminiferous tubules with complete loss of spermatogenesis and cytoplasmic vacuolation of the Sertoli cells lining the tubules. There was no measurement of reproductive function. The LOAEL, based on the testicular effects (Sertoli cell vacuolation) was 38 mg/kg bw/d and the NOAEL was 3.7 mg/kg bw/d. While females were examined in this repeated dose toxicity study for overall toxicity, no effects are reported for the female reproductive system.

However a study with mice by Lamb et al., (1997), described in detail in the multigenerational studies section, showed that fertility parameters were affected in both females and males even though no histological lesions were seen in female mice. Complete infertility was noted when control males were mated to high dose females. The LOAEL was 0.1% (140 mg/kg bw/d) based on decreased fertility and the NOAEL was 0.01% (14 mg/kg bw/d).

In a study by David et al. (2000a), groups of 6-week old Fischer 344 rats (50-80 males/group) were fed diets containing 0, 100, 500, 2500, or 12 500 ppm DEHP (0, 5.8, 29, 147, and 789 mg/kg bw/d for males) for 104 weeks. Testes weight (absolute and relative) was reduced in rats of the high-dose group. Aspermatogenesis was observed in all rats in the highest dose group at study week 78 but not in rats treated with 2500 ppm or in the control group. At study week 105, the incidence of aspermatogenesis was significantly increased in rats exposed to 100 ppm and higher. The percentage of rats with aspermatogenesis from the control to high-dose group was 58, 64, 78, 74, and 97%, respectively. Aspermatogenesis was not observed at week 78 in 10 animals dosed with 147 mg/kg bw/d. However, at 78 weeks other lower doses were not tested, while the 10 animals at the highest dose of had 100% incidence of aspermatogenesis. The findings indicate a NOAEL for testis effects of 5.8 mg/kg bw/d, based on the dose-response increase in the proportion of animals showing aspermatogenesis after 104 weeks treatment. However, the testes fixing procedure used in this study has been criticized by the NTP Expert Panel (CERHR, 2000) as being suboptimal and might have obscured detection of early vacuolar lesions.

Akingbemi et al. (2001, 2004) compared effects of short and long-term DEHP exposure in prepubertal, pubertal and young adult Long-Evans rats. In the first study (Akingbemi et al., 2001) male rats were gavaged with 0, 1, 10, 100, or 200 mg/kg bw/d for two 14 day periods (short term treatment), prepubertal postnatal days (PND) 21–34 or pubertal PND 35-48. Rats were also dosed for longer 28 day periods, PND 21-48, or as young adults PND 62-89. In the second study (Akingbemi et al., 2004), prepubertal rats were gavaged daily with 0, 10 or 100 mg/kg bw/d DEHP for 70 or 100 days during PND 21–90 or 21-120, respectively. Within 24 hours of the final doses, measurements of luteinising hormone (LH), 17β-oestradiol (E2) and testosterone (T) in the plasma were made, the animals were sacrificed and testicular histology was evaluated. Leydig cells were obtained to evaluate basal and LH stimulated testosterone synthesis and E2 production. Expression of several proteins involved in cell cycle, androgen and oestrogen biosynthesis was also monitored in the Leydig cell preparations (Akingbemi et al., 2004). Leydig cells were also incubated with testosterone biosynthesis substrates and enzyme activity measured in the first study only.

No treatment-related effects on body weight gain or food consumption were observed in either study. Short-term, 14-day DEHP treatment in prepubertal and pubertal rats had no effect on serum testosterone or LH levels at any dose. However, Leydig cells isolated from rats that had been treated on PND 21–34 or 35-48 showed a decrease in basal and LH-stimulated testosterone production at 100 and 10 mg/kg bw/d, respectively. This effect correlated with the decreased activity of steroidogenic enzymes in the Leydig cell preparations from pubertal rats treated at PDN 35-48, where 17β-hydroxysteroid dehydrogenase was reduced 74% at 10 mg/kg bw/d compared to control with other enzyme activities significantly reduced at 100 or 200 mg/kg bw/d.

In contrast, longer, 28 day exposure of prepubertal rats continuing to pubertal age (PND 21-48) was associated with an increased Leydig cell synthesis of testosterone (basal and LH stimulated) at 10 mg/kg bw/d and above. Serum LH and testosterone levels and interstitial fluid testosterone were also increased in these animals with 10 mg/kg bw/d and 100 mg/kg bw/d.

DEHP treatment of young adults (PND 62-89) for 28 days had no effect on serum testosterone or LH or on in vitro Leydig cell steroidogenesis (Akingbemi et al., 2001), However, similar to the 28-day treatment of prepubertal rats (PDN 21-48), DEHP treatment for longer periods (70 and 100 days), starting at prepubertal age PND 21 was associated with increased serum LH and T levels (Akingbemi et al., 2004). In contrast to the 28 days treatment, prolonged treatment for 70 days with 10 and 100 mg/kg bw/d and for 100 days with 100 mg/kg bw/d was associated with decreased Leydig cell steroidogenesis in the adult animals in vitro, similar to the decrease observed in short term, 14 day treatment of prepubertal PND 21-34 and pubertal 34-48 animals. Paradoxically, the number of Leydig cells in the testes was increased after prolonged exposure to DEHP and this was associated with increased levels of proteins involved in cell proliferation (Akingbemi et al., 2004). These parameters were not monitored in the short-term exposure study.

Taken together, these studies suggest that younger rats are more sensitive to the effects of DEHP on steroidogenesis. The LOAEL was 10 mg/kg bw/d for increased serum LH and testosterone in rats exposed from PND 21-48. The NOAEL was 1 mg/kg bw/d.

Table 6.2: Summary of DEHP effects on fertility related endpoints in rats from Akingbemi et al., 2001 and 2004.

Age at start of treatment (PND)	Duration of Treatment (days)			
	14	28	70	100
21	LCT ↓ (100) ST nc E nm	LCT ↑ (10) ST ↑ (10)	LCT ↓ (10) ST ↑ (10) LCN ↑ (10)	LCT ↓ (100) ST ↑ (100) LCN ↑ (10)
35	LCT ↓ (10) ST nc E ↓ (10)			
62		LCT nc ST nc		

LCT- Steroidogenesis by Leydig cell ex vivo; LCN – Leydig cell number

ST- Serum Testosterone levels ; E - Steroidogenic enzymes activity

( ) – dose at which the effect was first seen in mg/kg bw/d; **nc** – no change; **nm**- not measured

Boxes shaded in grey indicate that the treatment was not included in the studies

In a study by Dostal et al. (1988) groups of Sprague-Dawley rats were given oral doses of 0, 10, 100, 1000, or 2000 mg/kg bw/d of DEHP (>99% pure) by gavage in corn oil for 5 days (7-10 animals per group) at 1, 2, 3, 6, and 12 weeks of age. Absolute and relative testis weights were significantly reduced at doses of 1000 mg/kg bw/d in 1, 2, 3, and 6- week-old but not in 12-week-old rats compared to controls of the same age suggesting differential age sensitivity to testicular effects of DEHP. Doses of 2000 mg/kg bw/d were fatal to suckling rats and caused decreased relative testis weight but no lethality in 6- and 12-week-old rats. At 1000 mg/kg bw/d, the number of Sertoli cell nuclei per tubule was reduced by 35% in neonatal rats. Two- and three-week old rats showed loss of spermatocytes but not of Sertoli cells at this dose. Loss of spermatids and spermatocytes in 6- and 12-week old rats at 1000 and 2000 mg/kg bw/d was observed. These results suggest that Sertoli cells are sensitive to DEHP during their proliferative stage.

Sjoberg et al. (1986) examined the age-dependent testis toxicity of DEHP (1000 and 1700 mg/kg bw/d in the diet for 14 days) in rats at 25, 40, and 60 days of age. Body weight gain was retarded in all dosed groups and testicular weight was markedly reduced in 25- and 40-day-old rats given 1700 mg/kg bw/d. Severe testicular damage was evident for the 25-day- and 40-day-old rats at both dose levels. No changes were found in the 60-day-old rats.

A single bolus dose of DEHP (20, 100, 200, and 500 mg/kg bw) was given in corn oil to five neonatal rat (three-day old, CD Sprague-Dawley) pups per dose group (Li et al., 2000). MEHP (393 mg/kg bw), 2-EH (167 mg/kg), or vehicle was administered by gavage to 4 pups per group. All pups were killed 24 hours after dosing. The doses of MEHP and 2-EH were molar equivalent to 500 mg/kg DEHP. A time-course study was also conducted following a single dose of DEHP (200 mg/kg bw), where the pups were killed after 6, 9, 12, 24, or 48 h. Morphological

examination revealed a dose-dependent presence of abnormally large, multi-nucleated germ cells (gonocytes) by 24 h post-treatment with higher doses of DEHP (100-500 mg/kg bw). Sertoli cell proliferation was dose-dependently decreased from 100-500 mg/kg bw but not 20 mg/kg bw DEHP. There was a rebound in Sertoli cell proliferation at 48 hours following treatment with 200 mg/kg bw DEHP. MEHP (single dose group) induced similar effects as DEHP. A NOAEL of 20 mg/kg bw DEHP was derived based on altered gonocyte morphology and decreased Sertoli cell proliferation in neonatal pups.

Two studies administered oral doses of DEHP to pre- and post-pubertal marmoset monkeys for varying durations. Reproductive outcomes in particular were assessed. Kurata et al. (1998*) administered groups of 4 male and 4 female 12–15 months old (post-pubertal) marmoset monkeys, doses of 0, 100, 500, or 2500 mg/kg bw/d DEHP in corn oil by gavage for 13 weeks. There were no treatment-related decreases in testis weight, serum testosterone or oestradiol levels. There were no testicular histopathological changes even at the highest dose. The NOAEL was 2500 mg/kg bw/d.

In a study by the Mitsubishi Chemical Safety Institute (2003*), DEHP was administered by gavage in corn oil to juvenile marmoset monkeys (9 males and 6 females) beginning at 90–115 days of age until 18 months of age (young adulthood) at dose levels of 0, 100, 500, and 2500 mg/kg bw/d. Both males and females were assessed with in-life hormonal assays and with histopathology at necropsy. The results suggest little effect on testicular structure or function. Mean serum testosterone levels were highly variable, but the data suggested the possibility of a delay in the onset of puberty in male marmosets with increasing DEHP dose. Body weights and male organ weights were not affected. The NOAEL was 2500 mg/kg bw/d.

In a study by Tomonari et al. (2006) groups of 90-115 day old marmoset monkeys (5-6/sex/group) were given 0, 100, 500 or 2500 mg/kg bw/d DEHP by gavage for 65 weeks. Blood samples were taken throughout the study and analysed for DEHP, MEHP, zinc and testicular enzyme activity. At the end of the study, the liver and the primary and secondary sex organs were weighed and examined histologically. There were no treatment-related changes in male organ weights, no microscopic changes in male gonads, secondary organs, Leydig, Sertoli or spermatogenic cells. No increases in hepatic peroxisomal enzyme activities were noted. The NOAEL was 2500 mg/kg bw/d.

Inhalation route

In a 4-week inhalation study conducted according to OECD guideline 412, male Wistar rats (10 rats per group) were exposed 5 days per week, 6 hours per day to 0, 0.01, 0.05, or 1 mg DEHP/L (0, 10, 50, or 1000 mg DEHP/m^3) (99.7% pure) as liquid aerosol (Klimisch et al., 1992*). The males were mated to untreated females. No effects on male fertility were reported 2 and 6 weeks after the end of exposure and no testicular toxicity was detected histologically. However, the results were inadequately presented.

Parenteral exposure

Sjoberg et al. (1985b) exposed 25 or 40 day old rats to 0, 5, 50 or 500 mg/kg bw intravenously every other day for 10 days (time-weighted average 0, 2.5, 25 or 250 mg/kg bw/d). There was no change in testes weight but vacuolization of the Sertoli

cells and spermatocyte degeneration were observed at 250 mg/kg bw/d. The NOAEL was 25 mg/kg bw/d.

In the second study, neonatal male rats or rabbits were injected intravenously with either 62 mg/kg bw/d DEHP or 4% bovine serum albumin during PND 3-21 (rats) or 14-42 (rabbits) (Baxter Healthcare Corporation, 2000*). Histopathological examination of the testes and other organs of DEHP-exposed animals revealed no alterations at this dose.

Cammack et al. (2003) conducted a 21-day repeat dose study of DEHP in neonatal (3- to 5-day old) rats. Rats were injected intravenously with 0, 60, 300 or 600 mg/kg bw/d or gavaged with 300 or 600 mg/kg bw/d. A second group of animals was dosed intravenously for 21 days then held for a recovery period until 90 days of age. At the end of the 21-day dosing period, testicular atrophy, decreased seminiferous tubule diameter and mild depletion of germinal epithelial cells were observed at 300 mg/kg bw/d and 600 mg/kg bw/d. Although testicular atrophy persisted at the end of the recovery period, histopathological changes were not seen in the recovery group previously exposed to a DEHP dose of 300 and 600 mg/kg bw/d for 21 days. At equivalent doses, oral exposure induced more significant changes in testicular weight and pathology. The NOAEL for intravenous exposure in the study was 60 mg/kg bw/d and the LOAEL was 300 mg/kg bw/d based on testicular effects.

Effects on development

Prenatal developmental toxicity studies

Dietary levels of 0, 0.025, 0.05, 0.10, or 0.15% of DEHP (0, 44, 91, 190.6, or 292.5 mg/kg bw/d) were administered to mice throughout gestation (GD 0-17) (NTIS, 1984*; Tyl et al., 1988). Reduced maternal body weight gain was noted at 0.1% and above, mainly due to reduced gravid uterine weight. Increased resorptions, late foetal deaths and malformed foetuses, and decreased foetal weight and viable foetuses were observed at 0.1% and above. Increased malformed foetuses were seen also at 0.05% and above. The external malformations included unilateral and bilateral open eyes, exophthalmia, exencephaly, and short, constricted or no tail. Visceral malformations were localised predominantly in the major arteries. Skeletal defects included fused and branched ribs and misalignment and fused thoracic vertebral centra. The NOAEL for maternal toxicity was 0.05% (91 mg/kg bw/d) and for developmental toxicity was 0.025% (44 mg/kg bw/d).

DEHP at doses of 0, 40, 200, or 1000 mg /kg bw/d was administered by gavage to pregnant mice (15/group) from GD 6 to 15 (Huntingdon, 1997*). At GD 17, decreased viable pups and increased resorptions and post-implantation loss were observed at 1000 mg/kg bw/d. Cardiovascular abnormalities, tri-lobed left lungs, fused ribs, fused thoracic vertebral centres and arches, immature livers and kidney anomalies were also observed at this dose. At 200 mg/kg bw/d, there was a slight increase in foetuses with intra-muscular or nasal haemorrhage or dilated orbital sinuses. There also were a small number of foetuses with anomalous innominate or azygous blood vessels at this dose level. A NOAEL of 200 mg/kg bw/d was established for maternal toxicity and 40 mg/kg bw/d for developmental toxicity.

Pregnant rats received DEHP by gavage at doses of 0, 40, 200, or 1000 mg/kg bw/d from GD 6 to 15 (BASF, 1995*; Hellwig et al., 1997). Reduced uterine weights and increased relative kidney and liver weights were observed in dams at

1000 mg/kg bw/d. Also at this dose, decreased viable foetuses and foetal body weights, and increased implantation loss, external and skeletal malformed foetuses (predominantly of the tail, brain, urinary tract, gonads, vertebral column, and sternum) and foetuses with soft tissue, skeletal variations and retardations were seen. The NOAEL for maternal and developmental toxicity was 200 mg/kg bw/d.

