Priority Existing Chemical Assessment Report No. 36

Australian Government

Department of Health
National Industrial Chemicals
Notification and Assessment Scheme

Dibutyl phthalate

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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME
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Editor's Note

This document is published in both electronic (soft) and print (hard) versions. Each has its own ISBN number. Owing to the different constraints and advantages of each format, there is the possibility that some items might appear on different page numbers in each format, and this could be reflected in the table of contents. However, the content in both formats is identical, except that the soft copy does not contain an index, as the document is searchable.

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 (Environment), 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 focusing 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 the public comment period 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 the 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
Fax: +61 (2) 8577 8888
Free call: 1800 638 528

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

- NICNAS service charter;
- information sheets on NICNAS company registration;
- information sheets on the Priority Existing Chemicals and New Chemical assessment programs;
- safety information sheets on chemicals that have been assessed as Priority Existing Chemicals;
- details for the NICNAS *Handbook for Notifiers*; and
- details for the *Commonwealth Chemical Gazette*.

More information on NICNAS can be found at the NICNAS web site: http://www.nicnas.gov.au

Other information on managing workplace chemicals can be found at the Safe Work Australia web site: http://www.safeworkaustralia.gov.au

Contents

Acronyms & abbreviations

2-EH	2-ethylhexanol
AGD	anogenital distance
AGI	anogenital index
AICS	Australian Inventory of Chemical Substances
ARMs	anorectal malformations
BBP	butylbenzyl phthalate
bw	bodyweight
CAS	Chemical Abstracts Service
CERHR	Centre for the Evaluation of Risks to Human Reproduction (US)
CHO	Chinese hamster ovary
CPSC	Consumer Product Safety Commission
d	day
DBP	di-n-butyl phthalate
DCHP	dicyclohexyl phthalate
DEHP	diethylhexyl phthalate
DEP	diethyl phthalate
DIBP	diisobutyl phthalate
DIDP	diisodecyl phthalate
DINP	diisononyl phthalate
DIOP	diisooctyl phthalate
DMP	dimethyl phthalate
DMEP	dimethylethyl phthalate
DMSO	dimethyl sulfoxide
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
EURAR	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
GLP	Good Laboratory Practice
h	hour
HPT	hypothalamic-pituitary-thyroid
HPVC	High production volume chemical
HVICL	High Volume Industrial Chemicals List
IPCS	International Programme on Chemical Safety
iv	intravenous
kg	kilogram
L	litre
LC_{50}	median lethal concentration
LD_{50}	median lethal dose

LH	luteinising hormone
LOAEL	lowest-observed-adverse-effect level
m^3	cubic metre
MBP	monobutyl phthalate
MBzP	monobenzyl phthalate
MCL	mononuclear cell leukaemia
MEHP	monoethylhexyl phthalate
MEHHP	mono (2-ethyl-5-hydroxyhexyl) phthalate
MEOHP	mono-(2-ethyl-5-oxohexyl) phthalate
MEP	monoethyl phthalate
mg	milligram
mg/cm^3	milligrams per cubic centimetre
mg/kg bw	milligrams per kilogram bodyweight
mg/kg bw/d	mg/kg bodyweight/day
MiBP	monoisobutyl phthalate
µg	microgram
mL	millilitre
MMP	monomethyl phthlate
MNG	multinucleated gonocyte
MOA	mode of action
MOE	margin of exposure
mPa	milliPascal
MRNA	Messenger ribonucleic acid
ND	new data
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
StAR	steroidogenic acute regulatory protein
USA	United States of America
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation
wt	weight

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

Overview

Background and scope of the assessment

Dibutyl phthalate (DBP) (CAS No. 84-74-2) 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:

- the ubiquitous use of phthalates, including DBP, as solvents and plasticisers in industrial and consumer products;
- consumer products such as soft plastic articles and cosmetics being potentially significant sources of repeated and long-term exposure of the public to DBP through migration and leaching;
- concerns regarding potential adverse health effects, particularly reproductive effects from DBP exposure; and
- current restrictions (interim or permanent) overseas for phthalate use, including DBP, in certain consumer products.

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

Manufacture and importation

Data collected through calls for information specific to the assessment of DBP and for other purposes (e.g. compiling the High Volume Industrial Chemicals List—HVICL) suggest that most of the DBP introduced into Australia (more than 350 tonnes in 2004) is for industrial applications. DBP is not included on the HVICL for 2002 or 2006 as the quantity introduced was below the annual reporting threshold of 1000 tonnes. DBP is imported in finished products or mixtures, and as a raw material for local formulation. Manufacture of DBP in Australia as a raw material was not reported.

The amount of DBP used for applications with the potential for public exposure, such as toys, childcare articles and cosmetics is likely to be significantly lower than for other industrial applications. One respondent who imports DBP as a raw material that can be used in these applications, indicated importation volumes of approximately 95 tonnes in 2005 and 80 tonnes in 2006.

Uses

Information on the worldwide use of DBP indicates that while it has widespread use as a plasticiser in a variety of industrial 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, DBP is mainly imported in the form of finished products or mixtures. It is also used industrially as a plasticiser in surface coatings (paints, pigments, floor coatings), in car mat backing, in polymer emulsions for adhesives, in PVC compounds (such as wire cable tubing and footwear), nitrocellular lacquers for automotive refinishes, epoxy sealant, leather paint, galvanised iron primer and texture finishes, screen printing inks and textile wet-processing products.

Consumer uses in Australia include fragrance bases for household, personal care and cosmetic products, with the highest concentrations reported for nail polish (7 %). DBP is present in exercise balls, hoses, rubber sheets and in children's toys, including those intended for children aged 0–6 years.

Health effects

DBP is rapidly absorbed and excreted after being ingested. The bioavailability in humans after ingestion is assessed to be 100 %. Bioavailability through skin absorption is lower (5 %). Data are limited on the absorption of DBP by breathing; a default of 100 % is therefore applied for the purposes of risk characterisation.

DBP has low acute toxicity through all routes of exposure; and low eye, skin and respiratory irritation and skin sensitising potential.

DBP is non-genotoxic in most in vitro and all in vivo tests performed to standard testing guidelines. Therefore, DBP is not considered to be genotoxic. No adequate long-term carcinogenicity studies with DBP in laboratory animals are available. DBP is not likely to be a genotoxic carcinogen. Furthermore, DBP is not considered to be a relevant human non-genotoxic carcinogen, because the effects associated with liver carcinogenicity in rodents were associated with the activation of peroxisome proliferator-activated receptor α (PPARα), which is a mode of action not considered relevant for human carcinogenicity.

Target organs affected by toxic effects related to repeated DBP exposure in rodents include liver (liver weight changes, degeneration of liver cells, activation of fatty acid metabolising enzymes, alterations in fatty acids associated with increased peroxisome proliferation); and the reproductive system, particularly in males (decreased weight of testes and accessory organs, spermatocyte depletion, sperm producing channels—seminiferous tubule—degeneration, and disturbances in serum and testicular testosterone).

The molecular mechanism associated with liver toxicity in rodents includes activation of PPARα. Studies in humans have shown a relative lack of responsiveness in the liver to peroxisome proliferators. Therefore, the mechanisms by which DBP and other peroxisome proliferators induce chronic hepatotoxicity in rodents are not considered relevant to humans.

Repeat-dose toxicity studies on multiple generations of rodents showed testicular toxicity. Both fertility and development are affected in the parental and following generations. The toxicity in males involves overt effects on the reproductive tract organs. However, in females, fertility is decreased even in the absence of obvious organ toxicity, although progesterone and oestradiol production appeared to be affected. DBP also affects testosterone synthesis in male rodents. This is particularly demonstrated in multigenerational and developmental studies where males have undergone gestational exposure to DBP.

Studies in humans are limited and often contradictory. They do not directly assess fertility but evaluate the association between indicators of DBP exposure, such as DBP or monobutyl phthalate (MBP, primary metabolite of DBP) levels in serum or urine, and parameters linked to (in)fertility, such as sperm quality, testosterone levels and endometriosis.

Overall, the toxicity of DBP on fertility in rodents is similar to the related phthalate DEHP (NICNAS 2010) as it is mediated through similar adverse effects onreproductive tract organs and perturbations in oestrogen and androgen synthesis; a mechanism of reproductive toxicity also relevant for humans.

DBP toxicity on foetal development also mainly manifests as toxicity to the male reproductive system including:

- decreased testicular testosterone levels in foetuses;
- delayed preputial (foreskin) separation;
- decreased anogenital distance (AGD);
- retention of nipples in male F1 offspring; and
- increase of testicular malformation in foetuses and F1 adults.

Even a short (two-day) exposure to DBP during a critical window of the development is sufficient to induce permanent developmental abnormalities such as adverse effects on the pituitary gland in female offspring.

The reproductive tracts in mice appear to be less sensitive to DBP toxicity than in rats. However, mice exposed to DBP during gestation show a significant increase in teratogenicity (non-closing eyelids, encephalocoele, cleft palate, spina bifida, and increased incidence of skeletal abnormalities) at dose levels associated with reproductive tract malformation and testicular toxicity in rats.

Marmosets also appear to show low sensitivity to DBP toxicity in the one available study that administered one treatment dose during gestation (week 7–15) to a small number of animals.

Studies in humans mostly examine correlations between maternal MBP levels (in urine or breast milk) and developmental parameters such as gonadotrophins and gonadal hormones, undescended testicles (cryptorchidism) or anogenital index (AGI). Behavioural and neuropsychological parameters have also been analysed. No significant association is reported between MBP levels in human breast milk and cryptorchidism.

MBP levels in the breast milk showed direct correlations with sex-hormone binding globulin (SHBG) and luteinising hormone (LH)/free testosterone ratio, but there was inverse correlation of MBP levels with free testosterone. Studies that focused on measuring the AGD in newborns found inverse correlation between AGI and the maternal urinary concentrations of MBP, but not MEHP, using one statistical methodology, or for both metabolites using another method. Overall, these studies on humans have limited significance for risk characterisation of DBP exposure, but indicate that more comprehensive and prospective studies are needed.

The exact mechanism(s) underlying the reproductive toxicity of DBP have yet to be fully explained. However, the previously mentioned reproductive tract malformations observed in rodents and malformed urethras (hypospadias), occurrence of Leydig cell hypertrophy/hyperplasia, and the decrease in androgen concentration, are consistent with endocrine dysfunction, particularly testosterone synthesis deregulation, as a major component of the MOA for DBP toxicity. Sertoli cell dysfunction and perturbations in the expression of genes required to synthesise proteins essential for proper testicular development, such as insl3, are also indicated as possible mechanism(s) of DBP reproductive toxicity in rodents.

Considering that the main components of the postulated modes of action in rodents are applicable to humans, the reproductive toxicity of DBP observed in rodents is regarded as relevant for humans. Therefore, for risk characterisation, a no observed adverse effect level (NOAEL) of 10 mg DBP/kg bw/d was established, based on the lowest level at which no significant testosterone synthesis perturbations were observed in foetal rats exposed to DBP in utero (Lehmann et al. 2004).

Public exposure and health risk

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

1. use of toys and childcare articles by children; and
2. use of cosmetic products by the general population.

For children, two routes of exposure to DBP were considered:

1. dermal exposure during normal handling of toys and childcare articles, and
2. oral exposure during intentional or inadvertent mouthing, sucking and chewing on these products.

The rates of leaching (migration rates) of DBP are based on overseas in vivo and in vitro studies conducted with PVC containing a similar phthalate (DINP) that is predominantly used as a

plasticiser in PVC toys. DINP migration rates from plastic articles are considered to be applicable to toys and childcare articles containing DBP.

Studies conducted overseas indicated that children's mouthing behaviour, and therefore the potential for oral exposure, is highest between six and 12 months of age. Based on these studies, a reasonable worst-case exposure scenario estimated a maximum mouthing time of 2.2 hours a day (h/d); and a typical exposure scenario estimated a mean daily mouthing time of 0.8 h/d.

The risk of adverse acute effects for children arising from handling toys is low for DBP, given the chemical's low acute toxicity, low skin and eye irritation and the absence of skin-sensitising potential.

The longer term health risks for children include potential reproductive effects associated with repeated combined handling and mouthing of toys containing DBP as a secondary plasticiser at concentrations up to 0.5 %. MOE assessments comparing the DBP dose at which no adverse reproductive effects were observed in experimental systems with estimated internal DBP doses for children using toys containing DBP, derived an MOE for the typical and worst case scenarios of toy use of 28571 and 4878, respectively.

Given that the MOE is two orders of magnitude above 100 for both exposure scenarios, an adequate safety margin exists for DBP-induced adverse effects from using toys and childcare articles containing DBP at the current reported levels.

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

The risk of adverse acute effects for consumers exposed to DBP through cosmetics is low, given the chemical's low acute toxicity, low irritation and low sensitisation potential.

Long-term health risks for the general population from repeated exposure to DBP in cosmetics can potentially affect reproductive systems. Estimation of MOEs comparing the DBP dose at which no adverse reproductive effects were observed in experimental systems with estimated internal DBP doses in individuals using cosmetics containing DBP, derived an MOE for a reasonable worst case scenario of 64.

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, e.g. pregnant and breastfeeding women.

The effect of cumulative exposures can arise from:

- using cosmetics containing multiple phthalates acting on the same biological targets;
- the combined effects of several phthalates in toys and childcare articles; and
- the combined exposure to a range of products containing phthalates.

The determination of risk from cumulative exposures to multiple phthalates will take into account any risk mitigation measures recommended in each PEC assessment. Risks from cumulative exposure of children to DBP in toys and childcare articles and several other management measures have been implemented for the use of DEHP in toys, childcare articles and cosmetics, and DEP in cosmetics.

Risks from cumulative exposure to DBP and other phthalates will be considered on completion of other phthalate PEC assessments and, if required, further mitigation measures will be recommended.

Conclusion

Although there have been risks associated with cumulative exposure to phthalates, risks from cumulative exposure of children to DBP in toys and childcare articles and several other phthalates (DEHP, DINP and DEP) in this assessment is not likely to be higher than those for DBP alone. Any specific circumstances that will change the risk associated with the use of DBP will be considered under the secondary notification assessment requirement.

Recommendations

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

Recommendation 1 to the Delegate for Chemicals Scheduling

It is recommended that the Delegate for Chemicals Scheduling consider listing DBP in Appendix C of the *Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP) to limit the potential exposure of the public, including young children, to DBP from use in cosmetics and personal care products.

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

- Estimates of the margin of exposure (MOE) for use of DBP in cosmetics indicate that the risk of reproductive toxicity for the general population from the use of cosmetics containing DBP is unacceptable.
- Reproductive toxicity induced by DBP might have serious long-term health effects and affect the development and reproduction of future populations if the exposure is within a critical window of human health development.
- A cautious approach to the potential risks associated with DBP is warranted, given the level of uncertainty regarding both the health effects and levels of exposure for different population groups.
- Currently there are no restrictions in Australia on the use of DBP in cosmetics and there is a potential for introduction and widespread use of cosmetic products containing the chemical.

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 DBP, specific circumstances include the following:

- additional information becoming available on the adverse health effects of DBP;
- DBP being used in children's toys and childcare articles at a concentration >0.5 %;
- additional sources of public exposure to DBP other than toys, childcare articles and cosmetics being identified; and
- additional information or events that change the assumptions for estimating the cumulative risk in this assessment.

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 specified circumstances of which the introducer has become aware.

1. Introduction

1.1 Declaration

Dibutyl phthalate (DBP) (CAS No 84-74-2) was one of nine phthalate chemicals declared as a priority existing chemical (PEC) under the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) on 7 March 2006 to be assessed for risks to public health from use in children's toys, childcare articles and cosmetics. The basis for the declaration was the actual and potential use of DBP in children's toys, childcare 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 DBP;
- determine the use and functions of DBP in Australia in the specific consumer applications of children's toys, childcare articles and cosmetics;
- determine any adverse health effects associated with exposure to DBP;
- determine the extent of exposure of children and adults to DBP from these applications;
- characterise the risks to humans posed by exposure to DBP from use in these applications;
- determine the extent to which any risk is capable of being reduced; and
- recommend appropriate risk mitigation measures.

These consumer applications are as defined below:

- Toys—products or materials designed or clearly intended for use in play by children of less than 14 years of age;
- Child care articles—articles designed for use by children to facilitate sleep, relaxation, hygiene, feeding, 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 on 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 the Australian industry and governments, overseas regulatory authorities and publicly available literature sources.

1.3.1 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 was requested from industry in Australia.

In March 2006, as part of the declaration of certain phthalates including DBP as PECs, importers and manufacturers of DBP as a raw material for use in children's toys, childcare articles and cosmetics, and importers of cosmetics containing DBP, were requested to apply for assessment and supply information on the use of DBP. Unpublished information on health effects of phthalates, including DBP, 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 DBP, used in these applications. Similarly, unpublished information on health effects and exposure to phthalates from migration and leaching from articles was requested.

1.3.2 Literature review

For this assessment, key reviews on DBP prepared by the European Chemicals Bureau (ECB, 2004), US Centre for the Evaluation of Risks to Human Reproduction (CERHR, 2003) and the World Health Organisation International Programme on Chemical Safety (WHO IPCS) program (IPCS, 1997) were consulted. Information from these documents was supplemented with new relevant data identified from thorough literature searches on Toxnet, PubMed, ScienceDirect, SciFinder, Embase, Canadian Centre for Occupational Health and Safety (CCOHS) references and the search engine Google Scholar. The last searches were conducted in January 2013.

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

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

1.4 Peer review

The report has been subjected to internal peer review by NICNAS during all stages of preparation.