Pregnant Wistar rats were gavaged from GD 7 to 21 with vehicle or 10, 30, 100 or 300 mg/kg bw/d of DEHP (Borch et al., 2006). No maternal effects were reported. Male foetuses were examined on GD 21. Testicular testosterone production ex vivo and testicular testosterone levels were reduced significantly at the highest dose. Histopathological effects on gonocytes were observed at 100 and 300 mg/kg bw/d. At the highest dose level Leydig cell effects and vacuolisation of Sertoli cell were observed. There was reduced testicular mRNA expression of the steroidogenesis related factors and reduced mRNA expression of a nuclear receptor involved in regulation of steroid synthesis at the two highest doses. Even at the highest dose, there was no change in PPARα mRNA expression. The NOAEL for developmental effects was 30 mg/kg bw/d and the LOAEL was 100 mg/kg bw/d based on testicular pathology.

In a study of several phthalates, DEHP was administered orally to Sprague-Dawley rat dams at 750 mg/kg bw/d from GD 14 to PND 3 (Gray et al., 2000). There was no overt maternal toxicity or reduced litter sizes. DEHP treatment reduced maternal weight gain, pregnancy weight gain and pup weights. Male, but not female pups in both DEHP and butyl benzyl phthalate (BBP) administered groups displayed shortened AGD and reduced testes weights. As infants, males had female-like areolas/nipples and increased incidence of reproductive malformations.

In rats exposed to DEHP by inhalation, there was no consistent evidence of any treatment-related prenatal or postnatal developmental effects in the offspring of females (25/group) exposed to up to 300 mg/m^3 DEHP (the highest dose tested), 6 h/d during the period of organogenesis (GD 6–15) (Merkle et al., 1988*). The number of live foetuses/dam was statistically significantly decreased and number of resorptions increased in the 50 mg/m^3 group but not at the next highest dose level.

There are insufficient data on the developmental toxicity of DEHP administered parenterally to identify LOAELs and NOAELs for these exposure routes. In the only published intravenous exposure study with pregnant rats (Lewandowski et al., 1980*), no foetal toxicity was observed, however the doses, 1 - 5 mg/kg bw/d were lower than those used in oral exposure studies. The lowest dose reported to produce foetal toxicity following intraperitoneal (IP) administration was 1970 mg/kg bw/d (Peters & Cook, 1973*). Of 10 dams dosed on GD 3, 6, and 9 only one survived to delivery. Singh et al. (1972*) administered 5 or 10 mL/kg bw (4930 and 9860 mg/kg bw) DEHP to groups of five Sprague-Dawley rats by IP injections on GD 5, 10, and 15. Maternal toxicity was not evaluated in this study. There was an increased frequency of resorptions at both doses and a decrease in foetal weights. Gross anomalies were only observed at the 9860 mg/kg bw/d dose. The IP studies are limited as only high doses were tested and group sizes were small.

Shiota and Mima (1985) compared the effects of oral and IP administration of DEHP and MEHP. DEHP was administered to pregnant ICR mice (9-11/group) orally at 0, 250, 500, 1000 or 2000 mg/kg bw/d and MEHP orally at 0, 50, 100,

200, 400 mg/kg bw/d on GD 7-9 (Shiota and Mima, 1985). A second group received 0, 500, 1000, 2000, 4000, 8000 mg/kg bw/d DEHP or 0, 50, 100, 200 mg/kg bw/d MEHP by IP injections on GD 7-9. In groups given DEHP orally, resorptions and malformed foetuses (anencephaly and exencephaly) increased significantly above 500 mg/kg bw/d. No teratogenic effects were revealed following IP doses of DEHP, or oral or IP doses of MEHP, although high doses were abortifacient and lethal to pregnant females. Thus, DEHP in this study was embryotoxic and teratogenic in mice when given orally but not by IP administration. This difference may be a result of differences in metabolism, disposition, or excretion due to the route of administration.

Postnatal developmental toxicity studies

Female rats received DEHP in the drinking water at 3.0-3.5 and 30-35 mg/kg bw/d from GD 1 to PND 21 (Arcadi et al., 1998*). Decreased pup kidney weights were observed at both doses, accompanied by histopathological findings (shrinkage of renal glomeruli with signs of glomerulonephritis, dilation of renal tubuli and light fibrosis) between weeks 0 and 4 of age. Lower testicular weights were observed, associated with severe histopathological changes which included only a few elongated spermatids in tubules showing a pervious lumen at low dose level and a generalized disorganization of the tubular epithelium with spermatogonia detached from the basal membrane, absence of elongated spermatids and spermatozoa, and with the tubular lumen filled with cellular deposits at high dose level. No NOAEL was established. There is doubt regarding the delivered dose in this experiment as DEHP is not soluble in water so was delivered as a suspension.

The effects of DEHP were studied on male reproductive parameters in Sprague-Dawley rats exposed to 750 mg/kg/d DEHP by gavage commencing on GD 14 and ending at PND 3 (Parks et al., 2000*). Dams were sacrificed at GD 17, 18, 20, and at PND 2 and pups examined at each time point. Ex vivo testicular production of testosterone, testicular content of testosterone, and whole-body testosterone concentration were significantly reduced at all time points, with maximal effects at GD 20 where a 90% reduction in ex vivo testicular production of testosterone was noted. Anogenital distance was reduced at PND 2 and testicular weight was reduced at GD 20 and PND 2. Histopathological examination of the PND 2-testes showed increased numbers of Leydig cell hyperplasias and of multinucleated germ cells. DEHP exposure resulted in decreased testosterone, Leydig cell hyperplasia and formation of multinucleated germ cells in male foetuses and offspring.

The effect of DEHP on Leydig cell function in male Long-Evans rats exposed in utero (GD 12-21), during nursing, or during post-weaning stages was evaluated by Akingbemi et al. (2001). DEHP was administered to dams by gavage in corn oil at 0 or 100 mg/kg bw/d. Males were obtained for evaluation on PND 21, 35, or 90 (7 dams/group/stage). There were no effects of treatment during gestation on dam weight or weight gain or on offspring weight. Offspring testis and seminal vesicle weights also were not affected by treatment during gestation. However, serum testosterone was reduced 31%–33% and serum LH was reduced 50%–64% in 21- and 35-day-old males exposed to DEHP during gestation. In contrast, the same treatment had no effects on serum testosterone or LH in 90-day-old males. However, gestational exposure to DEHP was associated with decreased testosterone production in cultured progenitor Leydig cells obtained from 21-day-old males, but not in cell preparations from more mature, 35- and 90-day-old

males. Basal testosterone production in the prepubertal offspring at PND 21 was reduced 47%, and LH-stimulated testosterone production was reduced 56%.

In the more recent study by Culty et al. (2008 **ND**), pregnant Sprague-Dawley rats were treated with DEHP doses of 58, 117, 234, 469, 700, 750, 938 and 1250 mg/kg bw/d by gavage from GD14 to parturition (PND 0). Offspring were analysed at PND3, PND21 and PND60. There was no effect on number of pups, percent of males per litter or body weight of pups at PND60. Statistically significant reduction of AGD was only observed in the PND60 adults at 1250 mg/kg bw. Testes weight was not affected at PND 21. At PND 60, testes weight was reduced (40% and more compared to the controls) for some animals (number not specified) in the 938 mg/kg bw/d and 1250 mg/kg bw/d treatment groups. Authors excluded the animals with atrophied testes from further analysis. Testosterone production was reduced in testicular cultures from PND3 offspring of the 938 mg/kg bw/d treatment group, similar to that observed prenatally in GD20 foetuses treated with DEHP from GD14-20 (see mode of action studies below for details on this reduction). However, the mRNA for the steroidogenic enzymes Cyp11a1 and Cyp17a1 appeared to be upregulated at PND3 and PND 20 for the 469 and 938 mg/kg bw/d treatment. No histomorphological effects were observed at any tested postnatal age at the dose 750 mg/kg bw/d and below.

In this study, serum testosterone levels were also reduced in the PND60 adults at 234 mg/kg bw/d and above (Culty et al., 2008 **ND**). The analysis of Cyp11a1 and Cyp17a1 mRNA at PND 60 included only non-atrophied testes and did not show statistically significant difference with the controls. The results of this study together with the analysis of the prenatal developmental effects described in the previous section indicate that DEHP treatment during embryogenesis may have different short- and long-term effects on the overall regulation of testicular steroidogenesis.

Offspring of dams treated with DEHP at PND 1-21 (lactational indirect exposure) showed no significant differences in any of the measured parameters except slightly reduced serum testosterone levels at PND 21 that were not associated with any change of the serum LH levels.

Female Wistar rats were given oral (gavage) doses of DEHP at 0.015, 0.045, 0.135, 0.405 and 1.215 mg DEHP kg/bw/d (low doses) or 5, 15, 45, 135 and 405 mg/kg bw/d (high doses) on GD6 to PND21 (Grande et al., 2006). Exposure continued through lactation. In dams, liver and kidney weights were significantly increased at the highest dose level (405 mg/kg bw/d). No other signs of maternal toxicity were evident. Litter sizes, sex ratios, postimplantation losses and numbers of viable pups were also unaffected. In offspring, a significant increase in liver weight was observed on PND 1 (but not PND 22) at 135 and 405 mg/kg bw/d. A significant delay in the age of vaginal opening was observed at 15 mg/kg bw/d and above. Anogenital distance and nipple development were unaffected in the female offspring. A NOAEL for maternal toxicity was established at 135 mg/kg bw/day and for teratogenicity at 5 mg/kg bw/day.

The effects of DEHP on the male Wistar offspring from the above study were analysed in a parallel study (Andrade et al., 2006). Nipple retention and reduced AGD were seen in males exposed to the highest dose (405 mg/kg bw/d). Delayed preputial separation was observed in animals exposed to 15 mg/kg bw/d and above. Testes weights were significantly increased at 5, 15, 45 and 135 mg/kg bw/d (but

not 405 mg/kg bw/d) on PND 22. Histopathological examinations of testes on PND 1 and 22 showed changes at the two highest doses (135 and 405 mg/kg bw/d). On PND 1, bi- and multinucleated gonocytes were evident. On PND 22, signs of reduced germ cell differentiation in seminiferous tubules were observed. The study concluded that DEHP acts as an anti-androgen in males at the highest dose level (405 mg/kg bw/d) but also induced subtle developmental effects at lower doses. A NOAEL for developmental toxicity was established at 1.215 mg/kg bw/day based on increased testes weight in pubertal (PND 22) rats.

Multigenerational reproductive toxicity studies

In a study by Lamb et al. (1987) male and female CD-1 mice (20 pairs per breeding group) were fed DEHP in the diet at dose levels of 0, 0.01, 0.1, or 0.3% (0, 14, 140, and 425 mg/kg bw/d) from a 7-day premating period to 21 days after delivering litters (14 weeks in total). Decreased litters and viable pups were observed at 0.1% and above. No pairs were fertile at 0.3%. Also at 0.3% (the only dose examined), increased liver weights and decreased weights of the reproductive organs in parental animals (testes, epididymes, prostate, and seminal vesicles) were evident. All but one of the high-dose males showed some degree of bilateral atrophy of the seminiferous tubules. This dose also caused decreased sperm motility and sperm concentrations and increased incidences of abnormal sperm. A subsequent cross-over mating trial showed that both sexes were affected by exposure to DEHP despite no histological lesions being seen in female mice. Complete infertility was noted when control males were mated to high dose females. The LOAEL was 0.1% (140 mg/kg bw/d) based on decreased fertility and the NOAEL was 0.01% (14 mg/kg bw/d).

In a study by Schilling et al. (2001), groups of Wistar rats (25 males and females in each group) were fed DEHP in the diet at concentrations 0, 1000, 3000, or 9000 ppm (0, 113, 340, or 1088 mg/kg bw/d) for two successive generations, from at least 70 days premating of the first parental generation. Increased focal tubular atrophy in the testis was observed in all treated groups (F0, F1 and F2). Decreased food consumption, body weight gain, testis weights and fertility index were seen in F0 and F1 adults at 9000 ppm. Decreased body weight gains, total number of pups, delayed vaginal opening and preputial separation, and increased numbers of stillborn pups were observed in F1 and/or F2 at 9000 ppm. Decreased AGD was observed from 1000 ppm and was statistically significantly different from 3000 ppm. Severe effects on testicular histology, sperm morphology, fertility, and sexual development of the offspring occurred in both generations at 9000 ppm. Reduced testis weights in F2 and focal tubular atrophy were observed in male offspring in F1 and F2 at 3000 ppm. Focal tubular atrophy also occurred at 1000 ppm. Vacuolisation of Sertoli cells was only observed in atrophic tubuli, which were present in all exposed groups. There was no indication that Sertoli cell vacuolation preceded focal or diffuse tubular atrophy and subsequent loss of sperm production. No NOAEL for fertility or development were established as Sertoli cell vacuolation was recorded in the F1 offspring generation from the lowest dose level, 1000 ppm (113 mg/kg bw/d).

In a study by Wolfe & Layton (2003) groups of Sprague-Dawley rats (17 males and females in each group) were fed DEHP in the diet at concentrations of 1.5, 10, 30, 100, 300, 1000, 7500, and 10 000 ppm (0.1, 0.5-0.8, 1.4-2.4, 4.8-7.9, 14-23, 46-77, 359-592, and 543-775 mg/kg bw/d) for two successive generations. The F0 generation began exposure as adults. Clinical signs were generally comparable

among all groups in all generations and were not treatment-related in incidence or severity. In the F0 adults, a decreased number of live pups per litter were noted at 7500 ppm (592 mg/kg bw/d) and above. The only reproductive effects in the F0 rats occurred at 10000 ppm and included decreases in sperm counts and velocity, reductions in testis and epididymis weights, and increased numbers of rats with small testes in association with minimal-to-marked atrophy of seminiferous tubules characterized by loss of germ cells. The lowest dose level producing effects in F1 offspring was 7500 ppm (391 mg/kg bw/d) and included decreases in number of live pups/litter, reduced male AGD, delayed testes descent, vaginal opening and preputial separation.

Fertility was compromised in the F1 rats in the 10 000 ppm group which did not produce any viable litters. Other reproductive effects observed in F1 parents were similar to those observed in F0 parents but usually occurred at lower dose levels. For example, minimal to marked seminiferous tubule atrophy was noted at 10 000 ppm in the F0 and F1 males, and at 7500 ppm in the F1 and F2 males. Minimal atrophy was noted in 1 of 10 F1 males at 100 and 300 ppm. In the non-mating F1 adult males of the 300 ppm group there was a small increase in the number of animals (3 of 45) with small testes and/or epididymides (none were observed in the F0 males). The effects were not observed at the next higher dose (1000 ppm), but small testes were observed in 10 of 30 males of the 7500 ppm non-mating group. In F2 non-mating males, small testes were also observed in 1 of 21 animals at 300 ppm and 1-3 animals at 1000 ppm. Small testes and epididymides were also observed at 7500 ppm in F3 males. While Sertoli cell vaculoation was observed in seminiferous tubules of the 1000 and 7500 ppm F1 males (not 10 000 ppm males), the vacuolation was similar to that in the controls. It was concluded that the observed vacuolation resulted from distortion during fixation and processing of the tissues (ECB, 2006 and 2008). This distortion could also have obscured any minimal toxic effects that may have been present.

Increased liver weights were observed at 1000 ppm and above with accompanying histopathological changes. Decreased terminal body weights were observed at 7500 ppm and above. There was no general toxicity observed at doses below 1000 ppm. For fertility effects, the NOAEL was 1000 ppm (46 mg/kg bw/d) and the LOAEL was 7500 ppm (592 mg/kg bw/d) based on impaired litter parameters (F1 pups). For developmental effects, the NOAEL was 100 ppm (4.8 mg/kg bw/d) and the LOAEL was 300 ppm (14 mg/kg bw/d) based on small testes size and minimal seminiferous tubule atrophy in the F1 and F2 generation. The case of a single male showing atrophy of seminiferous tubules in testis at 100 ppm was not considered significant, as there were no other accompanying findings.