1.5 Applicants

Following the declaration of DBP as a priority existing chemical, 11 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:

Apisant Pty Ltd
Unit 9/12 Victoria St
Lidcombe NSW 241

Avon Products Pty Ltd
120 Old Pittwater Rd
Brookvale NSW 2100

NSW Governent of the Environment and Heritage
PO Box A290
Sydney NSW 1232

Devcos International Pty Ltd
1/50 Glenferrie Rd
Malvern VIC 3144

International Flavours & Fragrances (Australia) Pty Ltd
310 Frankston-Dandenong Rd
Dandenong South VIC 3175

Nuvo Australia Pty Ltd
324/23 Milton Parade
Malvern VIC 3144

Sigma Aldrich Pty Ltd
12 Anella Ave
Castle Hill NSW 2154

The Kingsbury Group Pty Ltd T/A Lumineye Nailcraft Innovations
2/14 Mercantile Court
Ernest QLD 4214

Toyo Tyre & Rubber
137-149 Airds Rd
Minto NSW 2566

Vinyl Council of Australia
65 Leakes Road
LAVERTON VIC 3028

Waproo Pty Ltd
3/5 Canterbury Rd
Canterbury VIC 3126

2. Background

2.1. International perspective

Dibutyl phthalate (DBP) is a member of the group of esters of phthalic acid commonly known as phthalates, used ubiquitously as plasticisers worldwide.

The Phthalate Esters Panel of the American Chemistry Council (2001; 2006 revised) derived three categories of phthalates based on use, physicochemical and toxicological properties. Low molecular weight phthalates (LMW) were defined as those produced from alcohols with straight-chain carbon backbones of ≤C3. High molecular weight (HMW) phthalates were defined as those produced from alcohols with straight-chain carbon backbones of ≥C7 or a ring structure. A similar definition of HMW phthalates is used by the Organisation for Economic Cooperation and Development (OECD, 2004). Transitional phthalates were defined as those produced from alcohols with straight or branched chain carbon backbones of C4–6.

On the basis of the ester side chain length, DBP belongs to this mid-molecular weight phthalate group known as transitional phthalates.

DBP is used as a plasticiser in a diverse range of industrial and consumer products and applications mainly in resins and polymers. DBP is also used in adhesives, lacquers, varnishes and printing inks.

In consumer products, DBP is widely used in cosmetics as a solvent, fixative, suspension agent, lubricant, antifoamer, skin emollient and also as plasticiser in nail polish and fingernail elongators (ECB, 2004). The DBP content in various products in the European Union (EU) has been reported to be as high as 80–100 % (ECB, 2004).

The physicochemical properties of phthalates that impart usefulness as plasticisers also permit their migration and leaching from polymer matrices. In PVC plastics, DBP appears to be predominantly used as a secondary plasticiser, up to 0.5 %, in combination with high levels of other phthalates. The potential for leaching from plastics and the widespread use in a variety of consumer products including cosmetics, together with the reproductive toxicity profile for phthalates in general, have led to concerns over the potential for health impacts from exposure to DBP. Particular concerns exist when there is the potential for young children exposed to DBP in toys and childcare articles or potential prolonged exposure of the general population through cosmetics.

Historically, studies of the health effects of certain phthalate esters have identified reproductive and developmental toxicity to be of particular concern. Accordingly, several overseas jurisdictions such as the European Commission (EC), USA and Canada have taken regulatory action on a number of phthalates, including DBP, 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 DBP (and similarly for diethylhexyl phthalate (DEHP) and butylbenzyl phthalate (BBP)) in toys and childcare articles. In addition, Cosmetic Directive 76/768/EEC bans DBP (and DEHP and BBP) from use as an ingredient in cosmetic products (Article 4b introduced in 2004) based on the restrictions for cosmetic use of chemicals with known carcinogenic, mutagenic or reproductive (CMR) toxicity potential (SCCNFP/0474/01).

The following additional regulatory information on DBP was obtained from the European Chemical Substances Information System (EC, 2011):

- DBP is classified as a Reproductive Toxicant Category 1B requiring hazard statements: 'May damage the unborn child' and 'Suspected of damaging fertility'. This current classification under Regulation (EC) No 1272/2008 Annex VI, is in accordance with the criteria set out in Annex I of the Regulation (the Globally Harmonised System, GHS) as implemented within the EU from 1 December 2010;
- DBP has been reported by the EU as a High Production Volume Chemical (HPVC);
- DBP was included in a priority list under the European Economic Community (EEC) Council Regulation No. 793/93 for the evaluation and control of the risks of existing substances; and
- In January 2009, DBP together with DEHP and BBP, were proposed for inclusion on the Registration, Evaluation, Authorisation and Restriction of Chemical substances (REACH) List of Substances of Very High Concern (SVHC), which would be subject to authorisation (Annex XIV) by the European Chemicals Agency (ECHA). This proposal was accepted by the member state committee in May 2009 and adopted by EC in February 2011 (Commission Regulation (EU) No 143/2011). Authorisation under REACH means that companies producing or importing Annex XIV substances will be required to apply for authorisation to the European Chemicals Agency (ECHA) in order to continue to produce and market the substance, except for specific exempt uses (for example, DBP is exempt from its use in immediate packaging of medicinal products covered by EC regulations).

In September 2007, Health Canada completed a public comment consultation on a proposal for legislative action on DEHP under the *Hazardous Products Act* (http://www.hc-sc.gc.ca/cps-spc/advisories-avis/info-ind/ethylhexyle-eng.php). During the consultation process, requests were received to expand the action to include more phthalates, (e.g. DBP, BBP and DINP). This led to a proposal for inclusion of Phthalates Regulations in June 2009, which prohibit the presence of DBP, DEHP and BBP at concentrations greater than 1000 mg/kg (equivalent to 0.1 % by mass) in the plasticised material used in toys and childcare articles, when tested in accordance with a method that conforms to good laboratory practice (http://www.gazette.gc.ca/rp-pr/p1/2009/2009-06-20/html/reg3-eng.html).

In August 2008, the US Congress passed the *Consumer Product Safety Improvement Act (2008)* to restrict certain substances in children's products. Among others, the law enacts a permanent restriction on three phthalates (DBP, DEHP and BBP) comprising more than 0.1 % w/w of children's toys or childcare articles.

In the US, use of DBP (or DEHP) in personal care products was prohibited by legislation in the State of California, effective 1 January 2007.

In December 2009, the US Environmental Protection Agency (USEPA) released a Phthalates Action Plan covering eight phthalates, including DBP. According to the plan, because of concerns over toxicity and evidence of human and environmental exposures to these phthalates, the USEPA intends to initiate action to address their manufacturing, processing, distribution and/or use. The action is part of a coordinated approach with the Consumer Product Safety Commission CPSC and US Food and Drug Administration (FDA).

The USEPA stated that they intended to initiate rulemaking to include these phthalates on the Concern List under Toxic Substances Control Act (TSCA) section 5(b)(4) as chemicals that present, or might present, an unreasonable risk of injury to health or the environment. The USEPA also intends to assess the use and exposure of, and substitutes for, these phthalates, and to consider a cumulative assessment approach, under development by the CPSC, for multiple phthalate exposures. In particular, the potential for a disproportionate impact on children and other sub-populations is to be evaluated. It was envisaged that any rulemaking from these assessments was to be initiated in 2012. To date, there is no information available and/or updates reported on the Phthalates Action Plan.

Beyond the recent action in USA, EU, and Canada, there are no regulatory restrictions on the use of DBP 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 cosmetics and children's products that contain DBP being imported into Australia from countries with no restrictions.

2.2 Australian perspective

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

As a result of literature searches and calls for information from industry in 2004 and 2006, one terephthalate and 24 ortho-phthalates, including DBP, were identified as currently or potentially in industrial use in Australia. DBP, 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.

DBP is currently listed in Safe Work Australia's Hazardous Substances Information System—HSIS (Safe Work Australia, 2010) where it is classified as a Reproductive Toxicant based on an adopted classification from the EU (EC Regulation No 1272/2008, see previous section). The classification for DBP specifies: Reproductive Toxicant Category 2 requiring the risk phrase 'R61: May cause harm to the unborn child, and Reproductive Toxicant Category 3 requiring the risk phrase 'R62: Possible risk of impaired fertility'.

DBP is currently listed with a time weighted average (TWA) exposure standard of 5 mg/m^3 in Safe Work Australia's HSIS (Safe Work Australia, 2010).

DBP is not currently listed in the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP 3, 2012).

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

2.3 Assessments by international bodies

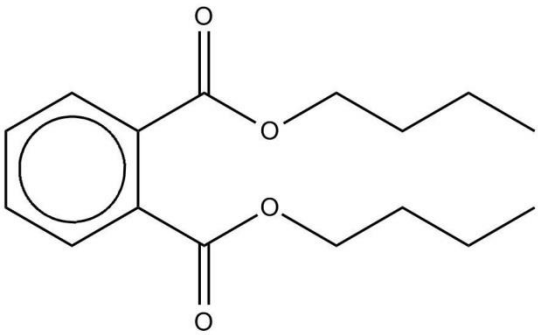
DBP has been assessed by several international bodies, which have reviewed and evaluated data pertaining to the health and/or environmental hazards posed by this chemical. Of these, the most noteworthy are:

- A European Union Risk Assessment Report (EURAR) on DBP (ECB, 2004);
- 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 dibutylphthalate within the OECD High Production Volume (HPV) Chemicals Program (OECD, 2001);
- US Agency for Toxic Substances and Disease Registry Toxicological Profile for di–n-butyl phthalate (ATSDR, 2001).
- US Center for the Evaluation of Risks to Human Reproduction, expert panel report on di-n-butyl phthalate (CERHR, 2000);
- IPCS program, Environmental Health Criteria (EHC) assessment 189 for di-n-butyl phthalate (IPCS, 1997); and
- Health Canada Assessment of DBP in 1994 when it was declared toxic under the Canadian Environmental Protection Act (CEPA), http://www.ec.gc.ca/substances/ese/eng/psap/psl1-1.cfm.

3. Identity, properties and analysis

Dibutyl phthalate (DBP) is listed on the Australian Inventory of Chemical Substances (AICS) as 1,2-Benzenedicarboxylic acid, dibutyl ester.

3.1 Chemical identity

Chemical Name (IUPAC):	Dibutyl-1,2- benzenedicarboxylate
CAS Nos.	84-74-2
EINECS No.	201-557-4
Synonyms:	DBP (ester)
	Di-n-butylphthalate
	Phthalic acid, dibutyl ester
	Bis-n-butyl phthalate
	Butyl phthalate
	Dibutyl o-phthalate
	Di(n-butyl) 1,2-benzenedicarboxylate
	n-Butyl phthalate
	Phthalic acid di-n-butyl ester
Molecular Formula:	$C_{16}H_{22}O_4$
Molecular Weight:	278.34
Structural Formula:	
Purity:	≥99.7 % w/w
Impurities:	ca. 0.01 % w/w butan-1-ol ca. 0.01 % w/w butyl benzoate

3.2 Physical and chemical properties

At room temperature, DBP is an oily colourless liquid with a slight characteristic odour.

Table 3.1 summarises the physico-chemical properties of DBP (adopted from ATSDR 2001; ECB 2004).

Table 3.1: Summary of physico-chemical properties

Property	Value
Melting point	−69 °C
Boiling point	340 °C (101.3 kPa)
Density	1045 kg/m^3 (20 °C)
Vapour pressure	97 ± 3.3 x 10^{-6} kPa (25 °C)
Water solubility	0.01 g/L (20 °C)
Partition coefficient n-octanol/water (log Kow)	4.57
Henry's law constant	4.5 × 10^{-6} m^3.atm/mole (25 °C)
	8.83 × 10^{-7} m^3.atm/mole (25 °C)
Flash point	157 °C

DBP is readily soluble in alcohol, ether, acetone, and benzene (ATSDR 2001).

Conversion factors: 11.36 mg/m^3 = 1 ppm

1 mg/m^3 = 0.088 ppm

4. Manufacture, importation and use

4.1 Manufacture and importation

DBP 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 that the chemical is manufactured in Australia.

The total volume of DBP imported into Australia for industrial uses in 2004, according to responses to a call for information on phthalates, was more than 350 tonnes. DBP is not included on the HVIC list for 2002 or 2006 as the quantity introduced by companies was below the reporting threshold of 1000 tonnes. The amount of DBP used in applications that have the potential for public exposure through use in toys, childcare articles and cosmetics, is relatively low (less than 100 kg reported for 2005 in cosmetics).

One respondent who imports DBP as a raw material that can be used in these applications indicated importation volumes of approximately 95 tonnes in 2005 and 80 tonnes in 2006. No further DBP-specific information is available on the introduction volume as either a raw material, or through imported finished products available to the public.

Information received in 2013 from Australian industry members indicated that DBP is not used as a primary plasticiser in vinyl products.

4.2 Uses of DBP

4.2.1 Use in Australia

DBP is mainly imported as finished products or mixtures.

According to information collected by NICNAS through calls for information from introducers of phthalates in 2004 and 2006, DBP is used industrially in Australia:

- as a plasticiser in surface coatings (paints, pigments, floor coatings);
- in car mat backing;
- in polymer emulsions for adhesives;
- in PVC compounds such as wire cable tubing and footwear;
- in nitrocellular lacquers for automotive refinishes;
- as an epoxy sealant;
- in leather paint;
- as galvanised iron primer and texture finishes;
- in floor polish and sealer;
- for textile wet processing products;
- as an ectoparisiticide for horses; and
- in screen printing inks.

Downstream products include safety glass, resins, adhesives, sealants, fragrance bases for household products, personal care and cosmetic products, nail polish, children's toys, exercise balls, hoses and rubber sheets.

In 2004, six companies indicated the import of DBP as a component of cosmetics and fragrances, toys and paint. The typical concentration of DBP in an unspecified set of products was identifed as 0.5 %. DBP was specifically identifed as used in playballs, hoppers, exercise balls, but the content was not specified. The typical concentration in personal care products (nail polish) was identified as 7 %.

In 2006 only one company indicated importing DBP as a raw material that could be used in toys, childcare articles and/or cosmetics.

The voluntary call for information on use of phthalates (including DBP) specifically in toys, also identified DBP as being in use, or with the potential for use, in a number of children's toys, some of which are intended for children aged 0–6 years.

Recently, a company reported that DBP is also imported for use as a laboratory research and development chemical in academic institutes and quality control laboratories. The total import volume of DBP for these applications was approximately 65 L between January 2010 and June 2013.

4.2.2 Uses overseas

DBP is used as a plasticiser in resins and polymers. It is also used as a softener in adhesives, lacquers, varnishes and printing inks. The ubiquity of DBP in consumer products is demonstrated by its wide usage in cosmetics as a perfume solvent and fixative, a suspension agent for solids in aerosols, a lubricant for aerosol valves, an antifoamer, a skin emollient and a plasticiser in nail polish and fingernail elongators (ECB 2004).

No data are available for estimating the worldwide production of DBP.

A number of international reports have given estimates of the quantitative usage distribution of DBP (as cited in ECB 2004). Based on 1997 data, on average around 76 % of DBP is used as a plasticiser in polymers, 14 % in adhesives, 7 % in printing inks and the remaining 3 % of DBP is used in miscellaneous other applications.

In the EU, the 1998 production volume of DBP was estimated at 26,000 tonnes, of which 8,000 tonnes was thought to be exported outside the EU (ECB 2004). No import of DBP into the EU was reported and a clear decreasing trend in the production of DBP was observed, with reduction from 49,000 tonnes in 1994 to 37,000 tonnes in 1997 (ECB 2004).

In the most recent technical report to ECHA on manufacture, import, export, uses and releases of DBP in the EU (ECHA 2009) the exact manufacture volumes are kept confidential, but the decreasing trend appeared to continue in recent years from >10,000 tonnes in 2005 to <10,000 tonnes in 2007 (ECHA 2009). The volume of DBP manufactured in the EU represents <1 % of the total volume of manufactured phthalates, estimated to be one million tonnes per year with ~900,000 tonnes being used as plasticisers for PVC (ECHA 2009).

The estimates of the export data for DBP include significant uncertainty as they may incorporate di-isobutyl phthalate (DIBP), a substance that is marketed as an alternative to DBP (ECHA 2009).

DBP is mainly used as a plasticiser in resins and polymers such as polyvinyl chloride. DBP is also used in printing inks, adhesives, sealants/grouting agents, nitrocellulose paints, film coatings, glass fibres and cosmetics (ECB 2004). The ECHA 2009 report indicates that polymer applications remain the major application area, noting that some of the nonpolymer applications, in particular adhesives, have increased (compared with data from the ECB 2004 report). The ECHA 2009 report notes that DBP is not permitted for use in cosmetics and toys (ECHA 2009).

Information from the Priority Substance List (PSL) report by Environment Canada (Government of Canada 1994) indicated that DBP was not manufactured in Canada. At the time, import volume of DBP—used mainly as a plasticiser in PVC emulsions—was estimated at about 540 tonnes a year. Importation of DBP in plastic products was not estimated. More recent and comprehensive information of DBP importation into Canada was not publicly available. However, a recent report (Koniecki et al. 2011) is available on the levels of DBP detected in a small sample of cosmetic products (252) tested in 2007. The sample included fragrances, hair care products (hair sprays, mousses, and gels), deodorants (including antiperspirants), nail polishes, lotions (body lotions and body creams), skin cleansers, and baby products (oils, lotions, shampoos and diaper creams). DBP was the second most frequently used phthalate after

DEP (103 of the 252 tested) higher than DIBP (9 products of 252), DEHP (8 of 252) and DMP (1of 252). The highest concentration of DBP was found in nail polish products (2.4 %), while in the other products it was 0.00036 % or less. DBP is not included on the Health Canada Cosmetic Ingredient Hotlist, which lists ingredients that are prohibited or restricted in cosmetics in Canada.

In the US, more than 8,500 tons of DBP was produced in 1994 (ATSDR 2001). More recent information from Massachusetts through the Toxics Use Reduction Institute (TURI) indicates a progressive decline in DBP use from 1996 onwards—total volume of DBP used in Massachusetts in 1996 was reported to be 679,177 pounds (~ 310 tonnes), about 400, 000 pounds (~180 tonnes) was reported for 2002 further declining to 28,811 pounds (~13 tonnes) in 2007. Manufacture of DBP was not reported by companies in Massachusetts. The uses of DBP were not detailed in this report (TURI 2010). http://turadata.turi.org/report.php?action=report_chemical_quantity_summary_all_years&cas_number=84742.