Co-administration studies (DEHP & DBP)

In a study by Howdeshell et al. (2007 **ND**) the effect of coadministration of DEHP and DBP were investigated. Pregnant (GD 14-18) Sprague–Dawley dams were gavaged with 500 mg/kg bw/d of each phthalate individually or together. Reproductive malformations were monitored in adult offspring and the levels of testosterone production and Insl3 mRNA were measured in ex vivo foetal testicular cultures. Maternal body weight gain was reduced compared to vehicle controls in dams treated with DEHP, DEHP plus DBP, but not DBP alone. DEHP and DBP co-administration significantly reduced litter size and increased foetal and neonatal mortality. DEHP alone and co-administered with DBP significantly increased the percent of male offspring with various reproductive malformations (significant

reduction of anogenital distance and nipples retention). The increase was significantly higher with co-administration. Analysis of the individual types of malformations indicated synergistic effect of DEHP and DBP, especially in the case of seminal vesicle agenesis where co-treatment was associated with an incidence of 63.1% ($p < 0.001$) while no malformations were found after treatment with DBP alone, and only 11.1% incidence (not statistically significant) after DEHP treatment alone. In addition, treatment with DEHP alone significantly reduced ex vivo testosterone production and mRNA synthesis for Insl3 and steroidogenic acute regulatory protein, StAR. However, the reductions were more pronounced when DEHP was co-administered with DBP, which by itself had no significant effect on insl 3. Individual DEHP and DBP treatments had no significant effect on the synthesis of P450 cyp11 mRNA. However, DEHP/DBP co-treatment significantly (58%) reduced cyp11 mRNA synthesis compared to controls.

Mode of action studies

In vitro mouse and rat cells

Tay et al., (2007 **ND**) examined in vivo and in vitro effects of MEHP on Sertoli cells in mice. Twenty-one-day old C57Bl/6N mice were given a single dose of 800 mg/kg MEHP by gavage and were sacrificed 24 h later for histological testicular analyses. Testes were also harvested from untreated mice for Sertoli cell cultures. Cultures were exposed to 0, 1, and 100 nmol/ml MEHP for 0, 3, 6, 12, and 24 h. Effects on Sertoli cell intermediate filament structure were monitored by antivimentin antibody staining. In addition, the presence of apoptotic cells was monitored by TUNEL (deoxynucleotidyltransferase-mediated dUTP nick end labeling) analysis. In vivo, MEHP treatment resulted in a dose- and time-dependent disappearance of vimentin from the testes of treated mice, as monitored by western blot analysis. The downregulation of vimentin (building block protein of intermediate filaments) correlated with disruption of the filament structure and increased number of apoptotic cells as monitored by immunohistochemistry and TUNEL analysis. Sertoli cell cultures treated in vitro showed dose- and time-dependent disappearance of vimentin associated with increased number and size of vacuoles in the Sertoli cell cytoplasm.

Zhang et al. (2008 **ND**) tested the effect of MEHP on Sertoli cells from normal 18-day-old Sprague–Dawley rats in polarized monolayer cultures. Test concentrations (10, 30, 150 and 600 μM) were chosen by the authors to correspond to the range of DEHP concentrations detected in the serum (0.03-22.78 mg/L) and semen (0.08-1.32 mg/L) of Chinese men. Pretreatment of the isolated cells with 600 μM MEHP resulted in Sertoli cells vacuolization and irregular intercellular membrane structures in the culture monolayers. Treatment of established Sertoli cell monolayers with MEHP for 24 h reduced transepithelial electrical resistance (TEER) in a dose dependent manner. Similarly, semi quantitative RT-PCR indicated that mRNA expression for the tight junction protein occludin was downregulated by the MEHP treatment in a dose dependent manner. In addition, distribution of F actin and another tight junction protein ZO-1, was reported to be affected in monolayers treated with 600 μM MEHP. The authors speculate that disruption of Sertoli cell tight junctions may be an underlying mechanism of DEHP induced reproductive toxicity in male rodents.

Downregulation of Insl3 mRNA was also reported in mouse Leydig cell cultures from newborns treated with 100, 200 and 500 mg/kg bw/d DEHP in utero from GD12 to PND3 (Song et al., 2008 **ND**). Similarly, Insl3 mRNA downregulation was reported for normal GD16 embryonal Leydig cell cultures treated in vitro with 50, 100 and 200 mg/L DEHP (Song et al., 2008 **ND**).

Nanomolar to micromolar concentration of DEHP, MEHP and two other oxidative metabolites of DEHP had no effect on basal or LH stimulated testosterone production in foetal (GD 14.5) testes preparations in vitro (Stroheker et al., 2006 **ND**). However, it is not clear how the concentrations tested in vitro relate to physiological concentrations in the tissues of foetuses exposed through the gestation period.

In vitro human cells

DEHP was not a competitive agonist at the oestrogen receptor (ER) in an in vitro competitive ligand-binding assay and did not induce ER-mediated gene expression in human breast cancer MCF-7 cells up to 10-5 M (Zacharewski et al., 1998). Similarly, DEHP (up to 10-5M) had no binding affinity for ERα or ERβ in vitro (Toda et al., 2004). DEHP, but not MEHP, demonstrated weak oestrogenic activities in a reporter gene assay in CHO-K1 cells cotransfected with either human oestrogen receptors hERα, hERβ or androgen receptor (AR) together with expression vector containing reporter gene under the regulation of ER or AR responsive elements. In this assay, DEHP showed hERα- but not hERβ-mediated oestrogenic activity as measured by activation of transcription of the reporter gene (Takeuchi et al., 2005). DEHP, but not MEHP, also demonstrated anti-oestrogenic activity via ERβ, but not antiandrogenic activity in hAR-transactivation assay (Takeuchi et al., 2005). Neither DEHP nor its metabolite MEHP displayed affinity for the human AR at concentrations up to 10^{-6} M in monkey COS cells transiently transfected with expression vectors for human AR (Parks et al., 2000).

DEHP (but not MEHP) increased proliferation of human breast cancer MCF-C7 cells (Okubo et al., 2003; Hong et al., 2005). However, the effects were not replicated in vivo as oral treatment with 600 mg/kg bw/d DEHP for 3 days did not increase expression of CaBP-9k mRNA (a gene highly regulated by 17β-oestradiol) in 7 day old female SD rats (Hong et al., 2005). DEHP did not induce oestrogenic responses in vivo in uterotrophic and vaginal cornification assays using immature and mature ovariectomised rats (Zacharewski et al., 1998).

In vivo studies in rats

DEHP, 500 mg/kg bw/d and above, administered orally to Sprague–Dawley rat dams on GD 14-18 significantly reduced both ex vivo testosterone production and Insl3 gene expression in foetal rat testes (Wilson et al., 2004; Howdeshell et al., 2007 **ND**) which are likely to result in gubernacular malformations and cryptorchidism in rats. The effect of DEHP on the incidence of gubernacular and epididymal lesions in Sprague–Dawley (SD) or Wistar rats was compared by administering of 750 mg/kg bw/d DEHP to dams during GD 14-18 (Wilson et al., 2007 **ND**). Incidences of epididymal lesions in the offspring were significantly higher in SD compared to Wistar rats, 67% and 8%, respectively. Conversely, gubernacular lesions were absent in SD rats but found at incidence of 64% in Wistar offspring. These effects were correlated with differences in the ratios between testicular testosterone (associated with epididymal lesions) and Insl3 mRNA (associated with gubernacular lesions) levels at GD18 in the two strains.

Culty et al. (2008 **ND**) treated pregnant Sprague-Dawley rats by gavage with 58, 117, 234, 469, 700, 750, 938 and 1250 mg/kg bw/d DEHP from GD14 to parturition (PND 0). Foetal testicular cells were isolated on GD 20 for analysis of ex vivo testosterone production or at GD 19 for mRNA analysis of steroidogenic enzymes by quantitative RT-PCR. Dose-dependent reductions in basal testosterone production were observed at 117 mg/kg bw/d and above, and downregulation of mRNA for steroidogenic enzymes (Cyp11a1 and Cyp17a1) was observed at 469 mg/kg bw/d and 938 mg/kg bw/d. Paradoxically, mRNA for Cyp11a1 (but not for Cyp17a1) appeared to be upregulated at 234 mg/kg bw/d. Expression of mRNA for insulin-like 3 peptide (Insl3) was downregulated at 469 mg/kg bw/d and 938 mg/kg bw/d but appeared to be upregulated at 234 mg/kg bw/d, the only lower dose tested.

The study by Li et al. (2000) where CD Sprague-Dawley pups were treated by gavage with single bolus doses of DEHP (20, 100, 200, and 500 mg/kg) or 393 mg/kg of MEHP (described in detail in section 6.2.7 *Oral*), also included biochemical analysis of the effects of DEHP and MEHP on Sertoli cells in neonatal rats. Results indicated that Sertoli cells proliferation was decreased by DEHP in a dose dependent manner as judged by decreased number of BrdU-labelled Sertoli cells. MEHP also caused a significant decrease in Sertoli cells BrdU-labelling as compared to controls. In addition, mRNA and the protein synthesis for the cell the cycle regulator, cyclin D2, was down-regulated by DEHP.

There are currently no studies examining biochemical effects of DEHP or MEHP on Sertoli cells of animals treated in utero.

6.3 Effects observed in humans

The majority of the information in this section was summarised and adopted from FDA (2002), ATSDR (2002), CERHR (2005) and ECB (2006).

6.3.1 Acute poisoning

Shaffer et al. (1945*) reported two adult male subjects who swallowed single 5 g and 10 g doses of DEHP. No symptoms resulted from the 5 g dose while the ingestion of 10 g caused mild gastric disturbances and “moderate catharsis”. Assuming 70 kg body weight, this equates to a dose of 140 mg/kg.

6.3.2 Irritation, sensitisation and allergen potentiation

In a study by Shaffer et al. (1945*), 23 human subjects were patch tested (skin of the back) with undiluted DEHP left in contact for 7 days and reapplied on the same spots 10 days later. There was no erythema or any other reaction at any time following application of DEHP.

A case control study was performed to assess the link between interior surface materials in the home and the development of bronchial obstruction (as an indicator for development of asthma) during the first two years of life (Jaakkola et al., 1999*). The results showed that the risk of bronchial obstruction was greater in the presence of PVC in the floor, but not wall materials. The risk of bronchial obstruction increased in relation to the amount of plasticiser-emitting materials in the home. In an earlier study by the same group (Oie et al., 1997), DEHP was predominant amongst different phthalates detected in sedimented dust samples

(69% of total phthalate) and suspended particulate matter samples (52% of total phthalate) taken from dwellings.

A risk assessment on indoor air quality by the EU Scientific Committee on Health and Environmental Risks (SCHER, 2007) which considered a number of various contaminants, found that DEHP is the dominant phthalate in house dust and indoor air. In a study where the concentrations of DEHP were measured (Bornehag et al., 2004*), DEHP was found associated with asthma in children at the highest exposure quartile. Long-term exposure to DEHP (Larsen et al., 2007*) and its metabolite, MEHP (Hansen et al., 2007*), together with a model allergen did not show promoting effects on the development of the allergen specific IgE antibodies. Therefore, based on the lack of mechanistic support and taking into account the low exposure level of phthalates by inhalation (Larsen et al., 2007*; Nielsen et al., 2007*), the SCHER did not find consistent scientific evidence to indicate that phthalates should be regarded as high concern chemicals in indoor air.

6.3.3 Case reports

Three preterm infants artificially ventilated through PVC respiratory tubes, developed unusual lung disorders resembling hyaline membrane disease during the fourth week of life. One infant died two weeks after birth; the other two were healthy at follow-up 20 months later. DEHP was detected in the lung after autopsy of the infant who died. The estimated inhalation exposure in the three infants ranged between 1-4200 μg/h based on the concentrations of DEHP in the condensate collected from the water traps of the respirator tubing. However, this is likely to be an over-estimate as infants were not exposed to the condensate. DEHP, but not MEHP, could be demonstrated in urine samples (Roth et al., 1988*).

6.3.4 Epidemiology studies

Polyneuropathy

Three separate studies reported the incidence of polyneuropathy in workers exposed to phthalates (including DEHP). Milkov et al. (1973*) conducted a morbidity study in the USSR on 147 workers at a PVC-processing plant. The workers were exposed to a mixture of phthalates, including DEHP as a minor constituent. The total phthalate air concentrations recorded varied between 1.7 and 66 mg/m^3. Polyneuropathy was evident in 47 workers (32%); the incidence increased with length of employment. Vestibular abnormalities were evident in 63 (78%) of 81 workers specifically examined. No reference group was included in the study.

In a cross-sectional study, symptoms and signs of polyneuropathy were reported in 12 out of 23 workers at a phthalate production plant in Italy (Gilioli et al., 1978*). The workers were exposed to a mixture of phthalates, including DEHP, but also to a lesser degree, to the corresponding alcohols and to phthalic anhydride. Total phthalate air concentrations varied between 1 and 60 mg/m^3. No referent group was included in the study.

In a study involving a Swedish PVC-processing factory, 54 male workers were examined for anomalous peripheral nervous system symptoms and clinical signs (Nielsen et al., 1985*). The workers were exposed mainly to DEHP, diisodecyl phthalate, and butylbenzyl phthalate. They were divided into three groups of approximately equal size and mean phthalate exposures of 0.1, 0.2, or 0.7 mg/m^3.

Peripheral nervous system symptoms and signs displayed were not related to the level of exposure.

Mortality/morbidity

A morbidity study was carried out on a group of workers (97 men and 4 women) employed in a German plant producing DEHP (Thiess et al., 1978c*). The average exposure period was 12 years (range: 4 months to 35 years). DEHP levels measured in ambient air were generally low (0.001-0.004 ppm, ~ 0.016-0.064 mg/m^3). Higher levels up to 0.01 ppm (~0.16 mg/m^3) were measured near the chemical reactor. Blood lipids, serum activities of liver enzymes and routine haematological tests were normal, and no excess of any pathological condition was found. There was no referent group.

A mortality study of 221 workers exposed to DEHP in the same plant was also conducted (Thiess et al., 1978b*). Eight deaths (including one carcinoma of the pancreas and one bladder papilloma) were observed (expected values of 15.9 and 17.0 from the city and county data, respectively) among the 221 workers exposed to DEHP for periods of 3 months to 24 years (average 11.5 years) (Thiess et al., 1978b*). No information about exposure levels were provided, however in two other reports by the same group, exposure levels in the plant ranged from 0.0006 to 0.01 ppm (0.01-0.16 mg/m^3) (Thiess & Fleig, 1978*; Thiess et al., 1978a*).

Chromosome aberrations

The frequency of chromosomal aberrations in blood lymphocytes was investigated in ten workers employed from 10-30 years in a DEHP production plant in Germany (Thiess & Fleig, 1978*). Exposure levels ranged from 0.0006 to 0.01 ppm (0.01-0.16 mg/m^3). There was no evidence of increased frequencies of chromosome aberrations in the exposed workers. The small number of workers examined and low levels of exposure limits the value of this study.

Cancer

Occupational exposure to polyvinyl chloride (PVC) and other products in the plastics industry was assessed in a case-control study of testicular cancer using self-administered questionnaires (148 cases and 315 controls) (Hardell et al., 1997*). An increased risk of testicular cancer was observed (as evaluated by an increased odds ratio (OR) of 6.6; 95% confidence interval: 1.4-32) for exposure to DEHP plasticised PVC, but not for other types of plastics. The authors discussed a potential oestrogenic effect of the chemicals used as plasticizers for PVC, and the increased risk of testicular cancer. However, considering that the design of the study (self administered questionnaires and occupational exposure to a number of different chemicals used in association with PVC plastics), no link could be established between testicular cancer and DEHP. This study was followed up by a larger case-control study taken from the Swedish Cancer Registry during 1993-1997 (Hardell et al., 2004). A total of 791 matched pairs completed a questionnaire regarding exposure. Overall exposure to PVC plastics gave an OR of 1.35 (confidence interval = 1.06-1.71). No dose-response relationships were found. There was no clear association between testicular cancer and exposure to PVC.

Fertility

Modigh et al. (2002*) evaluated time-to-pregnancy in the partners of men potentially exposed to DEHP by inhalation in their work environment. Median time-to-pregnancy was 3.0 months in the unexposed group, 2.25 months in the low exposure group (< 0.1 mg/m^3), and 2.0 months in the high-exposure group (0.1-0.2 mg/m^3). The authors concluded that there was no evidence of a DEHP-associated prolongation in time-to-pregnancy, although they recognized that there were few highly exposed men in their sample.

In a series of related human studies, spot urinary MEHP and semen and sperm motion parameters and sperm DNA damage were evaluated (Duty et al., 2003a; Duty et al., 2003b; Duty et al., 2004; Duty et al., 2005). The relationship between serum concentrations of reproductive hormones and MEHP urine concentrations was also assessed. Subjects included more than 150 men attending a clinic as part of a fertility evaluation. There were no significant associations between abnormal semen parameters, serum testosterone, sperm DNA damage and MEHP urine concentration above or below the group median. Jonsson et al. (2005) also studied semen parameters and urinary phthalate monoester levels in 234 military recruits. There were no significant associations between highest versus lowest urinary MEHP quartile and any of the dependent variables.

A recent pilot study with 45 male partners of subfertile couples in Michigen USA found no correlation between higher than median levels of DEHP metabolites MEHP, MEHHP or MEOHP in the urine, and sperm concentration, motility or morphology (Wirth et al., 2008 **ND**).

Endometriosis

Cobellis et al. (2003*) measured DEHP and MEHP concentrations in the plasma and peritoneal fluid of 35 women identified by laparoscopy as having endometriosis. There was no difference in the proportion of surgical patients compared to control women with detectable DEHP or MEHP (91.4% compared to 92.6% respectively). There was a significant difference in the median concentration of DEHP in the patients compared to control women (0.57 μg/mL compared to a control value of 0.18 μg/mL) but no difference in median MEHP concentration.

In another study of endometriosis, Reddy et al. (2006 **ND**) conducted an analysis of plasma phthalate levels in 85 infertile women with endometriosis compared to 135 age-matched fertile control women undergoing laparoscopic sterilisation in the same hospital. Mean plasma DEHP levels in women with endometriosis were at least 3 times higher than levels in controls. Differences were statistically significant.

Gonadotropins

Pan et al. (2006) measured the gonadotropins and gonadal hormone levels of 74 male workers exposed to elevated levels of DBP and DEHP in a PVC factory. Urinary MBP and MEHP levels (normalised to creatine) were significantly higher in exposed workers compared with 63 controls (MBP 644.3 μg/g versus 129.6 μg/g; MEHP 565.7 μg/g versus 5.7 μg/g). Circulating testosterone was significantly lower in exposed workers (8.4 μg/g) versus control workers (9.7 μg/g) and was negatively correlated with MBP and MEHP.

Thyroid hormones

Meeker et al. (2007 **ND**) reported an inverse correlation between urinary MEHP levels and blood levels of free thyroxine (T4), total triiodothyronine (T3) and thyroid stimulating hormone (TSH) in 208 men of subfertile couples seeking evaluation in one fertility centre in USA. However, no correlation between blood hormone levels was observed when analysis was restricted to late oxidative metabolites MEHHP and MEOHP. This study also investigated correlations between the blood hormone levels and the urinary levels of the primary metabolites of some other phthalates (DEP, DBP, BBzP). No positive or negative correlations could be established.

Overall, limited human studies do not identify significant associations between MEHP and adverse semen parameters, hormone levels, time-to-pregnancy, or infertility diagnoses in adults.

Reproductive system

There have also been several studies in humans where development of the male reproductive system and estimates of DEHP exposure during pregnancy or early childhood have been evaluated.

Colon et al. (2000) compared blood phthalate levels in 41 premature thelarche (beginning of breast development without other sexual development signs) patients and 35 controls. There was a statistically significant difference in average blood DEHP levels. DEHP was detected in 25 of the samples from premature thelarche patients at a mean concentration of 450 µg/L (187 - 2098 µg/L); MEHP concentration ranged from 6.3 to 38 µg/L. DEHP was detected in 5 of 35 blood samples from control patients at a mean concentration of 70 µg/L (276–719 µg/L). The reported levels in the control group were unusually high compared with the background MEHP concentration in urine in the normal population (mean 4.27, range 3.80–4.79 µg/L; Silva et al., 2004) and may reflect patient exposure to medical procedures within the hospital.

Cord blood samples were collected from 84 consecutive newborns (including a set of twins) delivered at an Italian hospital (Latini et al., 2003). DEHP and/or MEHP were detected in 74 of 84 cord blood samples with a mean (range) DEHP cord blood serum concentrations of 1.19 (0–4.71) µg/mL and MEHP of 0.52 (0-2.94 µg/mL). Mean gestational age, but no other parameter, was significantly lower in MEHP-positive neonates (38.16 weeks) versus MEHP-negative neonates (39.35 weeks). However, the levels measured in blood were unusually high compared to other studies.

Main et al. (2006) reported phthalate concentrations in pooled milk samples collected 1-3 months after birth from 65 Finnish and 65 Danish women as part of a study of cryptorchidism and hormone levels in male children. Phthalate monoesters mono-methyl phthalate (MMP), monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MBP), monobenzyl phthalate (MBzP), MEHP and mono-isononyl phthalate (MiNP) were measured in milk and gonadotropins, sex-hormone binding globulin (SHBG), testosterone, and inhibin B were measured in the serum of breast milk fed boys. Cryptorchidism was identified in 62 of the 130 children of these women. However, there was no significant association between milk phthalate concentrations and cryptorchidism.

Association between 11 maternal urinary phthalate monoester concentrations and genital parameters such as anogenital index (AGI) [i.e. anogenital distance (AGD) normalized for body weight] and testicular descent in children was investigated in 85 mother-son pairs (Swan et al., 2005). There was no significant association between maternal urinary MEHP concentration and infant AGI. However, urinary concentrations of four other phthalate metabolites MEP, MBP, MBzP and monoisobutyl phthalate (MiBP) were inversely related to AGI. This study has been criticised by McEwen et al. (2006) from the Cosmetic and Fragrance Associations of America and Europe. They suggested that AGD is more likely to be proportional to height rather than weight and that maternal phthalate urinary concentrations were not normalized for urine volume. The reliability of the measurement of AGD in humans has not been verified. One study of 87 neonates that assessed the correlation of AGD with body weight found in males a correlation of 0.48 and that body length may be a slightly better predictor for AGD than weight (Salazar-Martinez et al., 2004).

In a follow-up study, the daily phthalate exposure in the 214 mother-infant pairs from the Swan et al. (2005) study was estimated using pharmacokinetic models and measured urinary levels of phthalate metabolites (Marsee et al., 2006 **ND**). Estimated median and 95th percentile daily exposure to DEHP was 1.32 and 9.32 µg/kg bw/d, respectively.

The results from the study by Swan et al. (2005) were later subjected to a new statistical analysis including a total of 106 pairs and results from AGD measurements over two visits for 68 of the pairs (second visit 12.8 months post delivery) (Swan, 2008). In the new analysis anogenital index was calculated by dividing the measured AGD with the 95th percentile of the weight expected for the particular age of the infant in the US population instead of the weight in the original study. The authors believe that the 95th weight percentile is largely independent of weight and when included as a covariate in the analysis eliminates the confounding influence of weight. The new AG index was correlated with the maternal prenatal DEHP exposure estimated through urine metabolite measurements. It was found that there was a statistically significant inverse correlation of the AG index with the three DEHP metabolites measured. The infants cohort was also divided in three groups based on the difference between the AGD expected for the particular age/weight and measured AGD (longer, intermediate and shorter AGD) and the maternal metabolite levels were examined for each group. It was found that the levels of the three DEHP metabolites in the 'larger' AGD group was several times greater than that in the mothers of the 'shorter' group. The significance of this finding is not clear.

Rais-Bahrami et al. (2004*) examined onset of puberty and sexual maturity parameters in 14 to 16 year old adolescents (13 males and 6 females) who had been subjected to DEHP exposure via extracorporeal membrane oxygenation (ECMO) as neonates. Pubertal development was normal. Thyroid, liver and kidney function, LH, FSH, testosterone and 17β-oestradiol levels were normal for this stage of pubertal development.

In conclusion, a number of human studies have attempted to link maternal MEHP levels with gestation length, onset of puberty and AGD. Overall, these studies do not show effects of DEHP exposure on developmental parameters.

6.4 Summary

DEHP is rapidly and almost completely absorbed following oral or inhalation exposure. In contrast, bioavailability via dermal absorption is not likely to exceed 5%.

DEHP has low acute toxicity via all routes and low skin and eye irritation potential. There is no evidence of skin sensitization for DEHP in animals or humans.

Repeated exposure to DEHP in rodents is associated consistently with adverse effects on the liver (hepatomegaly, peroxisome proliferation and hepatocellular tumours), kidneys (increased weights, mineralisation of renal papilla, tubule cell pigments and chronic progressive nephropathy) and the reproductive organs mainly in males. Testicular toxicity manifests as decreased testes weights, testicular atrophy, increased bilateral aspermatogenesis, immature or abnormal sperm forms, seminiferous tubular degeneration, Sertoli cell vacuolation or complete loss of spermatogenesis.

For hepatotoxicity a NOAEL is established at 28.9/36.1 mg/kg bw/d for males and females respectively based on significant increases in serum albumin, absolute and/or relative liver weights and peroxisome proliferation at the LOAEL doses of 146.6/181.7 mg/kg bw/d for m/f in a 104-week rat dietary study. Knock-out mice studies demonstrate that the major molecular mechanism underlying hepatotoxicity of DEHP in rodents involves activation of peroxisome proliferator activated receptor alpha (PPARα).

The same study establishes the NOAEL for kidney toxicity at 28.9/36.1 mg/kg bw/d for males and females respectively based on increases in absolute and relative kidney weights at the LOAEL dose of 146.6 mg/kg bw/d. There is no information related to the mechanism underlying renal toxicity but it appears not to be related to activation of PPARα.

For testicular toxicity a NOAEL is established at 3.7 mg/kg bw/d from a 13-week rat dietary study based on increased incidence of Sertoli cell vacuolation at the LOAEL dose of 37.6 mg/kg bw/d.

Multigenerational studies reveal that DEHP exposure is associated not only with reproductive organ toxicity but also with adverse effects on reproductive outcomes in rodents. A NOAEL for fertility effects is established at 14 mg/kg bw/d in mice, based on a decreased number of litters and viable pups at the LOAEL dose of 140 mg/kg bw/d. Moreover, a cross-over mating trial at the highest dose of 425 mg/kg bw/d showed that fertility of both sexes was affected by the exposure to DEHP. Interestingly, while testicular histomorphology was affected at high doses in this study, fertility effects in females were not correlated with any obvious organ toxicity.

Parental and early-postnatal exposure to DEHP in rats also affects reproductive development of the progeny, particularly the males. A NOAEL for developmental toxicity in male rats is established at 1.2 mg/kg bw/d based on increased testes weight in the progeny at the LOAEL dose of 5 mg/kg bw/d. The NOAEL for female developmental toxicity is 5 mg/kg bw/d, based on significant delay in vaginal opening at the LOAEL dose of 15 mg/kg bw/d.

In a three-generational dietary study with rats a LOAEL for male developmental toxicity is established at 14 mg/kg bw/d based on decreased testes weight and seminiferous tubule atrophy in F1 and F2 generations. The NOAEL in this study is 4.8 mg/kg bw/d.

Biochemical analyses in rodents show that DEHP exposure is associated with alterations in Leydig cell steroidogenesis, serum levels of testosterone and luteinizing hormone (LH), and expression of genes crucial for development of the male reproductive system. A LOAEL of 10 mg/kg bw/d is established based on increased serum LH and testosterone levels in rats exposed to DEHP for 28 days during PND 21-48. The NOAEL in this study is 1 mg/kg bw/d.

Overall, rodent studies suggest that the type and severity of reproductive effects from DEHP exposures depend on the time and duration of dosing, and also the age at which effects are monitored. Generally, younger animals are more sensitive than older animals.

Lifetime dietary exposures to DEHP is associated with dose-dependent increases in the incidence of Leydig cell tumours in one rat study. However, overall, data are insufficient to determine an association between DEHP exposures and testicular neoplasms. Mononuclear cell leukaemia (MCL) was also observed inconsistently in rat studies.

In humans, studies of potential effects of DEHP on fertility and development are limited and generally based on examining correlations between urinary metabolite levels and reproductive parameters. Overall, available studies do not identify significant, consistent associations between DEHP exposures and reproductive parameters either in adults or children.

7. Human Health Hazard Characterisation

This section provides a brief overview of the main features of the available toxicity data, identifies the critical end-points and their no-observed-effect levels, and discusses the relevance of the effects observed in animal studies to humans.

7.1 Toxicokinetics

DEHP is rapidly and almost completely absorbed from the gastrointestinal tract following oral administration. The bioavailability of DEHP via the oral route is estimated to be 100% for both adults and children. In contrast, absorption of DEHP via the skin is significantly lower. The extent of dermal absorption in vivo was determined to be about 9% and 26% in rats and guinea pigs, respectively. Comparison studies in vitro demonstrate that human skin is significantly less permeable (4-fold) to DEHP than rat skin. Therefore, bioavailability of dermally applied DEHP in humans is not likely to exceed 5%. The mean dermal absorption rate of DEHP from PVC plastic film applied to rat skin was determined to be 0.24 $\mu g/cm^2/h$.

Case studies of transfusion and haemodialysis patients and occupationally exposed workers indicate absorption of DEHP can occur via both inhalation and parenteral routes, however, quantitative data for absorption of DEHP via the respiratory tract are not available. A substantial proportion of DEHP in aerosols may also become bioavailable via the gastrointestinal tract rather than the respiratory tract. The bioavailability of DEHP via the inhalation route in humans is estimated to be 100%.

Studies in rats and monkeys show the liver, kidney, testes and blood as the main sites of distribution following orally administered DEHP, however, DEHP and metabolites do not accumulate in tissues. DEHP and/or its metabolites have been detected in foetal tissues demonstrating that they can cross the placenta.

The first metabolic step is the hydrolysis of DEHP to MEHP and 2-EH by tissue lipases. MEHP is further metabolised via oxidative reactions resulting in the formation of numerous metabolites and a small amount of phthalic acid. Elimination of metabolites and minimal quantities of the parent DEHP occurs mostly via urine and faeces. A recent human study noted that 75% of orally administered DEHP was eliminated as metabolites via urine within 2 days.

7.2 Acute toxicity, irritation and sensitisation

In experimental animals, DEHP exhibits low acute oral, dermal and inhalation toxicity. Intravenous and intraperitoneal administration of DEHP results in higher acute toxicity than oral or dermal administration, however, the acute toxicity via these routes is still low. DEHP induced minimal skin and eye irritation in animals and did not induce skin irritation in human volunteers. Data are insufficient to determine the respiratory irritant potential of DEHP. DEHP is not a skin sensitiser in animals and limited data indicate no sensitisation reactions in humans. Human

studies indicate correlations between the risk of bronchial obstruction and plasticiser-emitting components of the indoor environment. However, there is currently insufficient evidence supporting a causal relationship between respiratory effects and DEHP.