On 31 December 2011, the Eastman Chemical Company in the US announced that it will discontinue the manufacture and supply of DBP (and DEP) as non-phthalate alternatives have become available (Eastman press release 16 March 2011).

4.2.3 Uses of phthalates and possibilities for substitution

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

The physicochemical factors expected to affect the choice of specific phthalates for particular uses include viscosity, water solubility and vapour pressure/boiling point. These properties alter with increasing molecular weight and side-chain length. As side-chain length increases from one to 13 carbons, phthalates exhibit a number of orders of magnitude decrease in vapour pressure. Water solubility is also inversely related to molecular weight and side-chain length (NICNAS 2008b). Viscosity varies from 9 mPa.s for DEP, 15 mPa.s for DBP, 52 mPa.s for DINP, 56 mPa.s for DEHP and up to 190 mPa.s for ditridecyl phthalate (Eastman 2002).

Thus, a HMW phthalate ester (for example, DINP) will be quite different from a LMW phthalate ester such as DMP. However, the difference in properties between two phthalate esters of similar molecular weight, such as DMP and DEP, would be expected to be much less. To the extent that these are the key considerations, substitution of a particular phthalate for 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 DMP.

Minimal information is publicly available in literature on the subject of substitutability of phthalates. A number of phthalates and their functions are listed in the International Cosmetic Ingredients (INCI) Dictionary and Handbook (Personal Care Products Council 2012). For example, DMP, DEP, DBP and DEHP all have listed functions as fragrance ingredients, plasticisers and solvents. DBP is also listed in the Compilation of Ingredients Used in Cosmetics in the United States (CIUCUS) (Bailey 2011). The CIUCUS provides a compilation of ingredients that have use in cosmetics documented by the FDA. However, the Scientific Committee on Cosmetic Products' (SCCP's) Opinion on phthalates in cosmetic products concluded that, among the phthalates found in a study of 36 perfumes (Peters 2005), only DMP (0.3 %) and DEP (up to 2.23 %) are likely to have been deliberately added, while DBP, DINP, DIDP, DIBP (a possible substitute for DBP), BBP and DEHP are likely to be present as traces and/or impurities leaching from plastic materials during production or storage (SCCP 2007).

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

Therefore, while it is clear that phthalates can be considered to be substituted with other phthalates of similar properties, there are likely to be limits on the extent to which dissimilar phthalates can be used. DMP is a LMW phthalate; therefore, it is not likely to be an appropriate substitute for DINP—a HMW phthalate commonly used as plasticiser in toys and childcare articles. In the absence of use data for DBP in the two scenarios considered, an assumption for complete substitutability was made. In this report, for example, migration or leaching rates reported for DINP are used to undertake an exposure assessment for DBP as part of a secondary plasticiser in mixed phthalate plasticiser (DINP + DBP) in relation to its use in toys and childcare articles.

5. Public exposure

Scope

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

- 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 DBP.

5.1 Methodology for assessing exposure

It is acknowledged that there are always uncertainties in deriving exposure estimates. Using measured data is always preferred in exposure assessments; however, modelled data might be used if measured data are not available. Australian data are also preferred, however if they are not available, overseas data might be used, provided that the scenarios represented by that 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 that represents an average for the target population.

5.1.1 Model for estimation of exposure of children from toys and childcare articles

Exposure to DBP in children's toys and childcare articles was estimated for children for both the oral and dermal routes. There is insufficient information available on the DBP content in toys in Australia. Therefore, the exposure estimate is based on international data related to the usage and concentration of DBP as part of a mixed phthalate plasticiser in toys and childcare articles in Australia.

Oral exposure was modelled using an estimate of:

- highest concentrations of DBP as a component of a mixed plasticiser in toys and childcare articles in Australia;
- the available fraction of DBP based on the results of overseas studies of children's mouthing behaviour and the extractability of phthalate plasticisers under mouthing conditions (see Section 5.2.1); and
- oral bioavailability of DBP (see Section 7.1)

Dermal exposure was modelled using:

- an estimate of the highest concentrations of DBP in toys and childcare articles as a component of a mixed plasticiser in Australia;
- default values for exposed surface area and body weight:
- an estimate of dermal contact time with toys and childcare articles; and
- an estimate of the migration rate of the mixed plasticiser from PVC matrix through the skin based on experimental studies (NICNAS 2010).

5.1.2 Model for estimation of exposure of the general population, including children, from cosmetics

Both dermal and inhalation exposure to DBP from cosmetics for the general population, including children, were estimated.

Dermal exposure was modelled using:

- the highest concentrations of DBP in cosmetic products for dermal application in Australia;
- default values for usage volumes and frequency for cosmetic products; and
- an estimate for dermal bioavailability of DBP (see Section 7.1).

Inhalation exposure was modelled using:

- the highest concentrations of DBP in cosmetic products applied by spraying in Australia;
- default values for usage volumes and frequency for cosmetic products;
- default values for inhalation rates and other parameters related to cosmetics using spray application; and
- an estimate for inhalation bioavailability of DBP (see Section 7.1).

International biomonitoring data provided an estimation of overall exposure of the general population, or specific subpopulations, to DBP. However, biomonitoring data do not allow contributions from specific exposure routes to be separately determined. Therefore, the available biomonitoring information was used to check whether the exposure estimates from the different routes were within the range of known population exposures, and whether they were likely to be major contributors to overall exposure.

The uncertainties in the exposure assessment are discussed in the context of the risk characterisation in 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 DBP, are used in children's plastic toys sold in Australia. However, data on the phthalate content of the toys were limited and import volumes relating specifically to toys were not available. Therefore, it was necessary to use overseas data to quantify the presence of phthalates in soft plastic toys and establish possible levels of exposure to children.

The limited Australian information, obtained through a voluntary call for information in 2004, showed one company importing articles for children aged four and above with a DBP content of 0.45 %. Another company reported importing playballs, hoppers and exercise balls containing 40 % total phthalates consisting of DBP, DIDP, DINP, DnOP, and DMEP. The concentration of DBP alone in the playballs was not provided. Since the information provided for the assessment only covered a small proportion of the toys available on the Australian market, available overseas data were also examined to establish a reasonable worst-case scenario of DBP exposure of children through the use of toys.

Stringer et al. (2000) investigated the composition of a range of plastic children's toys (71 toys, analysed as 76 different plastic components, 88.9 % of which were PVC or part-PVC and 11.1 % non-PVC) purchased in 17 countries, including five purchased in Australia. The country of origin was also stated, with 41 out of 71 toys purchased worldwide being made in China, including four of the five toys 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 (48 %) with concentrations ranging from 0.008 % to 35.5 % w/w. DBP was found in 12.5 % of the toys tested with concentrations ranging from 0.002 % to 0.18 %. Variations between batches in commercial and industrial mixes contaminated with other phthalates or other compounds were noted. Several phthalates were also found in concentrations too low to have a plasticising function. These phthalates could have been present as a

constituent or contaminant of other phthalates, a constituent of an ink or paint used on the toy, used as a processing aid or during manufacture of other products. The results indicated that the majority (72 %) of soft plastic toys contain substantial proportions of phthalates and that, in all of these, a single phthalate (normally DINP and occasionally DEHP or DIOP–diisooctyl phthalate) was dominant.

Rastogi (1998) performed an analysis of seven PVC toys and 10 non-PVC plastic toys to determine the phthalate content. DINP and DIDP were the predominant phthalates found in all of the seven PVC toys. DBP was found in only one toy (the doll head) at a maximum level of 0.01 %, this toy also had a DEHP content of 22.4 %.

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

In 2006, the Intergovernmental Forum for Chemical Safety (IFCS) published a paper: *Toys and chemical safety* (IFCS 2006), containing information on selected chemicals, including phthalates, in toys available in industrialised countries. The data presented in the report were compiled from a number of available studies on the different types of chemicals found in toys. This review indicated that DBP could be present in certain children's toys (e.g. teethers, play putty and bath toys) at weight concentrations up to 380 ppm (0.038 %). Most of the toys containing DBP also contained a mixture of phthalates, with high concentrations of DINP and DEHP.

The phthalate levels of toys available in the Indian market were investigated. Most of the toys analysed were for children aged three years and below. A total of 15 soft and nine hard plastic toys were tested. All were reported to contain phthalates. The predominant phthalates in the soft plastic toys were DINP and DEHP, with concentrations of up to 16.2 % DINP and 2.6 % DEHP. DBP was found in three out of the 15 soft toys (up to 0.1 %) (Johnson et al. 2011).

Chen (1998) conducted a study to identify phthalate-containing products (a total of 35 samples) that were likely to be mouthed by children in the US, to determine the amount of phthalate migration from these products using in vitro and in vivo tests. The products included soothers, teethers, nipples, pacifiers, books, handbags, 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 piston impact. For in vivo tests, human volunteers gently chewed/mouthed a polyethylene disk from a toy duck for four 15-minute intervals; saliva was collected after each chewing period. The study reported DINP to be the predominant phthalate found in children's toys with content ranging from 15–54 % by weight. DEHP and other phthalates, DIOP and di-n-nonyl phthalate (DnNP), were also found. DBP was not found in any of the samples tested.

Health Canada (2009—Canada Gazette) analysed 100 toys for phthalate content during 2007. Of these, 72 toys 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 four (7 %) contained DBP, while none contained DEP, BBP, DIDP or DnOP. The average concentrations were 12.5 % (DEHP), 21.9 % (DINP) and 0.08 % (DBP). Concentrations in individual toys were not reported. The results of this study were consistent with the results from Stringer et al. (2000), confirming that both DEHP and DINP were widely used, but with overall higher levels of DINP.

The overall findings from the above studies indicated that DBP was infrequently found in toys and, where present, at very low concentrations (up to 0.5 %) and in conjunction with higher levels of the predominant phthalates DINP and DEHP.

5.2.2 Concentration estimates for use in exposure assessment

Australian information on the concentrations of DBP in children's toys and childcare articles is restricted to one company, which provided information that DBP is imported as a component of articles for children aged four and above at a concentration of 0.45 %. The 40 % total concentration of mixed phthalates reported by an Australian company in 2004 is not considered in the exposure assessment, as the concentration of phthalate provided was for a mix of phthalates with an unspecified DBP content. Moreover, these products might not be suitable for mouthing by six-month-old infants. The limited reporting of DBP in toys and childcare articles in Australia, and the low concentrations reported overseas in the available published information, suggests that DBP is not normally used as a primary plasticiser in PVC due to its relatively high volatility (Wypych 2003; Chanda & Roy 2007), and that the main plasticisers used are DEHP and DINP, both of which have lower volatility. However, Chanda & Roy (2007) also indicated that due to the volatility of DBP, it has an application in PVC as a secondary plasticiser, and is a small component in a mixture of plasticisers used as a processing aid.

Using DBP as a secondary plasticiser is more probable than substituting DBP for DEHP or DINP as a primary plasticiser. This use scenario is consistent with the findings of the analytical studies described above (Section 5.2.1).

Therefore, calculating exposures to DBP is based on the assumption that the chemical is used as a secondary plasticiser at the maximum level observed (0.5 % w/w as a component of a mixture of plasticisers) in the analytical studies of toys.

5.2.3 Routes of exposure

There are two probable routes of exposure to DBP: plastic toys and childcare articles. Firstly, dermal exposure may occur during normal handling and, secondly, oral exposure may occur through chewing, sucking and biting these products, regardless of whether the products are intended to be mouthed. Inhalation exposure to DBP from these products is considered negligible due to the chemical's low vapour pressure.

When children mouth or chew childcare articles or toys, phthalate plasticisers can migrate into the saliva and be swallowed and absorbed in the gastrointestinal tract (GIT), or can be absorbed directly through the buccal mucosa (the mucous membrane lining the inside of the cheek). The amount of phthalate released from a product when it is mouthed or chewed is determined by the amount of time the product is in the child's mouth and the migration rate of phthalate from the product. The studies used for estimation of mouthing times and migration rates of phthalates from plastic articles under mouthing conditions are mostly performed on PVC that contains DINP and are summarised in the NICNAS PEC assessments on DEHP and DINP (NICNAS 2010, 2012). The results demonstrate that migration rate of phthalate plasticisers from plastic toys into saliva through biting and chewing is the combined effect of molecular diffusion and mechanical action, with the latter the likely dominating factor. The phthalate migration rate from articles appears largely determined by the magnitude of the mechanical force applied to an article and the properties of the PVC grade comprising the article; less so by the physicochemical characteristics or concentration of the particular phthalate. Therefore, although migration data that are specific for DBP and most phthalates are not available, the migration rates determined for DINP under chewing conditions can be extrapolated to other phthalates such as a mixture of phthalate plasticisers (i.e. primary and secondary plasticisers), which include DBP.

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

Oral exposure of children to DBP from mouthing toys was estimated by assuming that DBP is present in toys as a secondary plasticiser at a maximum concentration of 0.5 % and part of a phthalate plasticiser mixture containing DINP levels of up to 43 %, based on the weight of the

toy. A detailed calculation of exposure of children to DINP under this scenario explaining the derivation of all of the relevant parameters is given in the NICNAS PEC assessment of DINP (NICNAS 2012). The exposure estimate was made for a 6-month-old infant based on studies demonstrating that the maximum mouthing behaviour occurs between 6–12 months. The infants at six months have the minimum body weight in this calculation and therefore the highest systemic exposure.

The parameters considered in estimating the oral DBP exposure from mouthing toys and childcare articles were:

- a child of six months who weighs 7.5 kg;
- the surface area of the child's open mouth (10 cm^2);
- the time the child spends mouthing toys and childcare articles (typical value is 0.8 h/d and worst-case value is 2.2 h/d);
- phthalate oral bioavailability (100 %); and
- the migration rate of DINP from the toys and childcare articles under mouthing conditions (typical value is 26 $\mu g/cm^2/h$ and worst-case value is 58 $\mu g/cm^2/h$, based on studies using adult volunteers).

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

- reasonable worst-case extraction data from a study for DINP at a measured plasticiser concentration of 43 % (w/w) (NICNAS 2012);
- the extractability data for 43 % DINP are also applicable where the total phthalate concentration in the toys and childcare articles of 43 % (w/w) is comprised of 0.5 % (w/w) DBP and 42.5 % (w/w) DINP, i.e. 43 % of a mixed phthalate containing 1.16 % DBP and 98.84 % DINP; and
- the mixed phthalate is extracted under mouthing conditions without a change in composition.

The estimates for DBP are derived by multiplying the internal exposures from the total mixed phthalates by the proportion of the DBP content (1.16 %) in the mixed phthalates based on the parameters and assumptions stated above. These assumptions are the same as those used in the PEC assessment of DEP (NICNAS 2011), which is also used as a secondary plasticiser in toys and childcare articles.

Table 5.1: Estimated daily internal dose for total phthalate and DBP from oral exposure to children mouthing toys and childcare articles

Type of exposure	Total phthalate $D_{int.oral}$ (μg/kg bw/d) (NICNAS 2010)	DBP $D_{int.oral}$ (μg/kg bw/d)
Typical	27.8	0.32
Reasonable worst-case	169.9	1.97

$D_{int.oral}$—Internal dose via the oral route

The reasonable worst-case internal DBP exposure is estimated by considering the worst-case values of mouthing time and migration rate, which takes into account some individuals having higher exposures than others.

The EU risk assessment report (ECB 2004) stated that DBP in children's toys and childcare articles could be present as a by-product or impurity. The report also estimated an oral exposure of 0.81 μg/kg bw/day from DBP in these items assuming an 8 kg infant mouthing a toy with an area of 10 cm^2 for six hours every day. The value was estimated using a migration rate of 0.11 $\mu g/cm^2/h$ obtained from a Danish study. Oral exposure estimated in the EU report is within

the range of the typical and reasonable worst-case estimates in Table 5.1. The migration rate used in this assessment is the highest in vivo migration rate observed for DINP in a well-conducted study (Chen 1998) from the evaluation of several extractability studies of phthalate plasticisers (NICNAS 2010). In addition, the migration rate from a Danish study of 0.11 μg/cm^2/h used by the ECB (2004) has not been able to be reproduced by other laboratories (Chen 1998; Wilkinson & Lamb 1999).

5.2.5 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. Dermal exposure to DBP is estimated assuming that DBP is present as a secondary plasticiser in the toys at a maximum concentration of 0.5 % based on the weight of the toy. A detailed calculation of exposure of children to DBP under this scenario explaining the derivation of all relevant parameters is given in the NICNAS PEC assessment of DEHP (NICNAS 2010). This calculation is assumed to be applicable for a mixed phthalate containing DBP. The estimate is made for a 6-month-old infant, as the combined dermal and oral exposure is expected to be highest for this age group.

The parameters considered in estimating the dermal DBP exposure from toys and childcare articles were the following:

- a child of six months who weighs 7.5 kg;
- the contact surface area based on exposure to lips and hands (100 cm^2);
- the time the child spends handling the toys (typical value is 0.8 h/d and worst-case value is 2.2 h/d); and
- the dermal absorption rate of DEHP from a PVC film (0.24 μg/cm^2/h) (NICNAS 2010).

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

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

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

Table 5.2: Estimated daily internal dose for total phthalate and DBP from dermal exposure to children from toys and childcare articles

	Total phthalate $D_{int.dermal}$ (μg/kg bw/d) (NICNAS 2010)	**DBP $D_{int.dermal}$ (μg/kg bw/d)**
Typical exposure scenario	2.6	0.03
Worst-case exposure scenario	7.0	0.08

$D_{int.dermal}$—Internal dose via the dermal route

5.2.6 Combined exposure estimates for children from contact with toys and childcare articles containing DBP

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

Table 5.3: Estimated total internal exposure for children

Route of exposure	Typical D_{int} (μg/kg bw/d)	Worst-case D_{int} (μg/kg bw/d)
Oral	0.32	1.97
Dermal	0.03	0.08
Combined	0.35	2.05

D_{int}—Internal dose

5.3 Cosmetics and personal care products

5.3.1 Sources of exposure

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

Australian data (2004 and 2006) show that DEP, DBP, DMP, and DnOP are used, or have the potential for use in cosmetics and personal care products. However, DEP is the predominant phthalate reported to be used in cosmetics and is present in a number of cosmetic product types.