Therefore. DEHP is expected to have low acute toxicity in humans.

7.3 Repeated dose toxicity

The toxicity of DEHP has been evaluated in a number of animal species, in both short-term (few weeks) and life-time studies by several routes of exposure. The most pronounced effects are on the liver (hepatomegaly, peroxisome proliferation), kidney (increased organ weights, mineralisation of renal papilla, tubule cell pigments and chronic progressive nephropathy) and testes (atrophy, vacuolated Sertoli cells, multinucleated gonocytes, Leydig cell hyperplasia). Carcinogenicity studies in rodents reported statistically significant dose-related increases in the incidence of hepatocellular and Leydig cell tumours.

Exposure to DEHP during gestation and sensitive age periods in rodents also causes significant effects on reproductive parameters and development.

The effects of DEHP observed in animal studies and their relevance to humans are discussed in detail below.

7.3.1 Liver and kidney effects

Liver effects

Liver effects have been reported in several rodent species. In rats, hepatotoxicity was indicated by significant increases in serum albumin, absolute and/or relative liver weights and peroxisome proliferation at 146.6 mg/kg bw/d and above (Moore, 1996*). The NOAEL for these effects was 28.9 mg/kg bw/d. A similar NOAEL, 25 mg/kg bw/d, was established based on hepatic changes after sub-chronic intravenous exposure in rats (Sjoberg et al., 1985b*). The liver effects induced by oral administration of DEHP in rodents were not reported in oral administration studies with marmoset monkeys (Kurata 1998; Tomonari et al., 2006).

Further studies have shown that the liver effects induced by DEHP in rodents (hepatomegaly, peroxisome proliferation) are largely mediated through activation of peroxisome proliferator-activated nuclear receptor alpha (PPARα) (Ward et al., 1998; Lapinskas et al., 2005). In other species, such as Syrian hamsters, guinea pigs and monkeys, activation of PPARα by DEHP was significantly lower or not observed (Lake et al., 1984; Rhodes et al., 1986; Short et al., 1987).

Studies with hypolipidaemic agents in humans have provided no evidence of peroxisome proliferation or increased hepatocyte division (Bentley et al., 1993*; Ashby et al., 1994*; Cattley et al., 1998*). The comparative unresponsiveness of the primate liver to peroxisome proliferators has been explained on the basis of decreased tissue levels of PPARα, genotypic variations rendering the primate liver receptor less active compared to rodents, and species differences in phthalate hydrolysis and production of active phthalate metabolites (Tugwood et al., 1996; Palmer et al., 1998 and Woodyatt et al., 1999).

Overall, the mechanisms by which DEHP and other peroxisome proliferators induce chronic hepatotoxicity in rodents are not considered relevant to humans.

Kidney effects

DEHP-associated toxicity was consistently observed in kidneys of rats and mice (Moore, 1996*; Moore, 1997*). From the 104-week rat dietary study of Moore (1996*), a LOAEL was established at 146.6 mg/kg bw/d, based on increased absolute and relative kidney weights. Mineralization of renal papilla, tubule cell pigmentation and chronic progressive nephropathy was observed at higher doses. The NOAEL for kidney effects was 28.9 mg/kg bw/d.

No information related to kidney toxicity is available in monkeys.

Human studies on DEHP-induced toxicity to kidneys are not available.

The mechanism of DEHP-related toxicity to kidneys is not clear but it appears that it is not related to peroxisome proliferation as kidneys lesions were found in both PPARα-null and wild-type mice (Ward et al., 1998).

Given the lack of information on DEHP-induced kidney toxicity in primates (including humans), the relevance to humans of kidney effects observed in rats cannot be excluded.

7.3.2 Testicular effects

Testicular toxicity manifesting as decreased weights, testicular atrophy, increased bilateral aspermatogenesis, immature or abnormal sperm forms, seminiferous tubular degeneration, Sertoli cell vacuolation or complete loss of spermatogenesis was evident in repeated dose studies in rats. In a 13-week rat dietary study, a LOAEL of 37.6 mg/kg bw/d was established based on an increased incidence of Sertoli cell vacuolation (Poon et al., 1997). Significantly decreased absolute and relative testicular weights, mild to moderate seminiferous tubule atrophy and Sertoli cell vacuolation were observed at higher doses. The NOAEL was 3.7 mg/kg bw/d.

The consistent finding of testicular effects in rats and mice is in contrast to those from studies in marmosets (Kurata et al., 1998; Mitsubishi Chemical Safety Institute, 2003*; Tomonari et al., 2003* and 2006). In these studies, no significant treatment-related changes in testicular histology or more gross parameters were observed from oral exposures to DEHP of up to 2500 mg/kg bw/d. However these studies are very limited in number and may not cover critical windows for testicular toxicity especially in young and developing animals.

Therefore, although there were no reports of DEHP-induced testicular toxicity in primates, the relevance to humans of the effects observed in rats cannot be excluded based on the plausible mode of action discussed in detail below.

7.4 Carcinogenicity

7.4.1 Hepatocellular tumours

In mice and rats, DEHP induced significant dose-dependent increases in the incidence of hepatocellular tumours. At low doses, there was no evidence of liver toxicity or increase in hepatocellular tumours, suggesting a threshold for this effect (Moore, 1996*; Ito et al., 2007 **ND**).

The LOAEL and the NOAEL for tumour induction in rats were established as 146.6 mg/kg bw/d and 28.9 mg/kg bw/d, respectively (Moore, 1996*). In mice, the

LOAEL and the NOAEL for induction of liver tumours were 292 mg/kg bw/d and 98 mg/kg bw/d, respectively (Moore, 1997*).

The evidence suggests that, similar to chronic hepatotoxicity, peroxisome proliferation combined with suppression of hepatocellular apoptosis could also be the major mechanism for DEHP-induced hepatocarcinogenicity in rodents (Ward et al., 1998; Lee et al., 1995; Peters et al., 1997; Ito et al., 2007 **ND**). No studies showing an association between DEHP exposure and liver neoplasms in humans have been reported.

Klaunig et al. (2003*) analysed the relationship between animal bioassays of carcinogenicity mediated through PPARα and their relevance for human carcinogenicity. Species differences in reactivity to peroxisome proliferators with respect to hepatomegaly, peroxisome proliferation and tumour formation between rodents and primates have also been reviewed by O'Brien et al. (2005). Based on the overall information, the mechanisms by which DEHP and other peroxisome proliferators induce chronic hepatotoxicity and hepatocarcinogenicity in rodents are regarded as not relevant for humans.

7.4.2 Mononuclear cell leukaemia

Mononuclear cell leukaemia (MCL) was reported in one of two rat carcinogenicity studies (Moore 1996*) and in neither of two mouse carcinogenicity studies. This tumour type is well known to occur spontaneously with high incidence in F344 rats and is rare in other rat strains. This neoplasm has not been found in other mammalian species and has no histologically comparable tumour type in humans (Caldwell, 1999b).

Therefore, DEHP-induced MCL observed in rats is not considered relevant for humans.

7.4.3 Leydig cell tumours

In a single lifetime dietary study with Sprague-Dawley rats, DEHP was associated with increased incidence of Leydig cell tumours (Voss et al., 2005). In this study, the NOAEL for both hepatic tumours and testicular tumours was determined to be 95 mg/kg bw/d. However, the dose-related trend of increased Leydig cell tumours was observed commencing from the lowest dose of 30 mg/kg bw/d.

Leydig cell tumours were not reported in other studies with F344 rats even at higher doses (Moore, 1996*; Kluwe et al., 1982*; NTP, 1982*). Notably, spontaneous Leydig cell tumours are not common in Sprague-Dawley in contrast to F344 rats (Prentice and Meikle, 1995). DEHP does not appear to induce testicular neoplasias in B6C3F1 mice (Moore, 1997*; Kluwe et al., 1982*; NTP, 1982*).

The involvement of PPARα in DEHP-mediated testicular toxicity, including Leydig cell hyperplasia, is not considered very likely based on the occurrences of testicular toxicity in PPARα-null mice (Ward et al., 1998). In addition, several other phthalates that activate PPARα were not associated with testicular toxicity, suggesting that hepatic and testicular toxicity are mediated through different pathways that may under some circumstances share common cofactors or targets depending on their tissue distribution (Corton and Lapinskas, 2005).

Mechanisms for chemical induction of Leydig cell tumourogenesis in rodents and their relevance to humans were reviewed extensively by Cook et al. (1999). The review highlights that in rats, the majority of non-mutagenic chemicals associated with Leydig cell hyperplasia or tumours, ultimately result in increases in serum levels of luteinizing hormone (LH) and/or modulate Leydig cell responsiveness for LH-mediated processes, such as steroidogenesis. Such perturbations, if sustained, can cause Leydig cell hyperplasia and tumours. Paracrine factors produced by Sertoli cells and seminiferous tubules also appear to affect proliferation of Leydig cells and their precursors (Cook et al., 1999). Considering the similarities in regulatory pathways within the hypothalamic-pituitary-thyroid (HPT) axis of rats and humans, it was postulated that chemicals which induce Leydig cell tumours in rats by disrupting regulatory mechanisms within the HPT axis are likely to have similar effects in humans. However, susceptibility of humans to hyperplasia and tumours may be less than rodents, as human Leydig cells are less sensitive to the proliferative effects of LH (Cook et al., 1999).

Studies related to DEHP-induced testicular carcinogenicity in humans are limited and contradictory (Hardell et al., 1997*; Hardell et al., 2004). A single occupational case-control study (Hardell et al., 1997*) suggested an increased risk of testicular cancer from DEHP in the PVC industry. However, a larger follow-up study (Hardell et al., 2004) did not support this finding.

Overall, considering that DEHP-related testicular toxicity in rodents includes modulation of Leydig cell steroidogenesis, disruption of Sertoli cell structure/function and also effects on serum testosterone and LH levels, it is plausible that DEHP may also induce Leydig cell hyperplasia in humans, through disruption of the HPT axis. However, although the available data are inadequate to determine a reliable NOAEL for DEHP-induced Leydig cell tumours, the differences in sensitivity between rat and human Leydig cells, discussed above, suggest that the no observed effect levels derived from rat bioassays related to Leydig cell toxicity and steroidogenesis would provide adequate reference for examination of margins of exposure in humans, and they are further discussed in the following sections.

7.5 Reproductive toxicity

The reproductive and developmental toxicity of DEHP in rodents exhibited as perturbations in testicular structure and function, altered steroidogenesis and developmental malformations of the urinary tract and gonads. At high doses, other effects such as decreased anogenital distance (AGD) and nipples retention were also observed in males.

7.5.1 Fertility

Perturbations of testes structure and function are consistently observed in chronic studies examining the general toxicity of DEHP. In addition, numerous experimental animal studies, mostly using oral administration in rats, have been conducted to specifically examine the effects of DEHP on reproductive parameters.

For effects on fertility, a NOAEL of 14 mg/kg bw/d is derived from a continuous breeding study exposing both male and female adult CD-1 mice to DEHP via diet (Lamb et al., 1987). The LOAEL was 140 mg/kg bw/d based on decreased litters and viable pups. At this dose, no significant histological effects were observed,

however, at higher doses decreased weights of male reproductive organs including testes, epididymes, prostate and seminal vesicles, bilateral atrophy of the seminiferous tubules, decreased sperm motility, sperm concentrations and complete infertility were evident. Decreases in fertility outcomes were not necessarily linked only to male infertility. A cross-over mating trial at the highest dose of 425 mg/kg bw/d showed that both sexes were affected by exposure to DEHP.

Continuous breeding dietary studies in rats (Schilling et al., 2001; Wolfe & Layton, 2003) also demonstrated effects on fertility and development of offspring. No NOAELs for fertility or development were established in the study by Schilling et al. (2001) as Sertoli cell vacuolation was observed in F1 offspring from the lowest dose level of 113 mg/kg bw/d. The study by Wolfe and Layton (2003) demonstrated adverse effects on fertility in F0 adults at 592 mg/kg bw/d and above, manifesting as decreased number of live pups per litter. At higher doses, histopathological effects on the testes were apparent. However, similar reproductive effects were observed at lower doses in F1 generation parents. For fertility effects, the NOAEL was 46 mg/kg bw/d and the NOAEL for developmental effects was 4.8 mg/kg bw/d (discussed further below).

For testicular histopathology related to Sertoli cell vacuolation, a NOAEL and LOAEL of 3.7 and 38 mg/kg bw/d, respectively, were identified in a 13-week rat dietary study by Poon et al. (1997) based on a dose-dependent Sertoli cell vacuolisation in male rats. At the highest dose of 375.2 mg.kg bw/d, bilateral, multifocal, or complete atrophy of the seminiferous tubules with complete loss of spermatogenesis was also seen.

Studies in rats suggest that DEHP-mediated fertility effects may also result from alterations in Leydig cell steroidogenesis, which are dependent on the age of the animal and the duration of treatment. Akingbemi et al. (2001; 2004) demonstrated that younger Long-Evans rats appeared more sensitive than older postpubertal rats for DEHP-related perturbations in Leydig cell steroidogenesis and serum levels of testosterone and LH. From these studies, a NOAEL of 1 mg/kg bw/d was established based on increased serum LH and testosterone levels in rats exposed to 10 mg/kg bw/d for 28 days during PND 21-48. This effect correlated with increased basal and LH-stimulated testosterone production ex vivo in Leydig cell preparations from these animals.

Testicular effects were not observed in studies of DEHP in marmoset monkeys. However, it is noted that the number of studies examining fertility effects in marmosets are limited and that some effects on female reproductive organs have been reported in one study (MCSI, 2003*).

In humans, available studies on fertility effects of DEHP are limited, generally examining correlations between urine levels of DEHP metabolites and male and female reproductive health. Overall, these studies do not identify significant associations between MEHP and adverse semen parameters, hormone levels, time-to-pregnancy, or infertility diagnoses in adults. However, a single recent occupational study (Pan et al., 2006) suggests that circulating testosterone levels are reduced in male workers exposed to DEHP and DBP.

7.5.2 Developmental toxicity

Numerous single and multiple-generation studies show DEHP-related developmental effects in rodents.

DEHP induced overt structural malformations (predominantly of the tail, brain, urinary tract, gonads, vertebral column and sternum) in rats exposed to 1000 mg/kg bw/d during the critical period of development (GD6–15) (BASF, 1995*; Hellwig et al., 1997). More subtle effects, such as changes in AGD, were also recorded in a number of other studies. Based on reduced AGD, a LOAEL of 113 mg/kg bw/d was determined in rats (the lowest dose tested and was not maternotoxic) (Schilling et al., 2001).

In a postnatal developmental study with Wistar rats exposed to DEHP during gestation and lactation (GD6 to PND21), a NOAEL for developmental toxicity was established at 1.2 mg/kg bw/d, based on increased testes weight in prepuberal rats at 5 mg/kg bw/d. These weight increases were not associated with any histopathological or biochemical alterations (Andrade et al., 2006). In a continuation of the study, a NOAEL for female developmental toxicity was established at 5 mg/kg bw/d, based on a significant delay in vaginal opening observed at 15 mg/kg bw/d in female offspring (Grande et al., 2006).

Overall, the critical study for developmental toxicity of DEHP is a 3-generational dietary study in Sprague Dawley rats where a NOAEL of 100 ppm (4.8 mg/kg bw/d) was established based on decreased testes weight and seminiferous tubule atrophy at 1000 ppm (14 mg/kg bw/d) (Wolfe & Layton, 2003). At higher levels of exposure, decreased in utero survival, reduced AGD, undescended testes, retained nipples/areolae, incomplete preputial separation and disruption of spermatogenesis in the F1 and F2 generations were also observed.