Worldwide, the phthalates predominantly found in personal care and cosmetic products are DEP and DBP (Hubinger & Havery 2006*; US FDA 2008). Analysis of 48 cosmetic products available to consumers in the US showed high levels of DBP in nail enamel at concentrations up to 59,815 ppm (5.98 %) (Hubinger & Havery 2006*). A follow-up survey of 84 cosmetic and personal care products available in the US market reported concentrations of DBP at a maximum level of 62,607 μg/g (6.3 %) in nail polish (Hubinger 2010). DEP was the most frequently found phthalate in these surveys. In cosmetic products available in Korea, DBP was detected in 11 out of 42 perfumes and 19 out of 21 nail polish products at concentrations of up to 5051 ppm (0.5 %) (Koo & Lee 2004). DBP was found in 21 out of 36 perfumes tested in the EU with concentrations of up to 14 ppm (0.0014 %) (Peters 2005).

A more recent analysis of 252 cosmetic and personal care products, 98 of which were baby care products collected from retail stores in Canada, detected DEP, DMP, DIBP, DBP, and DEHP. DBP was detected in 15 products with a maximum concentration of 24,304 μg/g (2.4 %) in nail polish products. In the baby care products, DBP was only detected in baby shampoos at levels up to 1.8 μg/g (0.00018 %). DEP was the predominant phthalate detected in the baby care products (Koniecki et al. 2011).

Overall, the data suggest that the use of DBP in cosmetic and personal care products is mainly as a plasticiser in nitrocellulose nail polish films, rather than as a cosmetic solvent.

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

Mitani et al. (2003) analysed the amount of DEP, 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. DBP was detected in all of the four eye drops samples at a maximum level of 142.1 ng/mL ± 4.4 ng/mL.

The concentration of DBP arising from leaching from plastic containers is likely to be negligible. Moreover, the concentrations of DBP in cosmetic and personal care products are well above the measured values from a single study of phthalates in packaging.

5.3.2 Concentration estimates for use in exposure assessment

Australian information on the concentrations of DBP in cosmetic products includes one company in 2004 providing a typical concentration of 5 % DBP as a plasticiser in nail polish, and three companies in 2006 estimating a typical concentration of DBP as follows: 7 % in nail polish; 4.96 % in nail enamel; and <2 % as fragrance base. These concentrations are insufficient to determine the likely concentration of DBP across a range of types of cosmetic products for use in assessing exposure. The limited information from overseas sources may reflect the EU prohibition of DBP in cosmetics. However, due to the absence of restrictions on the use of DBP in cosmetics in Australia and many other countries, it is not possible to assume that all products marketed in Australia meet the EU standards.

Insufficient information on the actual concentrations of DBP in cosmetics in Australia and the assumption of complete substitutability of phthalates as 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 DBP based on the assumption that it could replace all DEP currently used in cosmetics. Therefore, the content of DBP in cosmetic products for the purposes of exposure assessment was assumed to be similar to the concentrations of DEP currently reported in different cosmetic product types in Australia. This provides a basis to estimate a potential level of exposure to DBP from cosmetic use. These values are used to calculate exposures for the different cosmetic product types (see Table 5.4).

5.3.3 Routes of exposure

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

Accidental oral exposure to phthalates from cosmetic and personal care products is unlikely to occur frequently (e.g. biting of finger nails that are painted with nail polish containing DBP) and would involve only very small amounts of the chemical. Current information does not indicate use of phthalates in oral cosmetic products that are likely to be subject to inadvertent ingestion such as toothpastes, mouthwashes, lipsticks and lip-glosses. Therefore, the potential for public exposure via this route is expected to be negligible and, hence, is not characterised further.

5.3.4 Estimates of dermal exposure

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

Dermal exposure to DBP 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 bio-availability of DBP. The rate of absorption was not used as it is considered that the total dermal bioavailability better reflects the absorption for a single dose than a prolonged exposure period.

No data on Australian use patterns (for example, typical amount used each application, frequency of use and exposure duration) were available for cosmetics or personal care products. However, data collected on typical use patterns of some classes of these products in Europe are provided in the *Technical guidance document on risk assessment* (TGD) of the European Chemicals Bureau (EC 2003) and the Scientific Committee on Consumer Safety's (SCCS) *Notes of guidance for the testing of cosmetic substances and their safety evaluation* (SCCS 2012).

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 DBP via the dermal route was assessed to be 5 % (based on a number of studies discussed in Section 6.1.1 and 7.1). The internal dose arising from dermal exposure to cosmetic and personal care products was estimated using Equation 1 below:

Equation 1

$$D_{int,derm} = \frac{A_{prod} \times n \times \frac{C}{100} \times \frac{B_{derm}}{100} \times RF \times CF}{BW}$$

Where:

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

The calculated daily internal DBP doses from the use of different product types are shown in Table 5.4 (page 31).

Not all product types reported by the Australian industry containing DEP, as summarised in the NICNAS PEC assessment of DEP (NICNAS 2011), have been included in this calculation. Some of the cosmetic and personal care products have interchangeable uses (e.g. hand wash and bar soaps) and, in these categories, only the product types with the higher DEP concentration have been used for the calculation.

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 DBP dermal exposure is determined to be 123.80 μg/kg bw/d.

The internal dermal exposures calculated using Equation 1 are frequently referred to as point estimates from a deterministic approach, using single values to represent each exposure variable to produce a single exposure estimate. An alternative method used in the exposure calculations is a probabilistic modelling approach, which uses the distributions around each variable as inputs, rather than single values, to generate an exposure distribution. Calculations therefore account for all the possible values of a variable in relation to the probability of each value occurring, generating a range of exposure estimates (WHO 2005).

In the case of the estimates for DBP internal exposure, the probabilistic approach was not conducted for all the cosmetic product types listed in Table 5.4, since the implementation of this distribution-based approach requires data obtained from a large sample size (IGHRC 2004).

Table 5.4: Typical use pattern and calculated daily internal dose from dermal exposure ($D_{int, derm}$) to various cosmetic and personal care products in adults

Product type	A_{prod}—n (mg/day)	RF	C^b (% w/w)	$D_{int,derm}$ (µg/kg bw/d)
Leave-on products				
Body antiperspirant roll-on / liquid	1500[a#]	1	1.13	12.11
Cologne / splash / aftershave	2400[a]	1	0.97	16.63
Nail polish	108[a]	1	25.0	19.13
Face cream / Moisturizer	1540[a#]	1	0.42	4.62
Body lotion	7820[a#]	1	0.25	13.96
Perfume spray[c]	3188[a]	1	2.5	56.92
Rinse-off products				
Soap bars	4800[a]	0.01	0.15	0.05
Shower products	10000[a]	0.01	0.48	0.34
Shampoo / conditioner	10460[a#]	0.01	0.05	0.04
Shaving products (cream, gel, stick, lather)	2000[a]	0.01	0.005	0.0007

a Typical values for use parameters derived from EU TGD (EC 2003) or **a#** are from SCCS (2012) with the higher value from the two references chosen for the calculations. A_{prod} = Amount of product applied daily (mg/event) and n = frequency of product application (event/day) are presented as a product of the two parameters. RF = retention factor.

b Concentrations of DBP derived from the maximum amount of phthalate (DEP) reported in these products in Australia.

c Dermal exposure estimated for perfume (A_{prod} = 750 mg/event and n = 5 events/day) assuming 85 % of the spray product amount ends up on the skin (Bremmer et al. 2006).

However, the values used for some of the parameters in Equation 1 were based on distribution data for typical cosmetic use levels (i.e. amount and frequency of use) for four cosmetic product types (liquid deodorant, face moisturiser, body lotion, and shampoo). The Scientific Committee on Consumer Safety (SCCS) adopted the findings of the European Cosmetic, Toiletry and Perfumery Association (Colipa) study in their latest cosmetics guidance (SCCS 2012) regarding the default values of exposure to certain cosmetic product types. Colipa investigated the probabilistic analysis of the use pattern based on distribution values from actual monitoring of some cosmetic products used by 44,100 households and 18,057 individual consumers in five European countries (Hall et al. 2007). The SCCS (2012) used the 90th percentile values of the Hall et al (2007) study.

Thus, the internal dose estimates presented in Table 5.4 are semi-probabilistic, based on the distribution values used for some cosmetic product types.

In Canada, the daily DBP internal exposure was only calculated for the nail polish and hair care products listed in Table 5.4, since the DBP levels in the other cosmetic product types were

below 10 μg/g. The dermal bioavailability used in the calculation was 0.5 % for nail polish exposure and 5 % for hair care exposure. Based on limited measured data, the daily DBP dermal exposure from fragrance, lotion, hair care, deodorant, and nail polish products was also analysed (Koniecki et al. 2011), and estimated as 0.36 μg/kg bw/d for female adults.

Dermal exposure in children

There are no available data on cosmetic product usage in children by age, or of differences in skin permeability between children and adults.

Using the model developed by NICNAS (NICNAS 2010), the quantity of whole body product applied to a child or infant can be estimated from the child or infant's ratio of body surface area compared with the adult. The systemic dose depends on the body weight of the child or infant, and therefore the systemic dose for any product used similarly in children and adults will vary according to the ratio of surface area to body weight, if the skin permeability is the same in adults and children. An estimate of the magnitude of the difference can be made using data issued by the SCCS on the Margin of Safety calculation for children (SCCP 2012). For children aged 0–10 years, the difference between the surface area to bodyweight (SA/BW) ratio is as follows: 2.3-fold at birth, 1.8-fold at six months, 1.6-fold at 12 months, 1.5-fold at five years and 1.3-fold at 10 years (SCCP 2012).

Assuming substitutability of phthalates, one type of cosmetic product that could contain DBP and is used on infants or children, is body lotions or creams. The maximum concentration for DBP in lotions and creams is 0.25 %. The internal dose for children up to 12 months using these products is calculated using the correction for the SA/BW ratio (SCCS 2012), and point estimates of product amount and frequency of use for the general population as described in ECB (2003), as these are considered more appropriate for children than the probabilistic data derived specifically for adult activities. These calculations and assumptions were previously used by NICNAS (NICNAS 2010; 2011). 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 ($D_{int,derm}$)to baby lotions

Infant age	Adult $D_{int,derm}$# (μg/kg bw/d)	SA/BW ratio	$D_{int,derm}$ (μg/kg bw/d)
Newborn	26.79	2.3	61.7
6 months	26.79	1.8	48.2
12 months	26.79	1.6	42.9

Derived on a deterministic basis for DEP (NICNAS 2011) modified for bioavailability

5.3.5 Estimates of inhalation exposure

Inhalation exposure to DBP from cosmetic and personal care products can occur through inhaling spray aerosols such as antiperspirant body sprays and/or perfume sprays.

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

- adult inhalation rate is 22 m^3/d (enHealth 2003);
- phthalate bioavailability through inhalation is 100 %;
- the average body weight is 70 kg (ABS 2005);
- room volume of 2 m^3 to represent the volume of air immediately surrounding the user (EC 2003); and

- assumed exposure duration is 3.17 minutes, consisting of 10 seconds for actual spraying of the product and a further three 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:

Equation 2

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

Where:

$D_{int,inh}$ = Internal dose via the inhalation route, μg/kg bw/d
A_{prod} = Amount of perfume spray, mg/event
n = Frequency of spray application, event/d
C = Concentration of DBP in product, %
B_{inh} = Bioavailability via the inhalation route, %
t = Time of contact (spray and exposure duration), minute
IR_{air} = Inhalation rate of person, m^3/d
CF_1 = Conversion factor (time), 1 d/1440 minutes
CF = Conversion factor (amount), 1000 μg/mg
V = Room volume, m^3
BW = Adult body weight, kg.

Data on the typical use pattern of these products can be found in the *Technical guidance document on risk assessment* (TGD) of the European Chemicals Bureau (ECB 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 DBP are the maximum phthalate concentrations reported in these products in Australia. The typical use pattern and calculations of DBP 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 ($D_{int,inh}$) to cosmetic and personal care products

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

a Typical values for use parameters derived from EU TGD (ECB 2003). A_{prod} = Amount of product applied daily (mg/event) and n = frequency of product application (event/day).

b Concentrations of DBP derived from the maximum amount 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/d. It is considered likely that only one of these two types of products listed in Table 5.6 would be used by an individual on a single day.

5.3.6 Combined exposure from contact with cosmetic products

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

Table 5.7: Total estimated exposure to DBP from cosmetic use

Route of exposure	D_{int} (µg/kg bw/d)
Dermal	123.8
Inhalation	32.4
Combined	156.2

5.4 Comparison with biomonitoring data

There have been some attempts to use biomonitoring data to estimate exposure to DBP through cosmetic and personal care product use. However, DBP is widely used in a range of cosmetic and non-cosmetic products; therefore, it is very difficult to specifically assess DBP exposure from these products unless there is information available on their phthalate content and use rates. One US study (Sathyanarayana et al. 2008a) monitored the presence of metabolites of nine phthalates, including DBP, in the urine of 163 infants in relation to the mother's reported use of five types of baby care products within the 24-hour prior to urine collection. The urine measurements were not used to determine doses. The study suggested that the level of phthalate metabolites in the infant's urine could be associated with the use of baby care products, and significant association was observed in younger infants (Sathyanarayana et al. 2008b). However, no information was available on the phthalate content of the products used in the study (tested or manufacturer-reported) and information on use was derived from self-reporting by the mothers, which did not include reporting on the amount of product used.

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

Biomonitoring data for the Australian general population or specific subpopulations are not available. Table 5.8 summarises several international biomonitoring investigations that provide exposure estimates for DBP as determined from the concentrations of monobutyl phthalate (MBP), the urinary metabolite of DBP.

Table 5.8: Summary of biomonitoring data estimating exposure to DBP

Study	Population group	Exposure (µg/kg bw/d)	
		Median	95th Percentile
Marsee et al. (2006)	214 mother–infant pairs observed for monoethyl phthalate (MEP) levels	0.99	2.68
Wormuth et al. (2006)	Compilation of several German studies for the general population	7.4 (children) 8.4 (females) 5.1 (males)	 24.4 (females) 17.4 (males)
Frederiksen et al. (2011)	129 Danish children and adolescents (6–21 years old)	4.29*	11.3*

Guo et al. (2011)	Adults (21–49 years old) from 7 Asian countries	110–822 μg/day

* Based on combined levels of MBP and monoisobutyl phthalate (MiBP) urinary metabolites analysed together.

There is a wide range between the median values in the large studies in Table 5.8. In the Wormuth et al. (2006) study, comparison of the median and 95th percentile values indicate that some members of the population may be exposed to much higher DBP doses than the population average. In addition, there is a discrepancy of exposure between adult males and females, as well as between adult males and children. The maximum calculated exposure from biomonitoring data from this study was 28.0 μg/kg bw/d for one female participant, compared with a median dose of 8.4 μg/kg bw/d for female adults and 5.1 μg/kg bw/d for male adults. This indicates that high exposure scenarios may be applicable to a subset of the population.

The calculated reasonable worst-case DBP exposure to cosmetics and personal care products is greater than the biomonitoring data of the DBP metabolite(s), due to the worst-case assumptions used. However, the estimates for cosmetic use for a single product such as body lotion are close to the 95th percentile and the maximum concentrations measured in these large biomonitoring studies. This indicates that the worst-case exposure scenarios considered in this assessment are applicable for highly exposed individuals. The results seen in the biomonitoring studies are also consistent with the basis of the exposure assessment of DBP, as they indicate that the general population exposure is much lower than the individual exposure, which can arise from these specific high-exposure scenarios. In comparison, the adult biomonitoring values for DBP were up to 41 times lower than the DEP concentrations in the Marsee et al. (2006) study and consistent with the expectation that DEP is more widely used in cosmetic products than DBP.

The Fourth National Report on Human Exposure to Environmental Chemicals (CDC 2009) is an ongoing evaluation of the US population exposure to environmental chemicals through cumulative biomonitoring studies. The median and 95th percentile urinary MBP levels decreased by up to 28 % from 1999–2000 to 2001–2002 and increased by up to 13 % from 2001–2002 to 2003–2004. The biomonitoring values from this report indicate that some age groups (6–11 years old) could have higher levels of DBP exposure compared with the general population. Corresponding dose estimates were not calculated for these results.

5.5 Cumulative exposure to multiple phthalates

Cumulative exposures to phthalates can arise from exposure to multiple phthalates used in cosmetics, toys and childcare articles, and from the exposure to a range of products containing phthalates. Further analysis of the combined exposures of DBP, DEHP, DINP, and/or DEP is discussed in Appendix 3.

6. Human health hazard assessment

NICNAS published the Existing Chemical Hazard Assessment Report on DBP in June 2008 (NICNAS 2008a) using as data sources the key international reviews prepared by the:

1. International Program on Chemical Safety—Environmental Helath Criteria No 189 (IPCS 1997);
2. European Chemicals Bureau (ECB 2004);
3. US Centre for the Evaluation of Risks to Human Reproduction (CERHR 2000); and
4. Agency for Toxic Substances and Disease Registry (ATSDR 2001).

This chapter of the PEC assessment report is largely based on the Existing Chemical Hazard Assessment Report (NICNAS 2008a), but has been supplemented with an evaluation of new relevant data identified from comprehensive searches of DBP related literature up to January 2013.

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

6.1 Kinetics and metabolism

6.1.1 Absorption

Absorption via the oral route

DBP is readily absorbed from the gastrointestinal tract (GIT). In studies with rats and hamsters treated orally with ^{14}C-DBP (dose not reported), between 63 % and ≥90 % of the applied dose was excreted in urine within 48 hours (Williams & Blanchfield, 1975*; Tanaka et al. 1978*; Foster et al. 1983*). Faecal excretion was low (1.0–8.2 %) (Tanaka et al. 1978*).