Strain specific differences are noted in the incidence of specific developmental malformations from DEHP exposure in rats (Wilson et al., 2007 **ND**). The same dose of DEHP was associated with a higher incidence of epididymal malformations in Sprague–Dawley rats while gubernacular malformations were more prevalent in Wistar rats.

One study in marmoset monkeys (MCSI, 2003*) suggested that increasing DEHP doses could be associated with delay in the onset of puberty in male marmosets. However, mean serum testosterone levels were highly variable, and minimal effects on testicular structure or function was reported. The NOAEL was the highest tested dose of 2500 mg/kg bw/d. The lowest tested dose was also relatively high 100 mg/kg bw/d. The exposure in this study was from 90–115 days (juvenile) to 18 months (young adulthood) and may not have been at the crucial age window for reproductive development in marmosets.

In humans, a number of studies have been conducted examining correlations between maternal MEHP levels and gestation length, onset of puberty and AGD. Overall, these studies do not provide convincing evidence of developmental effects from DEHP exposure in humans. This is related to the low power of studies due to small sample size, not representative sample (usually one study centre) and also uncertainties about the significance of the measured endpoints, for example AGD, as an indicator of developmental toxicity in humans.

7.5.3 Mode of action

Although DEHP appears to act as an anti-androgen in rodents, neither DEHP nor its metabolite MEHP displayed affinity for the oestrogen or androgen receptor in vitro (Zacharewski et al., 1998; Parks et al., 2000; Toda et al., 2004) suggesting that DEHP is not an androgen receptor antagonist.

The majority of data on the reproductive toxicity of DEHP and other related phthalates (reviewed most recently by Foster (2005), David (2006) and Ge et al., (2007)) supports a mode of action that includes effects on steroidogenesis and expression of genes critical for development of the reproductive system in rodents.

DEHP was shown to down-regulate testosterone production and/or alter mRNA synthesis for several proteins (StAR, Cyp11a1, Cyp17a1 and Insl3) involved in steroidogenesis and testicular development (Wilson et al., 2004; Howdeshell et al., 2007n; Song et al., 2008**ND**; Zhang et al., 2008 **ND**).

Toxicity to Sertoli cells through effects on proteins involved in cell cycle regulation is also indicated by some studies. In neonatal rats, DEHP down-regulated synthesis of the cyclin D2 mRNA and decreased Sertoli cell proliferation (Li et al., 2000). In addition, alterations in communication between Leydig and Sertoli cells may also play a role in testicular and developmental toxicity. In vitro treatment of rat Sertoli cells with MEHP resulted in cell vacuolization, perturbations of the intercellular membrane structures and distribution of tight junction specific proteins (Zhang et al., 2008 **ND**).

The exact mechanism(s) underlying reproductive toxicity of DEHP have yet to be fully elucidated. However, studies consistently demonstrate that the mechanism(s) ultimately lead to interference with endocrine function and thereby influence sexual differentiation and function. Therefore, considering that the components of the postulated mode of action in rodents are applicable to humans, the reproductive toxicity of DEHP observed in rodents is regarded as relevant for humans.

7.6 Summary

DEHP has low acute toxicity and is not a skin or eye irritant.

Significant toxic effects are related to repeated DEHP exposure and target organs include liver (hepatomegaly, peroxisome proliferation, hepatocellular tumours), kidneys (increased kidney weights, mineralisation of renal papilla, tubule cell pigments and chronic progressive nephropathy) and the reproductive system, particularly in males (testicular toxicity in males exposed to DEHP before and after birth resulting in effects on the development of reproductive system and also fertility as adults).

The molecular mechanism associated with liver toxicity in rodents includes activation of peroxisome proliferators activated receptor alpha (PPARα), a mechanism that is not considered relevant for humans.

The mechanism of DEHP-related toxicity to kidneys is not clear but it appears that it is not related to peroxisome proliferation and its relevance to humans cannot be excluded.

Effects on the reproductive system include: alterations in testes weights, testicular atrophy, increased bilateral aspermatogenesis, immature or abnormal sperm forms,

seminiferous tubular degeneration, Sertoli cell vacuolation or complete loss of spermatogenesis in repeated dose studies with young rats and rats treated during gestation and lactation. Decrease of AGD, undescended testes, retained nipples/areolae, incomplete preputial separation and disruption of spermatogenesis in progeny at higher doses also indicate effects on the development of the male reproductive system.

Effect on in utero survival of foetuses, number of litters and survival per litter demonstrate effects on parental reproductive functions.

Overall, the studies in rodents suggest that the type and severity of the observed effects of DEHP on parameters of reproductive toxicity depends on the time the dosing started, duration of treatment and also the age at which effects are monitored. Generally, younger rats are more sensitive than older and gestational exposure causes severe developmental effects with the reproductive system being affected at lowest doses.

Studies in humans did not report significant adverse association between DEHP exposure and parameters of reproductive system function or development in adults or neonates.

However, elements of the plausible mode of action for these effects, which includes perturbations of steroidogenesis and the expression of genes associated with the development of the male reproductive system, are common in rodents and humans. Therefore, the reproductive toxicity of DEHP is regarded as relevant for humans and is considered for risk characterisation in this assessment.

8. Human Health Risk Characterisation

8.1 Methodology

A margin-of-exposure 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 (MOE) 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 a Margin of Exposure (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 DEHP exposure through use of:

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

8.2 Critical health effects

Adverse effects to human health are characterised in detail in Section 7.

The most pronounced adverse effects associated with repeated exposure to DEHP in animals, predominantly rodents, include effects on the liver (hepatomegaly, peroxisome proliferation), kidney (increased organ weights, mineralisation of renal papilla, tubule cell pigments and chronic progressive nephropathy) and testes (atrophy, vacuolated Sertoli cells, multinucleated gonocytes, Leydig cell hyperplasia).

Hepatic toxicity of DEHP is not considered relevant to humans based on the plausible mode of action in rodents, which includes activation of peroxisome

proliferator-activated nuclear receptor alpha - PPARα, and the comparative unresponsiveness of the primate liver to peroxisome proliferators.

The most appropriate NOAEL for risk estimates of kidney toxicity in Section 8.3 is 28.9 mg/kg bw/d, identified in a 104-week dietary study with rats (Moore et al., 1996*)

Carcinogenicity studies in rodents show statistically significant dose related increases in the incidence of hepatocellular and Leydig cell tumours. However, DEHP-induced hepatic carcinogenicity is not considered a critical effect for this human health risk assessment as the mechanism of action is related to induction of peroxisome proliferation.

Non-mutagenic induction of Leydig cell tumourogenesis in rodents is generally associated with disruption of regulatory mechanisms within the hypothalamic-pituitary-thyroid (HPT) axis which are considered relevant to humans (Cook et al., 1999). Considering that DEHP-related testicular toxicity includes modulation of Leydig cell steroidogenesis and effects on serum testosterone and LH levels in rodents (Section 6.2.7 and 7.5), it is considered plausible that DEHP may also induce Leydig cell hyperplasia through disruption of the HPT axis in rodents and in humans although, the general susceptibility in humans appears to be lower than in rodents (Cook et al., 1999). Data are inadequate to determine a reliable NOAEL for DEHP-induced Leydig cell hyperplasia in animals or humans. However, the critical effect of DEHP on steroidogenesis and testicular function is considered further under the umbrella of male reproductive toxicity.

Reproductive toxicity of DEHP in rodents is associated with adverse effects on fertility and development with mice being less sensitive than rats. In particular, DEHP reproductive toxicity exhibited as perturbations in testicular structure and function, altered steroidogenesis and developmental malformations of the urinary tract and gonads after prenatal and early postnatal exposure (Section 6.2.7). Developmental effects of DEHP on the reproductive system of female rodents are also observed although the number of studies monitoring these effects in females is limited.

Reproductive toxicity following DEHP exposure of adults is followed by effects on fertility parameters from decreased litters and viable pups to complete infertility. Both sexes are affected as indicated in crossover studies of treated and untreated animals.

Table 8.1 summarizes the critical studies for reproductive and developmental toxicity in rodents.

The consistent finding of testicular effects in rats and mice is in contrast to those from studies in marmosets in which no significant treatment-related changes in testicular histology or more gross parameters were observed after oral exposures to DEHP of up to 2500 mg/kg bw/d (Kurata et al., 1998; Mitsubishi Chemical Safety Institute, 2003*; Tomonari et al., 2003* and 2006). However, it is noted that the number of studies examining fertility effects in marmosets are very limited and, in contrast to the vast number of rodent studies, the marmoset studies do not examine extensively the exposure at different stages which may be critical for reproductive and other aspects of the marmosets' development.

Available studies on fertility effects of DEHP in humans are limited (Section 6.3), and do not identify significant associations between urine MEHP levels and adverse semen parameters, hormone levels, time-to-pregnancy, or infertility diagnoses in adults. However, a single recent occupational study (Pan et al., 2006) suggests that circulating testosterone levels are reduced in male workers exposed to DEHP and DBP.

Developmental toxicity of DEHP in humans does not appear significant as determined by a number of studies examining correlations between maternal MEHP levels and gestation length, onset of puberty and AGD (Section 6.3).

Table 8.1: Critical studies for determination of NOAEL for risk characterisation

Toxicity observed	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Effect at LOAEL	Species and age at treatment	Reference
Testes/ Fertility	3.7	38	Sertoli cell vacuolation	Rat 4-6 weeks old	Poon et al., (1997)
Testes/ Development	1.2 (m)	5 (m)	↑ testes weight in F1	Adult rats (F0) and offspring (F1) exposed indirectly through lactation up to PND 21	Andrade (2006)
	5 (f)	15 (f)	delay in vaginal opening in F1		Grande (2006)
Testes/ Fertility	1	10	↑ LH and testosterone levels in serum for group treated PND 21-48	Rats treated at different stages from PND21-62	Akingbemi et al. (2001; 2004)
Fertility/ Development	14	140	↓ number of litters viable per litter in F0	Adult mice (F0)	Lamb et al., (1987)
Fertility/ Development	4.8	14	↓ testes wt, seminiferous tubule atrophy in F1 and F2	Adult rats (F0) and offspring (F1/F2)	Wolfe & Layton (2003)

m-male; f-female

The exact mechanism(s) of DEHP induced reproductive toxicity have not yet been fully elucidated (Section 7.5.3). However, studies in rodents consistently demonstrate that DEHP can interfere with components of the endocrine system and thereby influence sexual differentiation and function. The components of the postulated mode of action in rodents are applicable to humans and therefore, DEHP reproductive toxic effects seen in rodents are regarded as relevant for humans.

Taken together, noting effects of dose spacing and inherent biological variability, the studies summarized in Table 8.1 support a NOAEL for fertility and developmental effects from DEHP in the dose range of 1–10 mg/kg bw/d. Within this range, the most appropriate NOAEL for risk estimates in adults and children is

considered to be that determined from the multigenerational study by Wolfe & Layton (2003) of 4.8 mg/kg bw/d.

8.3 Risk estimates

8.3.1 Risk estimate related to use of toys and childcare articles

The two dominant routes of exposure to DEHP through the use of plastic toys and childcare articles are dermal exposure during normal handling of toys and childcare articles and oral exposure during intentional or inadvertent chewing, sucking and biting of these products.

The combined internal dose for children, arising from contact with toys and childcare articles is discussed in Section 5.2.5 and summarised in Table 8.2. Two major exposure scenarios are considered for children using toys and childcare articles, a "typical" and a reasonable "worst-case" scenario. The reasonable worst-case scenario takes into account the maximal mouthing time of 3 hours per day identified for children aged 6-12. The typical scenario considers the mean daily mouthing time of 0.8 h/day 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 month 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, it is assumed that the mouthing behaviour of Australian children is similar. Additional assumptions considered are as follows:

- Maximal and typical migration rate for DEHP 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 57.93 μg/cm^2/h. The mean migration rate, which is applied to the typical exposure scenario, is 26.03 μg/cm^2/h (Chen, 1998)
- Bioavailability of DEHP via the oral route is assumed to be 100%
- Dermal absorption of DEHP from PVC matrix is 0.24 μg/cm^2/h.

Table 8.2: 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	27.8	231.7
Dermal	2.6	9.6
Combined	30.4	241.3

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

Estimation of margin of exposure

Risk estimates take into account the likelihood for renal and reproductive effects at future life stages related to long term exposure through repeated handling and mouthing of toys. Table 8.3 provides the margins of exposure (MOE) estimated

from the internal DEHP dose in children and the dose at which no adverse effects were observed on the kidneys or the reproductive systems in animal systems, i.e. the NOAEL.

Table 8.3: Calculated MOE in children for chronic effects from estimated exposure to toys and childcare articles

Toxicity	NOAEL mg/kg bw/d	MOE for typical scenario exposure	MOE for worst case scenario exposure
Reproductive	4.8	157	20
Kidney	28.9	950	120

The risk estimates for kidney toxicity, in both scenarios of toy use by children derive MOEs above 100 (Table 8.3) and hence indicate low risk of adverse effects on kidneys. The risk characterisation for DEHP exposure of children from use of toys and childcare articles indicates that under typical conditions of toy use the MOE for children for reproductive toxicity is marginally above 100 (Table 8.3). However, the MOE for the worst case scenario (Table 8.3) is significantly less than 100 indicating a risk of adverse effects in this scenario.

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 DEHP arise mainly from inadequate data and include:

- absence of DEHP-specific data for migration from PVC
- absence of Australian-specific data on DEHP content in toys
- absence of Australian-specific data on children's mouthing behaviours, and
- lack of data on the health effects of DEHP in young and/or adult humans following repeated exposure.
- lack of extensive studies in primate animal models such as marmosets.

These data gaps contribute to the uncertainty in the risk estimates of DEHP toxicity in humans. In addition, this risk estimate does not take into account possible cumulative toxic effects from co-exposure to other phthalates with similar modes of action.

Areas of concern

The MOE for the worst case scenario (Table 8.3) is significantly less than 100 which represents a concern for individual children for whom toy use patterns and total contact with DEHP from toys are higher than typical. Considering the uncertainties within the risk estimates in this assessment, the type of the toxicity and the specific sensitivity of developing reproductive organs during the first few months after birth, there is a risk of reproductive toxicity in young children even for the typical exposure scenario with a MOE marginally above 100. Exposure to DEHP from application of personal care products such as baby lotions and creams

(Table 5.5) would contribute to further decrease of MOE estimated for exposure through the use of toys and childcare articles.

A MOE of 100 or greater is usually not regarded as an indication of concern as it encompasses the conservative default uncertainty factors of 10 each for intraspecies and interspecies variability used for risk characterisation. Specific uncertainty factors are not generally recommended for risk estimates for newborn and/or children, however, it is recognized that for substances that directly affect the developing foetus, and those affecting developing organ systems such as reproductive development in pre-puberty, special consideration may be needed on a case by case basis (ECETOC, 2003).

The WHO IPCS Environmental Health Criteria 170 also indicates that margins up to 10 000 can be applied based on the type and level of uncertainties of the assessment such as the adequacy of the overall data base or nature of toxicity (IPCS, 1994). An additional "safety factor" of up to 10 can be incorporated in calculations of tolerable intakes in cases where the NOAEL is derived for a critical effect which is a severe and irreversible phenomenon, such as teratogenicity or non-genotoxic carcinogenicity, especially if associated with a shallow dose-response relationship (IPCS, 1994).