Tomita et al. (1977*) reported oral absorption of DBP in humans after detecting increases (cf. controls) in blood levels in 13 individuals who had ingested food contaminated with DBP from plastic packaging.

Absorption via the dermal route

Absorption via the dermal route and subsequent elimination was assessed after application of 43.7 mg/kg ^{14}C-DBP in ethanol (with occlusion) to the clipped skin of male F344 rats, followed by measurements of the excreted ^{14}C radiolabel. Over seven days, DBP was excreted in urine and faeces at a nearly constant rate of approximately 10–12 % of the applied dose each day. Around one third of the applied dose remained at the site of application (Elsisi et al. 1989).

In a comparative in vitro study, Scott et al. (1987) demonstrated that the rate of dermal absorption for DBP is about 40 times greater in rat than in human epidermal skin preparations (93.35 μg/cm^2/h and 2.40 μg/cm^2/h, respectively).

More recently, Janjua et al. (2007 **ND**, 2008 **ND**) examined systemic uptake and elimination of DBP after dermal application in human volunteers. About 40 g of a standard cosmetic lotion formulation without (during control week) or with 2 % DBP (during treatment week) was applied to the whole body of 26 adult males for five consecutive days. The volunteers did not use any phthalate containing cosmetics for three weeks before the treatment week. Serum and urine concentrations of the primary metabolite, monobutyl phthalate (MBP), were measured. Urine was collected as individual samples at different time points during the first day of the treatment week and as 24-hour pools on all consecutive days. Results demonstrated increases in MBP in serum and urine within a few hours of application. An average of 1.82 % (range 0.11–5.94 %) of the applied DBP dose was recovered in urine as MBP during the treatment week. Taking into consideration the studies in rodents that demonstrate absence of significant

bioaccumulation of DBP in any organs or tissues, the studies by Janjua et al. suggest that DBP absorption via the dermal route in humans under conditions of usual cosmetic application is low.

A study with hairless guinea pigs (Doan et al. 2010 **ND**) that compared in vivo and in vitro skin absorption of DBP from an oil-in-water emulsion, found that in vivo, 62.0 % ±2.0 % (mean of three animals ±SEM) of the applied dose (AD) was systematically absorbed. Most of this (60.4 ±1.8 %AD) was excreted in the urine and less than 2 % was found in other tissues (ovaries, kidneys, liver). The amount of applied dose retained in the skin after 24 hours was 2.2 ±0.3 % AD; 7.4 ±2.3 % AD was trapped as volatile material in the first hour after dosing. The amount of DBP absorbed in vivo after 24 hours closely agreed with the amount of DBP found in the receptor fluid in vitro after 72 hours, suggesting that in vitro DBP is a lipophilic chemical that can initially form a reservoir in skin, and can slowly diffuse out of the skin into the receptor fluid. The relative permeability of human and guinea pig skin for DBP from this particular oil-in-water emulsion has not been compared.

Absorption via the inhalation route

Absorption of DBP following inhalation exposure has not been evaluated. However, in one study examining tissue distribution of DBP (but not metabolites) after inhalation exposure in rats, increased levels of DBP were detected in some tissues (Section 7.2) indicating that DBP might be absorbed through inhalation (Kawano et al. 1980a*).

6.1.2 Distribution

No significant retention was seen in any organ after male Wistar rats were orally dosed with 0.27 or 2.31 g ^{14}C-DBP/kg bw/d in corn oil. Tissue distribution was similar at both dose levels. The highest radioactivity was recorded in the kidneys (0.66 %) and the lowest was recorded in the brain (0.03 %), four hours after administration. Radioactivity was detected at 0.4 % of the dose in the blood, at both dose levels, after 24 hours. Less than 0.01 % was detected in all tissues after 48 hours (Williams & Blanchfield, 1975*).

Tanaka et al. (1978*) determined retention in 14 different tissues after administering 60 mg ^{14}C-DBP/kg bw/d (in DMSO) orally to rats. At 24 hours after administration, no retention was seen in brain, heart, lung, spleen, testicles, prostate or thymus and only low amounts were detected in the following tissues: liver (0.06 %), kidneys (0.02 %), muscle (0.3 %), adipose tissue (0.7 %), intestines (1.53 %), stomach (0.01 %) and blood (0.02 %).

Tissue distribution monitored after dermal application of ^{14}C-DBP to F344 rats for seven days showed low accumulation in adipose tissue (0.41 %), skin (1.4 %), muscle (1.1 %) and all other tissues <0.5 %. A third of the applied dose (43.7 mg/kg bw/d without occlusion, or 157 µmol/kg bw/d with occlusion) remained at the site of application (Elsisi et al. 1989).

Kawano (1980a*) performed a study in rats to measure organ distribution of DBP after daily inhalation exposure to DBP at 50 mg/m^3 and 0.5 mg/m^3 for three and six months. The highest concentrations of DBP at both dose levels were found in the brain. At the higher dose, the maximum levels of DBP detected in the brain were 0.42–0.68 mg/kg and 0.54–1.46 mg/kg, after three and six months of exposure respectively. Accumulation in other organs was less marked. No metabolites were measured in this study.

In a placental transfer study, pregnant Sprague Dawley (SD) rats received an oral dose of 500 mg or 1500 mg ^{14}C-DBP/kg bw/d on gestational day (GD) 14. Maternal and foetal tissues were collected at intervals from 0.5 hours to 48 hours. Radioactivity in embryonic tissues was <0.12–0.15 % of the dose. Radioactivity in the placenta and embryo was less than or equal to one-third of that in maternal plasma. No accumulation of radioactivity was observed in maternal or embryonic tissues. DBP and the metabolites MBP and MBP-glucuronide were shown to rapidly transfer to embryonic tissues at levels that were consistently lower than those in

maternal plasma. Most of the radioactivity recovered in maternal plasma, placenta and embryo was attributed to MBP with intact DBP present at low levels (Saillenfait et al. 1998*).

Clewell et al. (2009 **ND**) also monitored distribution of MBP in pregnant SD rats following administration of a single oral dose of DBP (500 mg/kg) on GD 19 or repeated dose (50, 100, and 500 mg DBP/kg bw/ day) from GD 12–19. Tissue distribution of MBP and MBP-glucuronide were monitored by liquid chromatography/mass spectrometry in maternal and foetal plasma, placenta and amniotic fluid. MBP in maternal plasma, placenta, and foetal plasma was mostly eliminated after 24 hours. Both placenta and foetal serum kinetics closely followed the maternal plasma, though the foetal plasma showed a slight delay in the time to reach peak concentration. Amniotic fluid MBP levels were not linearly correlated with either the maternal or foetal plasma when examined across doses. Maternal and foetal plasma MBP levels were consistently lower at repeated doses compared with a single dose, suggesting that metabolism of DBP was induced with multiple exposures. MBP concentrations in the amniotic fluid were also reduced with repeated doses of 500 g/kg DBP, compared with the single administration.

6.1.3 Metabolism

After administering DBP orally to rats, MBP, MBP-glucuronide, various ω- and ω-1-oxidation products of MBP (more polar ketones and carboxylates) and a small amount of phthalic acid were detected (Albro & Moore, 1974*; Williams & Blanchfield, 1975*; Tanaka et al. 1978*; Foster et al. 1983*).

After administering 2 g DBP/kg bw/d orally to rats and hamsters, 37.6 % and 52.5 % of the dose, respectively, was recovered as MBP-glucuronide and 14.4 % and 3.5 %, respectively, as unconjugated MBP, in urine (Foster et al. 1983*).

In vitro studies with liver homogenates (rat, baboon and ferret), kidney homogenates (rat), and intestinal mucosal cell preparations (rat, baboon, ferret and humans) showed hydrolysis of DBP to MBP (Lake et al. 1977*; Rowland et al. 1977*; Tanaka et al. 1978*; White et al. 1980*). The rat liver microsomal fraction demonstrated rapid hydrolysis of DBP to MBP (73 % within two hours). The rate of hydrolysis in the rat GIT was most rapid in the small intestine and slower in the caecum and stomach. Overall phthalate diester hydrolase activity decreased in the order baboon>rat>ferret (Lake et al. 1977*; Rowland et al. 1977*).

An in vitro study using an everted gut sac preparation from rat small intestine showed only 4.5 % of DBP crossed the intestinal mucosa, with the remainder being hydrolysed by esterases in the mucosal epithelium, before reaching the serosal perfusion solution. Inhibition of esterases reduced DBP hydrolysis, but also significantly reduced DBP absorption, whereas MBP absorption was unaffected (White et al. 1980*).

The pharmacokinetics of MBP and MBP glucuronide were not influenced by the chemical (parent DBP vs metabolite MBP), vehicle (oil vs aqueous), dose level (10–50 mg/kg bw MBP vs 50–250 or 500–1500 mg/kg bw DBP), or route of exposure (oral vs intravenous) (Kremer et al. 2005). Following intravenous (iv) dosing with MBP (10, 30, or 50 mg/kg bw/d) on GD 19 in pregnant dams, MBP was metabolised to MBP glucuronide within five minutes, and MBP and MBP glucuronide disappeared from maternal and foetal plasma within 24 hours (Kremer et al. 2005).

The metabolism of DBP and DIBP was investigated in a male human volunteer after a single oral exposure of approximately 60 µg/kg of D^4-DBP and D^4-DIBP (in two separate doses). The dose is about 50 times higher than the typical background adult exposures of 1–5 µg/kg a day for DBP (Wormuth et al 2006; Clark et al. 2012*). The majority of the dose was excreted (92.2 % DBP and 90.3 % DIBP) in the urine in the first 24 hours, and <1 % was excreted in day 2. For DBP, the simple monoester MBP was the major metabolite detected (84 %). Approximately 8 % was excreted as various oxidised metabolites of DBP. MBP reached peak

concentrations 2–4 hours post exposure. The elimination half life of MBP was 2.6 hours, with a longer elimination half time of 2.9 to 6.9 hours for oxidised metabolites (Kock HM et al. 2012 **ND**).

6.1.4 Elimination and excretion

In studies with rats and hamsters treated orally with ^{14}C-DBP (dose not reported), between 63 % and ≥90 % of the applied dose was excreted in urine within 48 hours (Williams & Blanchfield, 1975*; Tanaka et al. 1978*; Foster et al. 1983*).

In addition to elimination through urine, DBP appears to be eliminated in bile. Tanaka et al. (1978*) reported 32.2 % and 56.7 % dose recovery over three days in the bile of two rats with a single oral dose of 60 mg ^{14}C-DBP/kg bw/d. DBP and MBP were the main products in the bile (ratio 1:1). However, it is likely the DBP was reabsorbed from bile and then ultimately excreted in urine, as faecal excretion was low, 1.0–8.2 % (Tanaka et al. 1978*).

Kaneshima et al. (1978*) reported a recovery of 4.5 % of the dose in bile collected six hours after a single oral dose of 500 mg ^{14}C-DBP/kg bw/d in 50 % ethanol administered to male rats.

6.2 Effects on laboratory animals and other test systems

6.2.1 Acute toxicity

The acute toxicity of a single dose of DBP has been evaluated in a number of species using oral, dermal, inhalation and intravenous administration. However, most of the studies' reports contain limited information and were not Good Laboratory Practice (GLP) compliant.

LD_{50} values derived from these studies are shown in Table 6.1.

Table 6.1: Acute animal toxicity studies

Study	Species	Results (LD_{50}/LC_{50})	References
Oral	Rat	8000 mg/kg bw	Smith, 1953*
		6300 mg/kg/bw	BASF, 1961*
	Mouse	4840 mg/kg/bw	BIBRA, 1987*
Dermal	Rabbit	>20000 mg/kg/bw	Clayton & Clayton, 1994*
			RTECS, 1993b*
Inhalation	Rat	≥ 15.68 mg/L/4h	Greenough et al. 1981*
Other routes			
i.v.	Mouse	720 mg/kg/bw	RTECS, 1993c*
i.m.	Rat	>8000 mg/kg/bw	Smith, 1953*
i.p.	Mouse	3400–4000 mg/kg/bw	BASF, 1961*
			Calley et al. 1966*
			Lawrence et al. 1975*
i.p.	Rat	3178 mg/kg/bw	Singh et al. 1972*
i.p.	Rat	Ca. 4200 mg/kg/bw	BASF, 1958*
s.c.	Mouse	20800 mg/kg/bw	RTECS, 1993d*

Source (ECB 2004). Only Grenough et al. (1981) was GLP compliant.

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

Specific studies reported oral LD_{50} value for rats as 6300–8000 mg/kg bw (Smith, 1953*; BASF, 1961*) and 4840 mg/kg bw for mice (BIBRA, 1987*).

Dermal LD_{50} for rabbits was >20,000 mg/kg bw (Clayton & Clayton, 1994*).

In a GLP-compliant study by Greenough et al. (1981*), the inhalation LC_{50} in rats was estimated to be ≥15.68 mg/L/4h. SD rats (5/sex/dose) were exposed to 12.45, 15.68 and 16.27 mg DBP/L of air for four hours, and observed for 14 days. Controls were air exposed. The respirable fraction was 64.4 %, 56.9 %, and 59.9 %, respectively. A reduction in respiratory rate was seen at 15.68 mg/L. Excessive grooming in surviving animals led to persistent poor coat condition throughout the study. Macroscopy of the lungs revealed the following anomalies: white foci in all lobes in one male and one female rat at 15.68 mg/L, dark red regions in two female rats at 12.45 mg/L, and one male and one female rat at 16.27 mg/L.

6.2.2 Skin, eye and respiratory irritation

Skin irritation

A study in rabbits with undiluted DBP (OECD Guideline 404) revealed slight erythema in 2 out of 3 animals immediately after exposure and after 24 hours. No oedema was seen. Erythema disappeared 48 hours after exposure. DBP was not considered a skin irritant (BASF 1990a*).

Greenough et al. (1981*) reported mild reactions 24 hours after 0.5 mL of undiluted Vestinol C (DBP trade name) was applied to intact and abraded rabbit skins. The positive control was reported as 10 % laurylsulphate. No reaction was observed after 72 hours at any treatment site. The irritation index was calculated as 0.54/8.

A study cited in an NTP-CERHR report on DBP (CERHR 2003) reported minor irritation in rabbit dermal occlusion studies at 520 mg/kg bw/d.

These studies suggest that DBP causes minimal skin irritation in rabbits.

Eye irritation

In a study in rabbits with undiluted DBP (OECD Guideline 405), prominent conjunctival redness was observed after one hour and 24 hours in all animals, which reduced in severity after 48 hours and was completely reversed by 72 hours. DBP was not considered an eye irritant (BASF, 1990b*).

Undiluted 0.1 mL of Vestinol C (trade name of DBP) was applied to rabbit eyes (3/sex), which were not rinsed post administration. After one hour, three out of six animals showed mild redness and the balance (three animals) exhibited extremely mild redness. After 24 hours, very mild redness was observed in two out of six animals. Iris or corneal effects were not observed. The irritation index was calculated as 0.11/110. DBP was not considered an eye irritant (Greenough et al. 1981*).

These studies suggest that DBP causes minimal eye irritation in rabbits.

Respiratory irritation

Irritation of nasal mucous membranes was observed in cats after 5.5 hours exposure to 1 mg DBP/L (1000 mg/m^3) and in mice after two hours' exposure to 0.25 mg/L (250 mg/m^3). No additional data were available (BIBRA, 1987*; BUA, 1987*).

A 28-day repeat-dose toxicity study using Wistar rats (Gamer et al. 2000*), described in detail in section 6.2.4, suggests that DBP has a low irritation potential. At the highest exposure concentration of 509 mg/m^3, red crust formation at the snout was observed after cessation of daily exposure, but the rats recovered within 18 hours. The epithelium in the respective areas of the nasal cavity was regular, the infoldings were absent, and signs of inflammation were missing in the whole nasal cavity.

These studies suggest that DBP causes minimal respiratory irritation in animals.

6.2.3 Sensitisation

Skin sensitisation

No signs of sensitisation were observed for DBP treatment in two guinea pig maximisation studies performed according to OECD Guideline 406 and a GLP-approved FDA recommended method (Greenough et al. 1981*; BASF 1990c*).

Similarly, no sensitisation was observed in a non-GLP repeated patch test in rabbits (BASF 1957*).

Respiratory sensitisation

There are no data regarding the respiratory sensitisation potential of DBP.

6.2.4 Repeated dose toxicity

Oral route

DBP has been tested for repeat-dose effects via the oral route mainly in rodents. Key findings are summarised in Table 6.2.

Rats

In a study with F344/N rats (NTP 1995*) 10/sex/dose animals were given diet containing 0, 0.25, 0.5, 1.0, 2.0 or 4.0 % DBP (equivalent to 0, 176, 359, 720, 1540 and 2964 mg/kg bw/d for males and 0, 178, 356, 712, 1413, 2943 mg/kg bw/d for females) for 13 weeks. The following effects were reported:

- a statistically significant decrease in growth in males at ≥1.0 % and females at ≥2.0 %. Emaciation resulted from decreased food consumption in all animals at 4.0 %;
- an increase in relative liver and kidney weights (males at ≥0.5 % and females at ≥1.0 %), and decrease in testes weight (males at ≥2.0 % level; statistically significant);
- haemoglobin (Hb) values and erythrocyte counts were significantly decreased in males at ≥0.5 %. Haematocrit (Hct) values were decreased at ≥0.5 % but were statistically significant only at ≥2.0 %. Blood platelet counts were elevated to statistically significant levels in males at ≥0.5 %. Nucleated RBC levels were significantly increased in all animals at 4.0 %;
- cholesterol concentrations decreased significantly in both sexes at ≥2.0 %. Triglyceride levels decreased in a dose-dependent fashion; the decrease was statistically significant at all doses in males and at ≥1.0 % in females. Statistically significant increases in serum alkaline phosphatase (males at ≥2.0 %, females at ≥1.0 %) and bile acid concentration (males at ≥2.0 %, females at ≥0.5 %) were reported. Palmitoyl-CoA (PCoA) activity (an indicator of peroxisomal proliferation) was elevated in both sexes in a dose-related manner at ≥0.5 %;
- hepatocellular cytoplasmic alterations (indicative of glycogen depletion) were seen in both sexes at ≥1.0 %. Minor eosinophilic granulation and peroxisome proliferation were reported at 4.0 %. Dose-related germinal epithelium degradation was seen at ≥1.0 % levels with complete loss at 4.0 %;
- statistically significant decreases were seen in testicular Zn and serum testosterone levels (at ≥2.0 %) and serum Zn levels (at 4.0 %). Lipofuscin accumulation was seen at ≥1.0 %; and
- statistically significant decreases (at 2 %) in spermatid heads/testis and per gram of testis, epididymal motility and number of epididymal spermatozoa per gram of epididymis.

The NOAEL in this study was established at 0.25 % (177 mg/kg bw/d) and a LOAEL at 0.5 % (357 mg/kg bw/d) based on perturbations in haematological parameters and organ weight changes (NTP 1995* in IPCS 1997; ECB 2004; ASTDR 2001).

In a 3-month gavage study performed by Nikonorow et al. (1973*), Wistar rats (10/sex/group) received 120 or 1200 mg/kg bw/d of DBP. A statistically significant increase in liver weight was seen at all doses. The LOAEL was 120 mg/kg bw/d.

The same authors performed a 12-month dietary study in Wistar rats (20/sex/group) at 0 or 0.125 % DBP (62.5 mg/kg bw/d). Mortality in the control and treated groups was 10 % and 15 %, respectively. Clinical signs, pathological and haematological parameters were all normal. The NOAEL was 62.5 mg/kg bw/d, based on the lack of effects at the only dose tested. However, the study had only one dose group, amongst other limitations (Nikonorow et al. 1973*).

In a dietary study (Murakami et al. 1986*), Wistar rats (5 males/dose) were given 0.5 % and 5 % DBP in the diet (equivalent to 250 and 2500 mg/kg bw/d, respectively) over 34–36 days. Decreased body weight gains were seen at both dose levels and were statistically significant at 5 %. Various clinical parameters showed statistically significant changes at 5 %. Microsomal hepatic changes were seen at both dose levels. Peroxisome proliferation was observed at both dose levels, but was more pronounced at 5 %. The LOAEL was considered to be 0.5 % or 250 mg/kg bw/d (Murakami et al. 1986*). The study included several limitations in the reporting and magnitude of changes (ECB 2004).

In a 3-month dietary study on Wistar rats, performed according to OECD Guideline 408 (BASF 1992*), a NOAEL of 152 mg/kg bw/d was determined based on changes in haematological and clinical chemistry parameters. Testing protocols included dose levels of 0, 0.04, 0.2 and 1.0 % DBP in the diet (equivalent to 0, 30, 152, 752 mg/kg bw). At the highest dose, changes in the following parameters were observed: haematology (decreased Hb, Hct and erythrocyte counts); and clinical chemistry (decreased triglyceride levels, increased serum glucose and albumin levels). Statistically significant increases were seen in the activity of cyanide-insensitive palmitoyl-CoA oxidase (an indicator for peroxisome proliferation), and liver and kidney weights. Triiodothyronine (T3) levels decreased significantly. Histopathology revealed a reduction or absence of lipid deposition in hepatocytes at the highest dose. No effects on the testes were observed (BASF 1992*).

Mice

In mice administered with 2.5 % DBP in the diet (ca. 500 mg/kg bw/d—high dose group) for 86 or 90 days showed adverse effects including necrosis and notable vacuolar degeneration of hepatocytes; and cysts and degeneration of renal tubular epithelium. Parenchymal degeneration and minor histopathological changes in the liver were seen in the low dose group at ca. 500 mg/kg bw/d (Ota et al. 1973*, 1974*).

In a 13-week study (NTP 1995*) using B6C3F1 mice (10/sex/dose) treated with 0, 0.125, 0.25, 0.5, 1.0 or 2.0 % DBP in the diet (equivalent to 0, 163, 353, 812, 1601 or 3689 mg/kg bw/d in males and 0, 238, 486, 971, 2137 or 4278 mg/kg bw/d for females). The following adverse effects were reported:

- a significant decrease in growth in both sexes at 0.5 % and above;
- a significant increase in relative liver weights at 0.5 % and above;
- a significant increase in absolute and relative kidney weights in females at all doses (not significant at 2.0 %), and significant decreases in epididymal weights in males at the treatment doses that were examined (0.125, 0.5 and 2.0 %);
- the biochemical parameters were also affected—significant decrease of haematocrit (Hct) count in females was observed at 2.0 %;
- hepatocellular cytoplasmic alterations (indicative of glycogen depletion) were revealed in males at 1.0 % and above, and in females at 2 %. Peroxisome proliferation was observed in hepatocytes at 2.0 %. Lipofuscin accumulation was seen in the liver at 1.0 % and above;
- the serum testosterone levels were generally higher in treated groups but reached statistical significance only at 0.125 %. Testicular zinc concentrations were significantly higher at 0.5 % and above; and
- a significant increase in spermatid heads per gram of testis was reported at 2.0 %;

The NOAEL in males was established to be 0.25 % (353 mg/kg bw/d) and the LOAEL was 0.5 % (812 mg/kg bw/d) based on changes in growth and liver weight. A NOAEL could not be established in females because of organ weight changes (kidney) at all dose levels (NTP 1995*).

Other studies

Studies on peroxisome proliferation

Several studies specifically examined enzyme changes, histopathological and biochemical alterations in the liver. Activation of fatty acid metabolising enzymes, alterations of fatty acids associated with PPARαand increased peroxisome proliferation were observed.

Male and female F344 rats were given 0.6, 1.2 and 2.5 % DBP (equivalent to ca. 600, 1200 and 2100 mg/kg bw/d) in a 3-week dietary study. At the lowest dose (ca. 600 mg/kg bw/d) the activity of peroxisome-associated enzymes PCoA, LAH-11 and LAH-12 was increased as well as liver weight, while serum triglyceride and cholesterol levels were decreased. A NOAEL could not be established (BIBRA 1986*; Barber et al. 1987*).

Male F344 rats were given 0.05, 0.1, 0.5, 1 and 2.5 % DBP (equivalent to 51.5, 104, 515, 1040 and 2600 mg/kg bw/d) in a 4-week dietary study. A dose-dependent increase in liver weights was reported; the increase was statistically significant at all doses. Increase of PCoA activity was observed at 515 mg/kg bw/d and above (BIBRA 1990*).

Wistar rats (3/sex/group) received 400, 2000 or 10,000 mg DBP/kg of diet (equivalent to ca. 30, 152 and 752 mg/kg bw/d) in a 3-month study. The frequency and severity of peroxisome proliferation was measured by histochemical analysis of the number and size of peroxisomes. The NOAEL for peroxisome proliferation was established at 152 mg/kg bw/d (Kaufmann 1992*).

In a 2-week dietary study, male Wistar rats were given 20, 60, 200, 600 and 2000 mg DBP/kg of diet (equivalent to 1.1, 5.2, 19.9, 60.6 and 212.5 mg/kg bw/d). The NOAEL was 60.6 mg/kg bw/d for PCoA activity and 19.9 mg/kg bw/d for LAH-11 and LAH-12 (11- and 12- lauric acid hydroxylase, respectively) induction. Therefore, the overall NOAEL for induction of peroxisome-associated enzymes was 19.9 mg/kg bw/d (Jansen et al. 1993*).

Studies on testis and testicular functions

Several studies specifically examined the testicular effects of DBP in various experimental animals.

At doses of 500 mg/kg bw/d and higher, the following effects are reported in several repeat oral studies in rats: decreased weight of testes and accessory sex glands; spermatocyte depletion; seminiferous tubule degeneration; and decrease in testicular zinc and serum testosterone levels (Cater et al. 1977*; Oishi & Hiraga 1980a*; Gray et al. 1982*, Gray, Laskey, Ostby et al. 1983*; Srivastava et al. 1990*).

In young male rats, oral administration of 250, 500 or 1000 mg/kg bw/d DBP for 15 days showed a significant decrease in testes weight at 500 and 1000 mg/kg bw/d that was associated with marked degeneration in 5 % of seminiferous tubules. In all dose groups, a number of testicular enzymes associated with specific stages of the spermatogenesis were significantly altered (Srivastava et al. 1990*).

In guinea pigs, oral administration of 2000 mg/kg bw DBP for seven days revealed severe testicular changes manifested as reduced testes weight, severe tubular atrophy with loss of spermatids and a reduction in primary spermatocytes and spermatogonia (Gray et al. 1982*).

Oral administration of 2000 mg/kg bw/d by gavage to mice and hamsters for nine days, or 2 % DBP in the diet (ca. 2400 mg/kg bw/d), for seven days in mice was not associated with testicular effects (Oishi and Hiraga 1980b*; Gray et al. 1982*). However, in a subsequent mating study with male and female hamsters (strain unspecified), oral administration of 500

mg/kg bw/d and 1000 mg/kg bw/d for 35 days or 1000 mg/kg bw/d for 55 days was associated with a marked effect on testes size and viability, and growth of offspring in the 1000 mg/kg dose (Gray, Laskey, Ostby et al. 1983*). The female reproductive system did not appear to be affected at any dose (Gray, Laskey, Ostby et al. 1983*).

The species-specific differences in testicular toxicity have been partly attributed to differences between rats and hamsters in the ratio of free unconjugated primary DBP metabolite MBP (MBP is considered to be the active component for toxicity) to glucouronated MBP (Tanaka et al. 1978*; Oishi & Hiraga, 1980c*; Foster et al. 1981*, 1983*; Zhou et al. 1990*).

Table 6.2: Summary of significant studies of oral repeat-dose toxicity of DBP

Species	Type and duration of dosing	Results	References
General			
Rat	Diet, 13 weeks	NOAEL ca. 177 mg/kg bw/d. LOAEL ca. 357 mg/kg bw/d, ↑ liver and kidney weights, haematological and clinical chemistry effects.	NTP 1995*
Rat	Gavage, 3 months	LOAEL 120 mg/kg bw/d, ↑ relative liver weights.	Nikonorow at el. 1973*
Rat	Diet, 1 year	No adverse effect at ca. 62.5 mg/kg bw/d (only dose tested).	Nikonorow at el. 1973*
Rat	Diet, 34–36 days	LOAEL ca. 250 mg/kg bw/d, ↓ body weight gain.	Murakami et al. 1986*
Rat	Diet, 90 days	NOAEL ca. 152 mg/kg bw/d. LOAEL ca. 752 mg/kg bw/d, ↑ liver and kidney weights, haematological and clinical chemistry effects, and histopathological changes in liver.	BASF 1992*
Mouse	Diet, 86 or 90 days	LOAEL 500 mg/kg bw/d, degeneration of liver parenchyma.	Ota et al. 1973*, 1974*
Mouse	Diet, 13 weeks	NOAEL (males) ca. 353 mg/kg bw/d. LOAEL (males) ca. 812 mg/kg bw/d, ↓ body weight gain, ↑ relative liver weights and testis zinc levels. LOAEL (females) ca. 238 mg/kg bw/d, ↑ kidney weights.	NTP 1995*
Liver effects			
Rat	Diet, 3 weeks	LOAEL ca. 600 mg/kg bw/d, ↑ activity of PCoA, LAH-11 and LAH-12, and ↑ liver weights.	Barber et al. 1987*; BIBRA 1986*
Rat	Diet, 4 weeks	LOAEL ca. 51.5 mg/kg bw/d, ↑ liver weights (no NOAEL). At 515 mg/kg bw/d and above ↑ activity of PCoA.	BIBRA 1990*
Rat	Diet, 3 months	NOAEL ca. 152 mg/kg bw/d. LOAEL ca. 752 mg/kg bw/d, ↑ peroxisome proliferation.	Kaufmann 1992*

Rat	Diet, 2 weeks	NOAEL 19.9 mg/kg, ↑ activity of LAH-11 and LAH-12.	Jansen et al. 1993*
Testicular effects			
Rat	Diet, 15 days	LOAEL 250 mg/kg bw/d, enzymatic and histopathological testicular effects.	Srivastava et al. 1990*
Mice	Gavage, 9 days	No effects on testes. Single dose tested 2000 mg/kg bw/d.	(Gray et al. 1982*; Oishi & Hiraga 1980b*)
Guinea pigs	Gavage, 7 days	Severe testicular changes. Single dose tested 2,000 mg/kg bw tested.	(Gray et al. 1982*)
Hamsters	Oral, 9 days males only	No effects on testes. Single dose tested 2000 mg/kg bw/d.	(Gray et al. 1982*)
Hamsters	Oral, mating study 35 days at 500 mg/kg bw/d and 1000 mg/kg bw/d 55 days 1000 mg/kg bw/d	Marked effect on testes size and viability and growth of offspring at t1000 mg/kg bw/d.	(Gray, Laskey, Ostby et al. 1983*).

↑ = increased, ↓ = decreased
LAH-11 and LAH-12 = 11- and 12-lauric acid hydroxylase (indicators for peroxisome proliferation)
PCoA = cyanide-insensitive palmitoyl-CoA oxidase activity (indicator for peroxisome proliferation)
Source: ECB (2004)

Dermal route

In the only available 90-day dermal study, rabbits received dermal applications of 0.5, 1.0, 2.0 or 4.0 mL DBP/kg bw/d to the clipped intact skin (Lehman, 1955*). Slight irritation and dermatitis were reported without information on the dose level at which it was observed. Slight renal damage was reported at 4.0 mL/kg bw/d. This study had severe limitations and was poorly documented (strain of rabbits not identified, number and sex of animals, duration of daily application, dose levels at which effects were seen was not reported).

Inhalation route

Male Wistar rats (11–14/sex/dose) were exposed to DBP mist at 0.5 or 50 mg/m^3 for six months, 6 d/w, 6 h/d (three hours for Saturday). Growth was reduced, and elevated relative brain, kidney, lung and testes weights were observed at 50 mg/m^3 (statistically significant for brain and lung weight only). Absolute weights were not reported. Haematology revealed decreased levels of lymphocytes and elevated neutrophil counts at both doses, but the effects were not dose-dependent. Clinical chemistry revealed changes in certain parameters (mild increases in ALT, AST and SAP activities, serum glucose and triglyceride levels; decrease in serum cholesterol) at both doses at random time points (not dose-dependent). Gross and histopathology examinations were not performed. The NOAEC in this study was 0.5 mg/m^3 (Kawano 1980b*).

In another study, SD SD(15 males/dose) were exposed 6 h/d to 0, 0.5, 2.5 and 7.0 ppm DBP (ca. 0, 6, 28 and 80 mg/m^3) in a 5-day inhalation study. Body, lung and liver weights were unaffected. Microsomal cytochrome P-450 (Cyt. P-450) levels were markedly affected in the lung at 28 mg/m^3 and above (unaffected in the liver). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity and serum albumin levels were significantly increased at 80 mg/m^3. Serum alkaline phosphate (SAP) activity and serum total protein levels remained normal (Walseth & Nilson 1984*, 1986*).

In an inhalation study performed according to OECD Guideline no. 412 and 407 (for clinical and neurofunctional examinations and pathology) Wistar rats (5/sex/dose) were head–nose exposed to air containing 0, 1.18, 5.57, 49.3 or 509 mg DBP/m^3 as liquid aerosol for 6 h/d, 5 d/week, for four weeks (Gamer et al. 2000*). There was no animal mortality. Red crust formation at the snouts (recovery within 18 hours) was seen at 509 mg/m^3 in a maximum of four animals. The maximum duration of the effect was 13 to 27 days.

Functional observations revealed no treatment-related findings by open-field observations, home cage observations, sensorimotor/reflex tests or motor activity measurements. Statistically significant increase in rearing of males was reported at 49.3 mg/m^3. However, since a dose-response relationship was not evident and no other abnormalities were observed during the functional observations, this finding was considered as incidental.

Statistically significant decreases in food and water consumption were seen intermittently in only one sex (not specified) and did not show a dose-relationship. These changes were considered minor. No significant deviation of mean body weights was seen. Haematology, clinical chemistry and urinalysis parameters were normal with the exception of a statistically significant decrease in serum sodium levels in females at 509 mg/m^3. However, this was considered minor given the effect was sex-specific.

Absolute lung and testes weights were significantly affected at the lower doses. However, these effects were considered incidental as they were not dose-dependent.

Histopathology revealed a dose-dependant increase in the incidence of mucosal cell hyperplasia in the nasal cavity. The severity ranged from grade 1 (minimal) to grade 2 (slight). The epithelium was normal and inflammation was absent in the entire nasal cavity. A dose-related increase in the incidence of squamoid metaplasia (minimal degree) was seen (0, 1, 3, 4 and 5 males and 0, 1, 3, 5 and 4 females at 0, 1.18, 5.57, 49.3 and 509 mg/m^3, respectively).

No systemic effects (including neurotoxicity) were seen at up to and including the highest dose of 509 mg/m^3. Since dose-dependent changes were localised in the nasal cavity and can be considered adaptive, the systemic NOAEC was established as 509 mg/m^3. The LOAEC for local adaptive effects in the upper respiratory tract was 1.18 mg/m^3 (Gamer et al. 2000*).

6.2.5 Genotoxicity

In vitro

Mutagenic potential of DBP has been evaluated in a battery of in vitro tests, with and without metabolic activation, and reviewed in the available international assessments (IPCS 1997; ECB 2004). Tests included:

- gene mutation and/or DNA repair assays in bacteria (*S. typhimurium*, *E. coli*, *E. coli*, *B. subtilis)*;
- gene mutation in yeast *(S. cerevisiae*);
- gene mutation with mouse lymphoma cells (L5178Y TK± and L5178 TK±); and
- chromosomal aberration and/or sister chromatid exchange assays with Chinese hamster ovary (CHO) cells and human leucocytes.

The majority of the tests yielded negative results except for the following: an equivocal result in a bacterial gene mutation assay in *S. typhimurium* (TA100) in the absence of metabolic activation (S9 fraction); weak positive results at cytotoxic doses in another gene mutation assay (TA100) in the absence of S9 fraction; and a positive result in a gene mutation assay with mouse lymphoma cells in the absence of S9 fraction at cytotoxic doses (no testing was performed in the presence of S9 fraction). However, in another independent study with the same lymphoma cell line, results were negative in the absence, and positive in the presence, of S9 fraction.