By comparison, the California Office of Environmental Health Hazard Assessment (OEHHA) established a maximum allowable dose levels (MADL) for DEHP by the oral route (unspecified use applications) of 5.8 µg/kg bw/day for neonatal and infant boys (OEHHA, 2005) which provides a MOE of 1000 relative to NOAEL of 5.8 mg/kg bw/d established by David et al. (2000a) (Section 6.2.7). OEHHA applied the same criteria for adults (OEHHA, 2005). The EU Risk assessment for DEHP (ECB, 2006 and 2008) applied a margin of safety factor of 200 for infants and 100 for children and adults, based on the seriousness of the effect, sensitivity of infants to the effect and the likelihood of exposure of infants through several different routes (e.g. through baby food and breast milk).

Although children have been identified as a sensitive subpopulation in this assessment, additional uncertainty factors are regarded as not necessary for assessment of the risk for kidney toxicity in children, as systems involved in xenobiotics metabolism including metabolic enzymes and kidney function, quickly mature in infants to the point where by 6-12 months they are at least equivalent to those of adults (ECETOC, 2003).

8.3.2 Risk estimate related to use of cosmetics

The main route of exposure to DEHP 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 most prone to accidental oral ingestion such as toothpastes, mouthwashes, lipsticks and lip-glosses.

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

daily use of various DEHP-containing cosmetic products is estimated to be 154.7 μg/kg bw/d (Section 5.3.5) considering a "worst-case" scenario of daily use of all (leave-on, wash-off and spray application) cosmetic products, as outlined in the Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation (SCCNFP, 2003 and SCCP, 2006) and EU TGD (EC, 2003). Additional assumptions are as follows:

- DEHP content in cosmetics is similar to that reported for DEP in a limited number of cosmetic products in Australia
- Bioavailability of DEHP via the dermal route is 5% and via the inhalation route is 100%.

Estimation of margin of exposure

Table 8.4: Calculated MOE for critical health effects of DEHP from estimated aggregate exposure to cosmetic products for the general population

Type of toxicity	NOAEL mg/kg bw/d	MOE for reasonable worst case exposure scenario
Reproductive	4.8	31
Kidney	28.9	187

The estimated MOE for reproductive toxicity in the general population is less than 100 (Table 8.4). This indicates that the risk for the general population of reproductive toxicity from simultaneous use of multiple cosmetic products containing DEHP is high.

The risk estimate for chronic effects to kidneys derives a MOE above of 187 indicating low concern for kidney toxicity in the general population using multiple cosmetic products containing DEHP.

Exposure to DEHP from use of personal care products was also estimated specifically for children (Section 5.3.3, Table 5.5). Based on these estimates MOE for reproductive effects of DEHP exposure was found to be below and marginally above 100.

Table 8.5: Calculated MOE for reproductive effects of DEHP for children

Infant Age	$D_{int,derm}$ (μg/kg bw/day)	MOE
Newborn	61.7	77
6 months	48.2	99
12 months	42.9	105

Uncertainties in the risk estimate

Uncertainties involved 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 DEHP, have been used to determine the risk to consumers.

In addition, Australian-specific data are not available on typical or maximum DEHP content of specific types of cosmetic products. Therefore, for this risk characterisation, the DEHP content in products is assumed to be similar to that currently reported for DEP across different cosmetic product types in Australia. This assumption is based on the potential for phthalate substitution in products, and, compared with the reported typical level of DEHP in cosmetics of 0.05% provided by one company, may overestimate the current exposure to DEHP from cosmetics. However, the extent to which this assumption of substitution overestimates DEHP exposure via cosmetics currently, or in the future, is not known. Due to the ban on DEHP use in cosmetic products in the EU, only trace amounts of DEHP have been found in a limited number of perfumery products in the EU (Peters, 2005). DEHP has also been found in a very small number of products available in Korea at concentrations up to 18.3 mg/kg in perfumes and up to 25.1 mg/kg in nail polish (Koo et al., 2004). It is also identified as an ingredient in a mascara product available currently in USA and internationally through internet sale (EWG, 2009) No other data are available on the use or presence of DEHP in cosmetic products manufactured in countries where use of DEHP in cosmetics has not been restricted. However, the possible use of DEHP as a fragrance ingredient, plasticiser and solvent for cosmetic use is indicted in the INCI (International Nomenclature Cosmetic Ingredient) Dictionary.

The exposure and MOE estimates assume a reasonable but worst-case scenario where all possible DEHP-containing cosmetic products are used daily. However use patterns of cosmetic products are likely to vary greatly among individuals. For some adult consumers, this assumption may lead to an overestimation of risk. In addition, the MOE estimate does not consider specific subpopulations such as children and teenagers, which may have significantly different use patterns for cosmetics products. Use of several products from one preferred manufacturer with DEHP as an ingredient in their formulations may also contribute to increased exposure and a decrease of MOE in subpopulations inclined to brand loyalty.

There is a high degree of uncertainty associated with the exposure estimates in the children, as it is not known whether DEHP has been used in baby lotions or creams. In addition, information related to use pattern and/or levels of personal care products for babies and children is not available.

The lack of extensive studies in the primate animal models such as marmosets is also acknowledged as a data gap and contributes to the uncertainty in the risk estimate of DEHP toxicity in humans. The lack of human data on the health effects of DEHP in young and/or adult humans following repeated exposure also represents an additional uncertainty factor in these risk estimates.

Areas of concern

Considering the current absence of restrictions on DEHP use in cosmetics in Australia and other countries with the exception of the EU and USA, the potential for introduction of cosmetic products containing DEHP with widespread use and exposure cannot be excluded. Therefore, given the low MOE of 26.6 and the nature of the reproductive toxicity with a potential for serious long term and irreversible effects especially on the offspring of pregnant and breastfeeding women, potential exposure to DEHP from use in cosmetics is of concern.

Similarly, for young children undergoing critical developmental processes there is a concern for reproductive developmental toxicity from potential DEHP exposure through use of baby lotions and creams based on the MOE estimates which are below or close to 100 (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. In addition, the sensitivity of individuals and subpopulations to the critical health effects associated with exposure to DEHP may vary significantly as indicated by the studies in animals demonstrating that developing foetuses and young adults are most sensitive to the DEHP toxicity to reproductive system. Determination of the level of exposure to DEHP for the different subpopulations that may be at highest risk in the cosmetic use scenario is difficult. However, the results of the large biomonitoring studies (Section 5.5) where substantial difference was detected between the average levels 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 DEHP doses than the population average. In particular, a maximum exposure has been calculated for female adults (Wormuth et al., 2006). This raises concerns that the high exposure scenarios with MOE extremely close to or below 100 may be applicable to the subpopulation which is most at risk for reproductive developmental effects in their progeny i.e. pregnant and breastfeeding women.

9. Current Human Health Risk Management

9.1 Current public health risk standards

Currently in Australia there are no restrictions on the use of DEHP in consumer products including toys. DEHP is not listed in the *Standard for the Uniform Scheduling of Drugs and Poisons* (SUSDP, 2008) and is not included in the Australian/New Zealand Standard AS/NZS ISO 8124 *Safety of Toys*.

9.1.1 Toys and childcare articles

In Australia, DEHP was identified as being in use or with the potential for use in children's toys and childcare articles potentially including pacifiers, teething rings and squeeze toys. Two toy companies specified that the DEHP content in their products designed for children age 3 years and older, and not intended to be mouthed, did not exceed 0.1%.

There are currently no restrictions on the use of DEHP in toys and childcare articles in Australia.

In contrast, current EU and USA legislation restricts the use of DEHP to less than 0.1 wt % of the plastic used in any type of toys and childcare articles. Canada is in the process of implementing similar restrictions.

9.1.2 Cosmetics

Limited Australian information shows that DEHP is introduced as a raw material with potential downstream use in cosmetic industry, but also as a component of cosmetic products (mot specified) and fragrances with typical concentration of 0.05%.

There are currently no restrictions on the use of DEHP in cosmetics in Australia.

Current EU legislation prohibits the use of DEHP in cosmetic products. In the USA, use of DEHP in personal care products was prohibited by legislation in California.

As of September 2009, DEHP has been added to the Health Canada List of Prohibited and Restricted Cosmetic Ingredients (The Cosmetic Ingredient Hotlist), to reflect a declaration of 'toxic' under the *Canadian Environmental Protection Act* due to health concerns (Health Canada, 2009).

Labelling for consumer products

As DEHP is not listed in the SUSDP, there are no specific labelling requirements for consumer goods that contain the chemical. However, disclosure of the presence of DEHP is required on the packaging or on the product itself for cosmetics and toiletries in accordance with the Trade Practices (Consumer Product Information Standards) (Cosmetics) Regulations 1991, as amended by the Trade Practices (Consumer Product Information Standard) (Cosmetics) Amendment Regulations

1998 (no.1) (the mandatory information standard) made under the *Trade Practices Act 1974.*

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 DEHP content.

Appendix

Mouthing time studies

Studies of mouthing behaviour in children provide information about the duration and frequency of potential oral exposure to a phthalate in children's toys and childcare articles.

In the Netherlands, Groot et al. (1998) investigated the mouthing behaviour of 42 young children aged between 3-36 months, for five categories of objects: pacifiers, teethers, fingers, toys and non-toys. Ten 15-minute observations of mouthing behaviour were conducted by parents over 2 days with a total of 42 children aged between 3-6, 6-12, 12-18 and 18-36 months. Of the 4 age-groups observed, children 6-12 months of age showed the greatest daily mouthing times for objects excluding pacifiers, averaging 44 minutes/day (range 2.4 – 171.5 minutes/day). The average mouthing time across the 4 groups was 26.7 minutes/day. Differences in mouthing times between individuals were large.

Health Canada (1998) estimated that the mean mouthing time for teethers and other mouthing objects (excluding pacifiers) was 2 hours (range 1-3 hours) per day for a child aged 3-12 months; and 2.5 hours (range 2-3 hours) per day for a child 12-36 months of age.

Juberg et al. (2001) reported an observational study of the mouthing behaviour of children in the USA with pacifiers, teethers, plastic toys and other objects. Children were observed in their homes by parents who documented behaviour via standard daily diary forms. In the first 1 day study, for 107 children up to 18 months of age, the average daily durations of mouthing were: pacifiers 108 minutes, plastic toys 17 minutes, teethers 6 minutes and other objects 2 minutes. In a second 1 day study, for 110 children between 19 and 36 months of age, the average daily durations of mouthing were: pacifiers 126 minutes, plastic toys 2 minutes, teethers 0 minutes and other objects 2 minutes. A final study with 168 children 3-18 months of age of mouthing of all objects excluding pacifiers over 5 non-consecutive observation days revealed an average daily mouthing time of 36 minutes. A small number of children, 5 out of 168, consistently mouthed objects for more than 2 hours per day. The report noted considerable variations in mouthing behaviour between children, and in day-to-day mouthing behaviour in individual children.

Kiss (2001) conducted an observational study of children's mouthing activity in the USA. A total of 169 children ages 3-36 months were studied by trained observers for a total of 4 hours on at least 2 different days. Three groups of children were studied, ages 3-12, 12-24 and 24-36 months. For all objects except pacifiers, the estimated average daily mouthing times were 70 minutes (95% confidence interval 60-80 minutes) for children ages 3-12 months, 47 minutes (40-57 minutes) for children ages 12-24 months, and 37 minutes (27-49 minutes) for children ages 24-36 months.

Greene (2002) conducted further statistical analyses of the data from Kiss' study (2001). The upper 95th percentiles for mouthing times across the 3 age groups ranged between 122 and 134 minutes/day whereas the corresponding upper 99th percentiles ranged between 153 and 180 minutes.

DTI (2002) presented the findings of an investigation into the mouthing behaviour of 236 children aged 1-60 months in the UK. The study found that nearly all items a child came into contact with were mouthed. Mean estimated daily mouthing time on toys and other objects (excluding pacifiers) peaked at age 6-9 months (at approximately 1 hour) and

decreased as children grow older. The maximum daily mouthing time for toys and other objects (excluding pacifiers) for children ages 6-9 months was 297 minutes.

The following table summarises the mean and maximum estimated daily mouthing data from the studies above.

Table A.1: Summary of minimum and maximum daily mouthing time from mouthing time studies

Study	Number of children	Age (months)	Object mouthed	Daily mouthing times (mins)		
				Mean	**Max**	**SD**
Groot et al. (1998)	5	3-6	Toys meant for mouthing, toys not meant for mouthing & non-toys & fingers (excludes pacifiers).	36.9	67.0	19.1
	14	6-12		44.0	171.5	44.7
	12	12-18		16.4	53.2	18.2
	11	18-36		9.3	30.9	9.8
Health Canada (1998)	Not reported	3-12	Teethers and other mouthing products (excluding pacifiers)	120	180	-
Juberg et al. (2000)	107	0-18	Plastic toys	17		-
			Teethers	6		-
			Other objects (excludes pacifiers & fingers)	9	NR	-
	110	19-36	Plastic toys	2		-
			Teethers	0		-
			Other objects (excludes pacifiers & fingers)	2		-
	168	3-18	All objects, excluding pacifiers	36		48
Kiss (2001)	169 (total)	3-12	All objects, excluding pacifiers	70		-
		12-24	All objects, excluding pacifiers	48		-
		24-36	All objects, excluding pacifiers	37		-
					NR	
DTI (2002)	236	1-3	Toys, other objects (excluding pacifiers and fingers)	5	29	-
		3-6	Toys, other objects (excluding pacifiers and fingers)	40	231	-
		6-9	Toys, other objects (excluding pacifiers and fingers)	63	297	-
		9-12	Toys, other objects (excluding pacifiers and fingers)	39	155	-

SD – standard deviation; NR – not reported

Selection of mouthing time for use in exposure assessment

Table A.1 reveals substantial variability in mouthing times among children ages 3-36 months. Also, several studies noted that mouthing times decrease with increasing age (Groot et al. 1998; Kiss, 2001).

Mouthing times were highest for children aged 6-12 months, with a maximum value of approximately 3 hours per day. The mouthing times then gradually decrease as the age of the child increases. Therefore, the mouthing time for children aged 6-12 months represents a reasonable "worst-case" estimate of the maximum mouthing time for use in exposure assessment.

For the 6-12 month age group, a mean daily mouthing time of approximately 49 minutes per day (0.8 h/day) was calculated by averaging results across the studies which gave results for this group, although it was noted that there was great inter-individual variation (Groot et al., 1998; Juberg et al., 2001). This mean daily mouthing time is regarded as representing a reasonable "typical" mouthing time estimate for exposure assessment. In the absence of Australian information, it is assumed that the mouthing behaviour of Australian children is similar to overseas children and therefore that these data are representative of Australian mouthing behaviour.

Extractability of phthalate plasticizers

Extractability of phthalates from plastic articles as a function of composition, weight, surface area and time (migration rate) has been studied in vitro by a number of groups using various mechanical methods including shaking, ultrasound, tumbling ("head over heels") and impaction (Babich, 2002). Studies using these different methods have generated a broad range of results depending on the experimental conditions.

In vivo, phthalate extractability has been studied using adult volunteers providing saliva samples during mastication of plastic articles to measure migration of the plasticizer into the saliva as a function of time (migration rate).

These studies allow a direct comparison of results from in vivo and in vitro mechanical methods. In the majority of the studies, results from the in vitro methods underestimate the migration of phthalates from chewed articles. The results for in vitro studies were therefore not considered to be as useful as those from in vivo studies in determining suitable migration rates for calculating systemic doses.

DINP is the most prevalent phthalate in children's toys and the migration of this chemical from plastics has been studied most extensively. The studies demonstrate that migration of phthalates from plastic products is determined more by the magnitude of mechanical action applied to the plastic rather than the chemical diffusive properties determined by the physicochemical characteristics of the substrate or concentration of phthalate. Due to the limited specific information for DEHP, the information for DINP is considered to be representative for DEHP as well.