Using an in vitro Comet assay with human mucosal cells from oropharyngeal and nasal tonsillectomy samples treated with DBP ex vivo, Kleinsasser et al. (2000) reported a significant increase of DNA damage (single-strand breaks) in cells from both samples compared with the negative DMSO control. The DNA damage was significantly lower than the positive control MNNG (N-methyl-N'-nitro-N-nitrosoguanidine).

In vivo

All available in vivo tests for assessment of genotoxic potential of DBP showed negative results (IPCS 1997; ECB 2004). The test included sex-linked recessive lethal test in *Drosophila* and micronucleus assay (according to OECD 474 and comparable standards) in NMRI and B6C3F1 mice.

6.2.6 Carcinogenicity

No adequate long-term carcinogenicity studies with DBP in laboratory animals are available.

As discussed in Section 6.2.5., DBP is not considered to be genotoxic and is therefore not likely to be a genotoxic carcinogen. Moreover, in several in vitro transformation assays DBP did not induce cell transformation. DBP was negative in a cell transformation assay with Balb/3T3 cells in the absence of metabolic activation (Litton Bionetics 1985*). Also, mouse Balb/c-3T3 cells exposed to DBP concentrations up to 82 nL/mL for a period of 72 hours and incubated over four weeks, did not result in a statistically significant increase of cell transforming in the same cell line (Barber et al. 2000).

A phthalate ester mixture containing 21.9 % DBP was tested in an in vitro mammalian C3H/10T1/2 cell transformation assay. The mixture did not induce cell transformation at doses ranging from 0.0195 µL/mL to 0.0025 µL/mL (Nuodex, 1982).

In vivo, sub-chronic exposure to DBP in rodents (Section 6.2.4 and Table 6.2) is associated with fatty acid metabolising enzyme activation and the altered metabolism of fatty acids, leading to activation of peroxisome proliferator-activated receptor α (PPARα) (BIBRA 1986*; Barber et al. 1987*; BIBRA 1990*; Kaufmann 1992*; Jansen et al. 1993*;). Lapinskas et al. (2005) demonstrated that DBP (and the DEHP-related phthalate) induced liver effects in mice such as hepatomegaly and fatty acid metabolising enzyme induction, are indeed mediated through PPARα as these effects were absent in a PPARα-null mice.

Activation of PPARα is associated with induction of hepatocellular tumours by certain non-genotoxic substances in rodents, but not in humans (Lee et al. 1995; Peters et al. 1997).

Based on the information available, DBP is not likely to be a genotoxic carcinogen. The NOAEL for peroxisome proliferation, the non-genotoxic effect associated with liver carcinogenicity of other non-genotoxic substances in rodents, is established at 19.9 mg/kg bw/d in rats (Jansen et al. 1993*).

6.2.7 Reproductive toxicity

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

In this section, summaries of the studies are organised on the basis of test procedure, mainly timing of exposure (adult, gestation or early postnatal). The effects on fertility (as adults) and

development (as foetuses or early in postnatal development) are then discussed separately, to the extent possible. The nomenclature in the summaries of the studies in this section that refer to the days during foetal/embryonic days and postnatal days and weeks, are kept as indicated in the original studies including: gestation day (GD), embryonic day (e), postnatal day (PND or d) and postnatal week (PNW). Similarly, designation of the generations in the multigenerational studies, F0 and P0, are retained from the original studies.

The effects of DBP on reproductive end points have been tested in rats, mice, hamsters and guinea-pigs. Overall, rats were the most sensitive to reproductive effects, followed by mice and hamsters. Key studies are described below; a summary of toxicity effects at the LOAEL and the corresponding NOAEL is presented in Appendix 1.

Fertility studies

Studies that specifically examine the effects of DBP on fertility in mature animals are limited. However, several repeated oral exposure studies with DBP (described in Section 6.2.4), show marked testicular toxicity of DBP, which is likely to lead to decreased fertility and adverse reproductive effects. Repeated doses of DBP of 500 mg/kg bw/d and higher resulted in distinct testicular changes in rodents, including decreased organ weight and histopathological perturbations in the testes, indicative of testicular degeneration (Cater et al. 1977*; Oishi & Hiraga, 1980a*; Gray et al. 1982*, Gray, Laskey, Ostby et al. 1983*; Srivastava et al. 1990*). In these studies, 15-day oral treatment with the lowest tested dose of 250 mg DBP/kg bw/d was also associated with changes in testicular enzymes, indicative of spermatogenic cell atrophy (Srivastava at al.1990*).

In a study specifically examining the reproductive effects of DBP on females, Gray et al. (2006) gavaged LE Hooded female rats (8–12/group) with 0, 250, 500 or 1000 mg/kg bw/d DBP from weaning through to puberty and young adulthood, then mated them with untreated males. Dosing continued through mating, pregnancy and lactation. Liver weight was increased at 1000 mg/kg bw/d with no effect on body weight. The percentage of females delivering live pups was reduced by more than 50 % at 500 mg/kg bw/d and by 90 % at 1000 mg/kg bw/d in the absence of overt toxicity, whereas the ages at vaginal opening and first oestrous, oestrous cyclicity and mating indices were not significantly affected. The litter sizes from mated females were also significantly reduced at 500 and 1000 mg /kg bw/day. Many females in the 500 and 1000 mg/kg bw/day groups that were pregnant, but did not deliver pups, displayed a constant leucocytic, pregnancy-like vaginal lavage for 21–29 days (indicator of pregnancy or pseudo-pregnancy) and blood was detected in the vagina at or after midpregnancy, suggesting miscarriage, although no live or dead pups were recovered.

After weaning the F1a generation, the same females (P0) were mated with untreated males for a second time and sacrificed at GD 13 for serum analysis of progesterone, testosterone, and oestradiol. In addition, ovarian cultured fragments were prepared for examination of human chorionic gonadotropin (hCG)-stimulated ovarian production of progesterone (P4), testosterone (T), and oestradiol (E2) production ex vivo. The numbers of live and dead foetuses were also counted.

On GD 13, several of the DBP-treated females had ovaries that contained grossly visible haemorrhagic corpora lutea and reduced serum progesterone levels, but this was statistically significant only at the highest level. Serum progesterone levels in pregnant females with dead foetuses were very low, approaching those seen in nonpregnant females. Ex vivo progesterone production in ovarian cultures from females with live foetuses was significantly decreased in the two highest dose groups, while oestradiol production was increased.

In this study (Gray et al. 2006), the NOAEL for fertility in female rats was 250 mg/kg bw/d and the LOAEL 500 mg/kg bw/d, based on decreased fertility in the P0 generation. The findings show that DBP has an adverse effect on female fertility. Further, the effect is mediated through hormonal changes in the absence of any overt morphological toxicity to the reproductive organs.

Such an effect was also indicated in an earlier crossover study with LE rats by Wolf et al. (1999; see Effects on development—Multigenerational reproductive toxicity studies later in this section).

In a study by Mahood et al. (2007 **ND**), primarily aimed at assessing development (described in more detail in the following section), fertility was evaluated for Wistar rat male offspring treated with 0, 4, 20, 100, or 500 mg/kg/d DBP during gestation (GD 13.5–21.5). Following a treatment-free period from GD 21 to PND 90, adult male offspring were housed for one week with untreated females of known fertility. Males were classed as fertile if offspring were produced. A statistically significant increase in infertility (75 %) was observed at 500 mg/kg bw/d, as assessed by the number of infertile versus fertile animals/litter and overall. Testis weight of the F1 adults was also significantly decreased (~50 %) at 500 mg/kg bw/d.
A NOAEL for male fertility of the F1 generation cannot be established with certainty, as increased infertility was observed at lower doses (33 %, 14 % and 22 % at 100, 20 and 4 mg/kg bw/d, respectively), and although not statistically significant and without clear dose response at the lower doses, it was correlated with significant testicular adverse effects in the offspring starting at 100 mg/kg bw/d (described in the prenatal developmental toxicity studies).

Several earlier continuous-breeding studies with rats, described in more detail in the multigenerational reproductive toxicity studies, also indicate that DBP exposure significantly affects fertility indices, or fertility related end points, in the F1 generation. In these studies, the lowest NOAEL for reproductive toxicity was 50 mg/kg bw/d in Charles River COBS CD rats, based on reduced testicular weight and testicular lesions observed in the F1 males at the LOAEL of 500 mg/kg bw/d—a dose that was also associated with maternal toxicity (IRDC 1984*).

However, in a continuous-breeding dietary study with SD rats (NTP 1995*; Wine et al. 1997) the LOAEL for fertility and embryotoxicity was at the lowest tested dose of 52–80 mg/kg bw/d (males–females), based on a decreased total number of live pups in each litter following breeding of F0. This effect was observed in the absence of maternal toxicity (observed at the highest dose, 509–794 mg/kg bw/d) and histopathological changes in the reproductive tract in F0 males or females, even at the highest dose, and without effect on average number of litters per pair at any dose. The body weight of the live pups (adjusted for litter size) was decreased at the highest and mid doses (256–385, 509–794 mg/kg bw/d). In this study, fertility indices (percentage of females with plug, pregnant and fertile) were significantly decreased at the highest dose for the F1, but not for the F0 generation, suggesting greater sensitivity of the F1 generation, dosed from gestation, compared with F0, dosed seven days premating at adulthood.

Crossover mating of F0 males and females treated with the highest dose (509m–794f mg/kg bw/d) to control animals, indicated that this DBP effect on the first generation is mediated through toxicity to females rather than males, based on decreased adjusted weight of live pups observed in the crossover mating of treated females with control males only (NTP 1995*; Wine et al. 1997).

In a multigenerational dietary study with CD-1 mice (20/sex/group), doses of 0, 0.03, 0.3 and 1.0 % DBP (ca. 0, 40, 420 and 1410 mg/kg bw/d) were administered (Lamb et al. 1987*; Morrissey et al. 1989*) over a week-long premating period, during a 98-day mating period (as pairs), and after mating until the offspring were at least 21 days old. A week-long crossover mating trial was performed between P0 control animals and F1 animals from the highest (1 %) dose group.

Effects in the P0 1 % dose group included:
- significantly decreased growth in males;
- significantly increased liver weights in females;

- significant decrease in the:
  - ~ percentage of fertile pairs;
  - ~ number of litters/pair;
  - ~ number of live pups/litter; and
  - ~ number of pups born alive.

These effects were absent at the lower dose levels. In the crossover mating trial (using dosed females and control males), a statistically significant decrease was seen in the percentage of fertile pairs, number of live pups/litter, pups born alive and live pup weight, indicating that the effects were dam-mediated. The NOAEL for fertility, parental and embryotoxicity in this study was 0.3 % (420 mg/kg bw/d).

Developmental studies

Prenatal developmental toxicity studies

Two older studies with mice are available. Only a summary was available from a study by Hamano et al. (1977*) in which ICR/JCL mice were given 0.005, 0.05 or 0.5 % DBP in the diet (ca. 10, 100 and 400 mg/kg bw/d) from GD 1–18. At 0.5 %, the following effects were seen: for maternal toxicity, increased kidney weights; and for embryotoxicity, a reduced number of live offspring. DBP also had teratogenic effect on foetuses that exhibited as a significant increase in incidence of non-closing eyelids, encephalocoele, cleft palates and spina bifida; and increased incidence of skeletal abnormalities. The NOAEL for maternal and foetal toxicity was 100 mg/kg bw/d.

In another study with ICL/ICR mice (Shiota et al. 1980), dams were dosed during GD 1–18 with 0.05, 0.1, 0.2, 0.4 or 1.0 % DBP in the diet (ca. 80, 180, 350, 660 and 2100 mg/kg bw/d). Maternal growth was significantly reduced at 1.0 %. Foetal mortality and the number of resorptions were higher at 0.1 % and above (statistically significant at 1.0 % but without dose-response). Foetal weights were decreased at all doses (significant at 0.4 % and above). An increased incidence of skeletal variations (lumbar ribs) was seen at all dose levels (not statistically significant) and there was a significant reduction in number of ossified coccygia (dose-responsive and significant at all doses). It is likely that the decrease in ossified coccygia observed in the DBP-treated mice is an indirect effect of decreasing body weight. A study examining relationships between the foetal body weight of Wistar rats at term and the extent of skeletal ossification found that the number of ossified sacrococcygeal vertebrae decreased with body weight (Chahoud & Paumgartten 2005). The NOAEL for maternal toxicity in mice was 0.4 % (660 mg/kg bw/d). The NOAEL for foetal toxicity was at 0.2 % DBP (350 mg/kg bw/d) and the LOAEL was at 0.4 % DBP (660 mg/kg bw/d) based on decreased pup weight.

DBP is also embryotoxic in rats.

Treatment of pregnant Wistar rats (10/dose) by gavage with 0, 120 or 600 mg /kg bw/d DBP in olive oil on GD 0–21 was associated with a significant decrease of placental weight at 120 mg/kg bw/d and above (Nikonorow et al. 1973*). Increase in the number of resorptions and decrease in the number of foetuses and foetal weight were significant at 600 mg/kg bw/d. The NOAEL for embryotoxicity was 120 mg/kg bw/d.

In a study with Wistar rats (Ema et al. 1993*), pregnant dams were gavaged with 500, 630, 750 or 1000 mg/kg bw of DBP on GD 7–15. Maternal toxic effects included dose-related increases in incidences of facial fur staining (reddish-brown) and piloerection, and dose-related decreases in maternal body weight gain (significant $\geq$ 630 mg/kg bw/d). Embryotoxic effects were also observed at the maternally toxic doses that included: increased incidence of resorptions (significant $\geq$ 630 mg/kg bw/d), increased number of dead foetuses per litter and increased post implantation loss per litter; and increased malformations at $\geq$ 750 mg/kg bw/d (increased incidence of cleft palate). The NOAEL for maternal toxicity and foetal toxicity was 500 mg/kg bw/d.

In a follow up study, pregnant Wistar rats were gavaged with 750, 1000 or 1500 mg DBP/kg bw/d during GD 7–9, 10–12 or 13–15 (Ema et al. 1994*). Dams were sacrificed on GD 20. At 750 mg/kg bw/d and above, post implantation loss was significantly increased for all dosing periods. At 750 and 1000 mg/kg bw/d, dose-related increases in the number of external and skeletal malformations (cleft palate and fusion of sternebrae) were seen for treatments during GD 7–9 or GD 13–15 but not GD 10–12. A NOAEL could not be established.

Single oral doses of DBP (0, 500, 1000, 1500 or 2000 mg/kg bw/d) were given to pregnant SD Dawley rats on GD 14; dams were sacrificed on GD 21 (Sallenfait et al. 1998*). Higher incidences of skeletal variations were seen at 1000 mg/kg bw/d and above. At 1500 mg/kg bw/d and above, significantly decreased maternal body weight gain, increased incidence of resorptions and reduced foetal body weights were observed. Foetal mortality per litter increased at 2000 mg/kg bw/d. There were no increases in post implantation losses. No developmental effects were reported at 500 mg/kg bw/d.

In a dietary study by Ema et al (1998*), pregnant Wistar rats received 0, 0.5, 1.0 or 2.0 % DBP (ca. 0, 331, 555 or 661 mg/kg bw/d, respectively) from GD 11–21. Dams were sacrificed on GD 21. At 1.0 % and above, the following effects were reported: significant and dose-related decrease in body weight gain and food consumption in dams; increased number of male foetuses with cryptorchidism and decreased urogenital distance. At 2 %; significant decrease in foetal weights, increased incidence of foetuses with cleft palate and fusion of sternebrae were observed. The NOAEL for maternal and foetal toxicity was 0.5 % (ca. 331 mg/kg bw/d). Embryotoxic effects were observed only at maternally toxic doses.

Gavage treatment of pregnant LE Hooded rats with 500 mg/kg bw/d DBP from GD 14–19 was associated with statistically significant reduction of the anogenital distance (adjusted for body weight) and seminal vesicle weight in male offspring compared with controls. The frequency of retained thoracic nipples was increased (Wolf et al. 1999).

Mylchreest et al. (1999) gavaged SD pregnant CD rats with 100, 250 or 500 mg DBP/kg bw/d on GD 12–21. The following effects were observed in male offspring at:

- 100 mg/kg bw/d and above, delayed preputial separation;
- 250 mg/kg bw/d and above, retained thoracic nipples and decreased anogenital distance; and
- 500 mg/kg bw/d, hypospadias, cryptorchidism and degeneration of the seminiferous epithelium.

No reproductive or developmental anomalies were detected in female pups. A NOAEL for developmental toxicity could not be established as delayed preputial separation was induced in the male pups at the lowest dose tested (100 mg/kg bw/d).

In a similar study with lower DBP doses, SD CD rats (19–20/group) were gavaged with 0, 0.5, 5, 50, 100 or 500 mg/kg bw/d DBP on GD 12–21 (Mylchreest et al. 2000). No effect on maternal body weight gain or food consumption was observed. At 100 mg/kg bw/d, there was a statistically significant increase of seminiferous tubule atrophy and retained nipples in the male offspring at PND 14. At 500 mg/kg bw/d, a significant decrease of anogenital distance in male pups at birth and increased frequency of male reproductive organ malformations (hypospadias, absent or partially developed epididymis) were observed. At the same dose, the weights of testes, prostate, epididymis and seminal vesicles were decreased at PND 110. The NOAEL for developmental effects was established at 50 mg/kg bw/d based on increased seminiferous tubule atrophy and retained nipples at 100 mg/kg bw/d.