Chen (1998) conducted an in vivo study in the US with adult volunteers and an in vitro study using impaction methods and saliva simulants. In the in vivo study, two plastic disks (each with a surface area of approximately 10.3 cm^2) were cut from each of 5 identical PVC toy ducks each containing 43% DINP by weight. Ten US Consumer Product Safety Commission (CPSC) staff volunteers were asked to gently chew the disks for four 15-minute intervals. Saliva samples were collected after each chewing interval and analysed for DINP. Migration rates varied substantially from individual to individual. The average DINP migration rate across all time periods from volunteers was 26.03 $\mu g/cm^2/h$ (range 6.14 –

57.93 μg/cm^2/h). In vivo migration rates also averaged 39.5 times higher than rates obtained from the in vitro impaction study. In vitro impaction studies of phthalate release rates (range 0.1 to 4.4 μg/cm^2/h) from samples of children's toys or childcare products showed poor correlation between release rates and the amount of phthalate present in samples.

Meuling and Rijk (1998) conducted an in vivo study in the Netherlands with 20 adult volunteers and an in vitro study with a simulant of saliva using shaking, head over heels mixing and ultrasound methods. In the in vivo study, three specimens were used: a standard PVC disk (38.5% DINP), part of a PVC teething ring (43% DINP), and a disk punched from the same teething ring (43% DINP). Each specimen had a surface area of 10cm^2. Initially all 20 volunteers were asked to suck and bite on the standard PVC disc for four 15-minute intervals. Saliva samples were collected after each biting interval and analysed for DINP. Subsequently, the volunteers were divided into two groups of 10. One group repeated the test using part of the teething ring while the other group used the disk punched from the teething ring. In the in vivo study, the mean release rates were: 8.28 μg/cm^2/h (range 1.8 – 49.8 μg/cm^2/h) for the standard PVC disc, 14.64 μg/cm^2/h (range 5.4 – 53.4 μg/cm^2/h) for the teething ring and 9.78 μg/cm^2/h (range 5.4 – 34.2 μg/cm^2/h) for the disc punched from the teething ring. The researchers noted that the amount of DINP released into saliva exceeded its expected solubility and that mechanical force was required in the in vitro studies in order to attain migrations rate comparable to that obtained from the in vivo studies.

Fiala et al. (1998) conducted an in vivo study in Austria with nine volunteers and an in vitro study with a simulant of saliva using shaking or ultrasound methods. In the in vivo study, PVC sheets (32% DEHP) and parts of PVC teethers (36% DINP) were used separately. Each specimen had a surface area of 10-15 cm^2. The volunteers were asked to suck only or chew the samples separately for 1-3 hours. Saliva samples were collected and analysed. For DINP, the mean release rate (sucking for 1 hour) was 8.33 μg/cm^2/h (range 2.97 - 14.52 μg/cm^2/h). Higher values were recorded from chewing. The mean release rate for DINP (chewing for 1 hour) was 13.3 μg/cm^2/h (range 7.68 – 21.52 μg/cm^2/h). This study also showed that migration rates were substantially higher in the in vivo chewing study than those obtained in the in vitro studies.

Niino et al. (2001) conducted an in vivo study in Japan with 4 volunteers and an in vitro study with a simulant of saliva using shaking methods. In the in vivo study, two PVC ball samples were used: sample A contained 10.0% DBP and 18.5% DEHP, and sample B contained 25.6% DINP. Each specimen had a surface area of approximately 15 cm^2. Four volunteers were asked to gently chew each of the specimens for four 15-minute intervals. Saliva samples were collected after each chewing interval and analysed for phthalate content. In contrast to previous studies, the in vitro study of phthalate migration showed a substantially higher mean migration rate at approximately two orders of magnitude higher than the human in vivo study.

In a follow-up study, Niino et al. (2002) conducted an in vivo study with 4 volunteers and an in vitro study with a simulant of saliva using shaking methods. In the in vivo study, samples of a PVC plate and toys (including pacifier, teether, rattle, ball, soft doll, containing 16.0%-58.3% DINP) were tested separately. Each specimen had a surface area of approximately 15 cm^2. Four volunteers were asked to chew each of the specimens for four 15-minute intervals. Saliva samples were collected after each chewing interval and analysed for DINP. The average migration rate across all samples was 16.4 μg/cm^2/h (SD 2.8 μg/cm^2/h). The highest migration rate was for the PVC plate sample at 32.6 μg/cm^2/h (SD 2.6 μg/cm^2/h). The authors noted that DINP contents in the toy products did not correlate

with the amount of in vivo migration. The in vitro migration studies showed consistently higher mean migration rates than the in vivo studies.

The results of the five in vivo studies are summarised in Table A.2.

Table A.2: Summary of migration rates for phthalate plasticizers from in vivo testing

Study	PVC Product	Phthalate	Wt.%	Test Condition	Migration rate (SD) ($\mu g/cm^2/h$)	
					Mean (SD)	Maximum
Chen (1998)	Toy ducks	DINP	43	Chewing	26.03 (15.35)	57.93
Groot et al. (1998)	Disk	DINP	38.5	Sucking and biting	8.28	49.80
	Teething ring	DINP	43	Sucking and biting	14.64	53.40
	Teething ring	DINP	43	Sucking and biting	9.78	34.20
Fiala et al. (1998)	Sheet	DEHP	32	Sucking	2.64	NR
	Teethers	DINP	36	Sucking	8.33 (3.97)	14.52
	Teethers	DINP	36	Chewing	13.30 (5.17)	21.52
Niino et al. (2001)	Toy ball A	DBP	10	Chewing	1.17 (0.98)	NR
		DEHP	18.5	Chewing	4.44 (1.23)	NR
	Toy ball B	DINP	25.6	Chewing	7.80 (2.89)	NR
Niino et al. (2002)	Plate	DINP	16-58.3	Chewing	32.6 (2.6)	NR
	Pacifier	DINP	58.3	Chewing	20.0 (6.0)	NR
	Teether	DINP	38.9	Chewing	12.5 (1.9)	NR
	Rattle	DINP	38	Chewing	21.9 (2.6)	NR
	Ball	DINP	25.5	Chewing	7.8 (2.9)	NR
	Soft doll	DINP	16	Chewing	3.8 (0.9)	NR

SD – standard deviation; NR - not reported

Selection of migration rate for exposure assessment

As the results from the in vitro studies do not reproduce the in vivo findings for the same systems, the results from only in vivo studies are used in the exposure assessment. The following conclusions can be drawn from the above five in vivo studies:

- Within studies, migration rates vary substantially from individual to individual, even though the same action (e.g. chewing) is involved;
- Migration rates have little direct relationship with the phthalate content of an article in the tested phthalate range of 15%-58% by weight, indicating that differences seen between different test articles may depend more on the properties of the PVC grade comprising the article;

- The amount of phthalate released into saliva through biting and chewing exceeded its expected solubility in water in all in vivo studies, indicating that migration is not merely a simple diffusion process;
- Migration rates are proportional to the amplitude of mechanical action ie. chewing results in a higher migration rate than mouthing or sucking alone.

Based on the above conclusions, it is evident that migration 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 likely to be the dominating factor. The migration rate of phthalates 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 affected by the physicochemical characteristics or concentration of a particular phthalate.

Limited migration data specific for DEHP and some other phthalates are available (Table A.2) and demonstrate that migration of DEHP is comparable to that of DINP under the conditions tested. However, the data for DINP are more robust and coves a wide range of extraction conditions and concentrations in PVC. Therefore, the migration rates for DINP under chewing conditions are extrapolated to DEHP and some other phthalates assuming similar product uses and concentrations in products.

In these studies, the use of adults in in vivo studies as a surrogate for the activities of children is accompanied by several uncertainties. Firstly, the level of mechanical force applied to the plastic toys may differ. Therefore, the use of adults in the in vivo studies might lead to an overestimation of phthalate migration from toys. Also, children do not swallow all the saliva, which means that estimates of exposure from adult in vivo studies where all saliva harvested is assumed to be swallowed, may again overestimate the oral exposure of children. Finally, absorption through the oral mucosa is not accounted for in migration measurements in adults in vivo. However, compared to potential oral ingestion, mucosal absorption is likely to be very low.

The highest in vivo migration rate observed for DINP in a well conducted study was 57.93 $\mu g/cm^2/h$ from an article with 43% DINP content (Chen, 1998). Assuming similar uses for DEHP and comparable concentrations of DEHP in products, this migration rate is therefore applicable for a worst case exposure assessment for children from the use of DEHP in toys. The mean migration rate for DINP in this study was 26.03 $\mu g/cm^2/h$ (Chen, 1998), which is similar to the highest mean migration rate of 32.6 $\mu g/cm^2/h$ (Niino, 2002), in a study using a smaller number of volunteers. The mean migration rate determined by Chen (1998) is regarded as applicable for typical exposure assessment of DEHP in toys.

Glossary

NICNAS uses the IPCS Risk Assessment Terminology (IPCS, 2004) glossary which includes Part 1: IPCS/OECD Key Generic Terms used in Chemical Hazard/Risk Assessment and Part 2: IPCS Glossary of Key Exposure Assessment Terminology. The IPCS Risk Assessment Terminology can be accessed at:

http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf

Acute exposure	A contact between an agent and a target occurring over a short time, generally less than a day. (Other terms, such as "short-term exposure" and "single dose" are also used).
Adverse effect	Change in the morphology, physiology, growth, development, reproduction, or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences.
Analysis	Detailed examination of anything complex, made in order to understand its nature or to determine its essential features.
Assessment	Evaluation of appraisal of an analysis of facts and the inference of possible consequences concerning a particular object or process.
Assessment end-point	Quantitative/qualitative expression of a specific factor with which a risk may be associated as determined through an appropriate risk assessment.
Bioavailability	The rate and extent to which an agent can be absorbed by an organism and is available for metabolism or interaction with biologically significant receptors. Bioavailability involves both release from a medium (if present) and absorption by an organism.
Childcare 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
Chronic exposure	A continuous or intermittent long-term contact between an agent and a target. (Other terms, such as "long-term exposure," are also used.)
Concentration	Amount of a material or agent dissolved or contained in unit quantity in a given medium or system.
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.
Dose	Total amount of an agent administered to, taken up or absorbed by an organism, system or (sub) population.
Dose-effect relationship	Relationship between the total amount of an agent administered to, taken up or absorbed by an organism, system or (sub) population and the magnitude of a continuously-graded effect to that organism, system or (sub)population Related terms: *Effect assessment, Dose-response relationship, Concentration-effect Relationship*.
Dose-related effect	Any effect to an organism, system or (sub) population as a result of the quantity of an agent administered to, taken up or absorbed by that organism, system or (sub) population.
Dose-response	Relationship between the amount of an agent administered to, taken up or absorbed by an organism, system or (sub) population and the change developed in that organism, system or (sub) population in reaction to the agent. Synonymous with *Dose-response relationship*. Related Term: *Dose-effect relationship, Effect assessment, Concentration-effect relationship*.
Dose-response curve	Graphical presentation of a dose-response relationship.
Dose-response relationship	Relationship between the amount of an agent administered to, taken up or absorbed by an organism, system or (sub) population and the change developed in that organism, system or (sub) population in reaction to the agent. Related Terms: *Dose-effect relationship, Effect assessment, Concentration-effect relationship*.
Effect	Change in the state or dynamics of an organism, system or (sub) population caused by the exposure to an agent.
Expert judgement	Opinion of an authoritative person on a particular subject.
Exposure	Concentration or amount of a particular agent that reaches a target organism, system or (sub) population in a specific frequency for a defined duration.
Exposure assessment	Evaluation of the exposure of an organism, system or (sub) population to an agent (and its derivatives). Exposure Assessment is the third step in the process of Risk Assessment.
Exposure concentration	The exposure mass divided by the contact volume or the exposure mass divided by the mass of contact volume depending on the medium.
Exposure duration	The length of time over which continuous or intermittent contacts occur between an agent and a target. For example, if an individual is in contact with an agent for 10 minutes a day, for 300 days over

	a 1-year time period, the exposure duration is 1 year.
Exposure event	The occurrence of continuous contact between an agent and a target.
Exposure period	The time of continuous contact between an agent and a target.
Exposure route	The way an agent enters a target after contact (*e.g.* by ingestion, inhalation, or dermal absorption).
Exposure scenario	A set of conditions or assumptions about sources, exposure pathways, amount or concentrations of agent(s)involved, and exposed organism, system or (sub) population (i.e. numbers, characteristics, habits) used to aid in the evaluation and quantification of exposure(s) in a given situation.
Fate	Pattern of distribution of an agent, its derivatives or metabolites in an organism, system, compartment or (sub) population of concern as a result of transport, partitioning, transformation or degradation.
Hazard	Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub) population is exposed to that agent.
Hazard assessment	A process designed to determine the possible adverse effects of an agent or situation to which an organism, system or (sub) population could be exposed. The process includes hazard identification and hazard characterisation. The process focuses on the hazard in contrast to risk assessment where exposure assessment is a distinct additional step.
Hazard characterization	The qualitative and, wherever possible, quantitative description of the inherent properties of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose-response assessment and its attendant uncertainties. Hazard Characterisation is the second stage in the process of Hazard Assessment, and the second step in Risk Assessment. Related terms: *Dose-effect relationship, Effect assessment, Dose-response relationship, Concentration -effect relationship.*
Hazard identification	The identification of the type and nature of adverse effects that an agent has inherent capacity to cause in an organism, system or (sub) population. Hazard identification is the first stage in hazard assessment and the first step in process of Risk Assessment
Intake	The process by which an agent crosses an outer exposure surface of a target without passing an absorption barrier, i.e. through ingestion or inhalation.
Margin of exposure	Ratio of the no-observed-adverse-effect level (NOAEL) for the critical effect to the theoretical, predicted or estimated exposure dose or concentration. Related term: *Margin of safety*

Response	Change developed in the state or dynamics of an organism, system, or (sub)population in reaction to exposure to an agent.
Risk	The probability of an adverse effect in an organism, system, or (sub)population caused under specified circumstances by exposure to an agent.
Risk assessment	A process intended to calculate or estimate the risk to a given target organism, system or (sub)population , including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system. The Risk Assessment process includes four steps: hazard identification, hazard characterization (related term: *Dose-response assessment*), exposure assessment, and risk characterization. It is the first component in a risk analysis process.
Risk characterization	The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system or (sub)population, under defined exposure conditions. Risk Characterization is the fourth step in the Risk Assessment process.
Risk management	Decision-making process involving considerations of political, social, economic, and technical factors with relevant risk assessment information relating to a hazard so as to develop, analyse, and compare regulatory and non-regulatory options and to select and implement appropriate regulatory response to that hazard. Risk management comprises three elements: risk evaluation; emission and exposure control; risk monitoring.
Source	The origin of an agent for the purposes of an exposure assessment.
Target	Any biological entity that receives an exposure or a dose (e.g., a human, human population or a human organ).
Threshold	Dose or exposure concentration of an agent below that a stated effect is not observed or expected to occur.
Time-averaged exposure	The time-integrated exposure divided by the exposure duration. An example is the daily average exposure of an individual to carbon monoxide. (Also called time-weighted average exposure.)
Toys	Products or materials designed or clearly intended for use in play by children of less than 14 years of age.
Toxicity	Inherent property of an agent to cause an adverse biological effect.

Uncertainty	Imperfect knowledge concerning the present or future state of an organism, system, or (sub)population under consideration.
Uptake (absorption)	The process by which an agent crosses an absorption barrier.
Validation	Process by which the reliability and relevance of a particular approach, method, process, or assessment is established for a defined purpose. Different parties define "Reliability" as establishing the reproducibility of the outcome of the approach, method, process, or assessment over time. "Relevance" is defined as establishing the meaningfulness and usefulness of the approach, method, process, or assessment for the defined purpose.

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