In a subsequent study, Mylchreest et al. (2002) investigated the effect of DBP exposure during the prenatal period on pathologic changes and alterations in androgen status in rat testis. Pregnant SD CD rats were gavaged with 500 mg/kg bw/d DBP on GD 12–21. Dams underwent necropsy on GD 14, 16, 18 or 21 and were examined for histomorphology and testosterone

levels of foetal testes. At GD 16–21 Leydig cell hyperplasia was observed with an increased number of proliferating cell nuclear antigen (PCNA) positive Leydig cells in focal areas of hyperplasia. Testicular testosterone was decreased on GD 18 and 21. At GD 21, testis atrophy was apparent, seminiferous cords were enlarged and contained PCNA-positive multinucleated gonocytes. The authors consider that Leydig cell proliferation is likely to be triggered as a compensatory mechanism to maintain testicular steroidogenesis. Overall, a persistent decrease in androgen concentration is likely to result in reproductive tract malformations as observed in the earlier studies. The multinuclearity and hyperproliferation of gonocytes was considered to indicate underlying Sertoli cell dysfunction.

Barlow et al. (2004) gavaged pregnant SD rats with 0, 100 or 500 mg DBP/kg bw/d on GD 12–21. Male offspring were sacrificed at 6, 12 or 18 months of age. At the highest dose, decreased anogenital distance was observed. Increased incidence of areolae retention was observed at both doses on PND 13, and only at the highest dose (500 mg/kg) at PND 180. The incidence of testicular lesions (testicular atrophy and occasional enlargement with oedema) was significantly higher at 500 mg/kg bw/d at all time points. Other effects at the highest dose included: significantly higher incidence of malformed epididymides; absent vas deferens; malformed or absent seminal vesicles; hypospadias and decreased prostate size. Histopathology revealed testicular dysgenesis and germ cell degeneration at 500 mg/kg bw/d. The NOAEL could not be established, although the LOAEL for developmental toxicity was 100 mg/kg bw/d.

Jiang et al. (2011 ND) studied the incidence of anorectal malformations (ARMs) in male rat offspring. Pregnant SD rats were dosed daily with 850 mg/bw kg/d DBP by gastric intubation during late gestation (GD 12–18). On PND 1, the incidence of ARMs in male offspring was 39.5 %. All abnormal pups had secondary megacolon complications. Body weight and AGD were significantly decreased. The serum testosterone concentration was significantly lower than the control group. On PND 7, histological analysis of the terminal rectum of the abnormal pups showed no clear anal structure and transitional zone. The blind side of the terminal rectum was covered with interstitial epithelium. On PND 35, pups displayed swollen abdominal features with the absence of a scrotum and testis in the perineum region. Necropsy analysis revealed enlargement of the colon and a large volume of faecal matter retained in the intestines. Relative weights of the brain, heart, liver, spleen, lung and kidney and reproductive organs (testes and epidydimis) were reduced.

Carruthers and Foster (2005) gavaged SD rats with 500 mg DBP /kg bw/d on GD 14–15, 15–16, 16–17, 17–18, 18–19 or 19–20 (9–11 rats/group). Anogenital distance measured at PND 90 was significantly decreased in the GD 15–16 and GD 18–19 exposure groups. Persistent areolar nipple retention was observed in male offspring following exposure on GD 16–17 and there was a significant increase in epididymal malformations and small testes at GD 17–18. The data suggest that even short two-day gestational exposure during a critical window (GD 16–18) of foetal development is sufficient to induce permanent developmental abnormalities.

Hutchison et al. (2008a **ND**) also examined the critical window of foetal testicular development most sensitive to DBP treatment in Wistar rats. Dams were gavaged daily with 500 mg/kg bw/d DBP from e13.5–e21.5 (full window) or from e19.5–21.5 (late window). Testis development, including Leydig cell differentiation, was monitored morphologically through the expression of cellular markers: anti-mullerian hormone (AMH, Sertoli cell marker), 3 β-hydroxysteroid dehydrogenase (3β-HSD, Leydig cell marker), and smooth muscle actin (marker of seminiferous tubules). Results indicate that Leydig cell aggregation occurred in foetal testes in most animals dosed with DBP through full window (up to e21.5) and only <10 % animals dosed with DBP through late window. Postnatally (days eight and 10), only full window administration was associated with focal dysgenetic areas (malformed seminiferous cords with intratubular Leydig cells) in about 60 % of the animals. The results indicate that full window administration of DBP, critically affects development of rat testes. Full window, but not late window administration was also associated with Sertoli cell occurrence outside the normal

seminiferous tubules and intermingled with Leydig and interstitial cells. In a parallel study, Hutchison et al. (2008b **ND**) found that full window treatment significantly reduced (50 %) the number of Sertoli cells at e21.5. However, the effect appeared reversible in the scrotal testes by postnatal day 25.

Similarly, Ferrara et al (2006 **ND**) examined developmental windows sensitive to DBP exposure in terms of the effect on germ cell (gonocyte) differentiation and proliferation. Pregnant Wistar rats were administered by gavage with 0 or 500 mg DBP/kg bw/d from e13.5 to e21.5, or only on e19.5 and e20.5, to allow discrimination between early (e13.5–e17.5) and late (e19.5–e20.5) effects on gonocytes. Testes were collected from foetuses for gonocyte analysis at e15.5, e17.5, e19.5, e21.5, and postnatally from rats at d4, d6, d8, d15, d25, and d90.

Immunohistochemical analysis of markers for specific stages of gonocyte differentiation (OCT4, a transcription factor expressed in totipotent embryonic stem and germ cells but not in the intermediate gonocytes; phosphorylated retinoblastoma tumour suppressor gene—Rb, a crucial regulator of the cell cycle at the G1/S phase) indicated that foetal DBP exposure induces a slight, but significant, delay of the early phase of gonocyte development in the foetal rat testis. At e15.5–e17.5, expression of OCT4 was temporarily prolonged in foetal testes of animals treated with DBP in utero. A similar effect was observed on the expression of phosphorylated-Rb.

At the histological level, changes in the frequency of multinucleated gonocytes (MNGs) in the seminiferous cords were observed. Cords with MNGs were absent or sporadic in control and DBP-treated animals from e13.5 to e17.5, but were significantly increased on e19.5 and e21.5 (~10 % and 35 %, respectively). In the control group the frequency of tubules with MNGs did not exceed 2 % at any time point, but in the treated animals it was still significantly higher (~22 %) postnatally on day four even though DBP treatment had ceased at e21.5. MNGs were no longer detectable in control group testes or DBP-exposed animals at, and beyond, PND 15.

Short-term exposure to DBP at latter developmental stages (e19.5–e20.5) also induced MNGs at e21.5 with a frequency similar to that induced by daily DBP treatment from e13.5, suggesting that that this effect is likely to be independent from the perturbations in the early process of gonocyte differentiation highlighted above.

The number of gonocytes in the testes of animals treated with DBP from e13.5 was significantly decreased at postnatal days four, eight and 15 (by 53 %, 79 % and 80 %, respectively), after which it gradually recovered to control levels by adulthood on PND 90. Short-term DBP treatment had no effect on gonocyte numbers at PND 4 (Ferrara et al. 2006 **ND**).

In a more recent study (Jobling et al. 2011), pregnant rats were treated daily with an oral dose of 500 mg/kg bw/d DBP from e14.4 to e21.5 for foetal tissue recovery, and from e13.5 to e21.5 for post natal tissue recovery. DBP was also administered to rats for early window (from e13.5 to e15.5) and for late window (from e19.5 to e20.5) observations. Germ cell numbers, proliferation, apoptosis, differentiation (loss of octamer-binding transcription factor—OCT4, doublesex and MAB-3-related transcription factor 1—DMRT1 expression, DMRT1 re-expression, germ cell migration) and aggregation were examined at various foetal and postnatal ages.

DBP exposure following testis differentiation in the rat (e13.5) caused reduction in foetal germ cell numbers of up to 60 % and delays in germ cell differentiation including delayed OCT4 and DMRT1 switching off; delayed entry to quiescence; delayed re-emergence from quiescence (after birth); and delayed re-expression of DMRT1. Occasional apoptopic germ cells were also reported. On the other hand, DBP exposure in a late gestation (e19.5–e21.5) showed central aggregation of germ cells or of multinucleated gonocytes. These findings suggest germ cell susceptibility when exposure to DBP during early gestation.

Jiang et al (2007 **ND**) administered DBP to pregnant SD rats with 0, 250, 500, 750 or 1000 mg/kg bw/d by gastric intubation from GD 14 to 18. Maternal body weight significantly decreased at 750 mg/kg bw/day after GD 14. Live pups per litter decreased significantly at 750 mg/kg bw/d and above.

In the offspring, cryptorchidism increased significantly at 250 mg/kg bw/d and above. Significant incidence of hypospadias was observed at 500 and 750 mg/kg bw/d (6.8 % and 41.3 %, respectively) compared with no hypospadias in controls. Reduced AGD was also observed at 500 mg/kg bw/d and above.

Serum testosterone (T) level, measured on PND 70 in the male rats, was decreased in a dose-dependent manner from 250 mg/kg bw/d. The serum T levels of hypospadiac rats were significantly lower at 500 and 750 mg/kg bw/d than in controls.

No NOAEL could be established. The LOAEL for developmental effects is 250 mg/kg bw/d based on increased incidence of cryptorchidism and decreased T levels.

Mahood et al (2007 **ND**) compared dose-sensitivity of foetal (prenatal) and adult (postnatal) end points related to testicular dysgenesis. Pregnant Wistar rats were gavaged from GD 13.5 to either GD 20.5 (for foetal samples) or GD 21.5 (for postnatal tissue) with 0, 4, 20, 100, or 500 mg/kg/d of DBP. Adults that had a treatment-free period from GD 21 were analysed at PND 90.

In foetuses, testis weight was decreased to about 70 % at 500 mg/kg bw/d compared with controls. Testosterone levels in foetal testes were significantly decreased at 100 and 500 mg/kg bw/d. Dysgenic areas in the foetal testes (evaluated by number and size of Leydig cell aggregation areas, and percentage of seminiferous tubules with MNGs) were significantly increased at 100 and 500 mg/kg/d, with increasing trend evident from 20 mg/kg bw/d.

In adults, testis weight was significantly decreased (~50 %) at 500 mg/kg bw/d. This decrease was found to be entirely due to the increased number of cryptorchid testes; 90 % incidence at 500 mg/kg bw/d compared with 0 % in the controls, as well as in the 4 and 20 mg/kg/d DBP treatment groups. Only one of 19 animals showed cryptorchidism at 100 mg/kg/d. The weight of scrotal testes was comparable to the controls. Dysgenic area increase (evaluated by the number of testes containing Sertoli cell-only seminiferous tubules, or areas with irregular staining for specific testicular proteins) were significantly increased in adults at 100 and 500 mg/kg/d. Anatomically, normal scrotal testes showed a statistically significant increase of dysgenic areas at the two highest doses.

The NOAEL for foetal end points is 20 mg/kg bw/d based on reduced testosterone levels correlated with significant testes dysgenesis at the LOAEL dose of 100 mg/kg bw/d. The authors concluded that foetal end points are more sensitive than adult end points. However, the adult end points showed significant perturbations at the same doses (100 and 500 mg/kg/d) as the foetal end points. Furthermore, fertility assessment of the adults (details of this study in the section: Effects on fertility) showed significant adverse effects at 500 mg DBP/kg bw/d with a trend apparent from 4 mg/kg/d. A similar trend is observed for the occurrence of dysgenic areas in the foetal testes from 20 mg/kg bw/d, shown by increased seminiferous tubules with MNGs, that was statistically significant from 100 mg/kg/d and above.

Considering all the results from the study and noting that, in fact, the animals analysed as adults had a treatment-free period from GD 21 to adulthood, the perturbations in the fertility and other end points tested in adulthood are significant and strongly suggest that the DBP doses that induce histomorphological and hormonal changes in the foetal testes also significantly affect reproductive end points in adults.

In a study by Guerra et al. 2010 **ND,** which focused on the effects of gestational DBP exposure to female offspring, Wistar rats were administered DBP by gavage at a single dose level of 100 mg/kg bw/d from GD 12 to GD 20 (to evaluate the effect on P0 females) or up to the end of the

lactation period (PND 21), to evaluate reproductive/developmental parameters in the F1 females. Only female foetuses and offspring were analysed for histomorphology of gonads and onset of puberty, oestrous cycle, sexual behaviour, or hormonal status. No significant treatment-related effects were reported for any of the parameters analysed in the females at this DBP dose, which is toxic to males. Parameters analysed in offspring included AGD; reproductive organ (uterus, ovaries) weights and histomorphology; oestrus cycle length; and hormonal status in puberty.

In a study with marmosets (McKinnell et al. 2009 **ND**), pregnant females were dosed from week seven to 15 of gestation with 500 mg/kg bw/day MBP. Male offspring were studied at birth (1–5 days; n = 6) or in adulthood (18–21 months of age; n = 5). This foetal treatment period was chosen as it appears to correspond to a time window in the rat that is critical for androgen-dependent programming of reproductive tract masculinisation. Treatment was not continuous to the time of analysis, leaving at least ~12 weeks of treatment-free period before newborns were analysed, and more in the case of adults. Control animals were combined from vehicle treated and untreated mothers.

For the effect of neonatal exposure, a separate group of five newborn co-twin pairs of males were dosed with 500 mg/kg/day of MBP, or with the vehicle starting at age of four days for 14 days. Animals were analysed four hours after the last neonatal treatment (McKinnell et al. 2009 **ND**).

Foetal exposure of marmosets to MBP did not affect gross testicular morphology, reproductive tract development or testosterone levels at birth or in adulthood. Germ cell numbers and proliferation were also not affected, nor were Sertoli cell numbers or the ratio of germ to Sertoli cells—assayed by immunocytostaining for protein markers for Leydig, Sertoli and germ cells. In two of six MBP-exposed animals analysed at birth, unusual clusters of undifferentiated germ cells were found, but their significance is unclear as similar, but more sporadic, clusters containing smaller number of cells were found in testicular sections in two out of 10 control animals. Foetal exposure to MBP did not affect testis size/morphology, germ cell numbers or fertility in adulthood. Fertility was assessed in three of five adult animals and no adverse effects were observed.

Neonatal MBP treatment did not affect germ cell numbers or differentiation assessed using immunocytostaining for specific cellular protein markers.

Overall this study (McKinnell et al. 2009 **ND**) does not indicate adverse developmental or reproductive effects from MBP (and by inference, of DBP) in marmosets. However, reliability of this study is limited considering that only one treatment dose was used, together with a small number of animals for which significant individual (for control and treated animals) variations were reported in some of the measured end points.

Postnatal developmental toxicity studies

Some of the studies described in this section include prenatal and postnatal treatment—the latter often only indirect through lactation, and can be considered trans-generational studies. However, mostly postnatal developmental end points in males are evaluated. Fertility end points are also reported in some studies. These have been included in the fertility studies detailed above.

In a study by Mylchreest et al. (1998), pregnant SD CD rats (10/dose) received DBP at 0, 250, 500 or 750 mg /kg bw/d in corn oil by gavage from GD 3 throughout pregnancy and lactation until PND 20. Dams were sacrificed on PND 21 (weaning) and pups on PND 100–105 (sexual maturity).

At the highest dose (750 mg/kg bw/d) observations included a significant decrease in the number of live births per litter, body weight gains of dams, and pup survival to weaning. There was no significant effect on the number of implantations or pup weight. Significantly decreased

mean kidney weights and a decrease in mean prostate weight (by 27 %) in the offspring were also observed at the highest dose.

At 500 mg/kg bw/d and above, the following effects were observed in male offspring: decreased anogenital distance; dose-related increase in frequency of malformations of genitalia; seminiferous tubule underdevelopment; and testes weight decrease.

At 250 mg/kg bw/d and above the following effects were observed: a dose dependent increase of hypospadias (3 %, 21 % and 43 % at 250, 500 and 750 mg/kg bw/d, respectively); underdeveloped/absent epididymis, frequently bilaterally, in 9 %, 50 % and 70 % offspring; atrophy of seminiferous tubules; and increase in the frequency of a dilated renal pelvis.

The following effects were seen in female offspring: an absence of vaginal opening in one out of 30 rats (1/8 litters) at 500 mg/kg bw/d and two out of nine rats (1/4 litters) at 750 mg/kg bw/d; absence of patent vagina, uterus or left kidney in animals with no vaginal opening at 500 mg/kg bw/d; uterine horn abnormalities in one female at each dose of 500 and 750 mg/kg bw/d.

A NOAEL could not be established for this study. The LOAEL was 250 mg/kg bw/d based on seminiferous tubule atrophy and hypospadias in the male offspring.

In another study with pregnant SD rats gavaged with 0 or 500 mg DBP/kg bw/d from GD 14-PND 3 (Wolf et al. 1999), anogenital distance reduction in male offspring was significant compared with controls after adjustment for body weight. In addition, there was an increased frequency of retained thoracic nipples, hypospadias, and testicular and epididymal atrophy. Ventral prostate and testes weight were also reduced.

In a study investigating postnatal development in both sexes following gestational and early indirect exposure to DBP (Lee et al. 2004), pregnant SD CD rats (6–8/group) were given a diet containing 0, 20, 200, 2000 and 10000 ppm (ca 0, 1.5–3, 14–29, 148–291, 712–1372 mg/kg bw/d males-females, respectively) from GD 15 to PND 21. Offspring were sacrificed on PND 21 and postnatal week (PNW) 11 and 20.

No significant signs of maternal toxicity were reported at any dose.

The following effects were observed in males:

- At 20 ppm and above, a reduction in testicular spermatocyte development was observed on PND 21;
- At 20 ppm and above, statistically significant effects on spermatocytes with increased severity in dose were observed;
- At 20 ppm and above, vacuolar degeneration of the alveolar cells of the mammary gland was observed at PNW 11 in the male offsprings, but there was no clear dose-response;
- At 20, 200 and 2000 ppm, significantly increased relative pituitary weights were detected at PNW 11 but not PNW 20;
- At 2000 ppm, aggregation of Leydig cells and decreased epididymal duct cross-sections were observed at PND 21; however, no significant adverse effects to Leydig cells were observed at PNW 11 and PNW 20, or in the epididymis at PNW 11 (PNW 21 not reported);
- At 10,000 ppm, decreased neonatal anogenital distance, retention of nipples and decreased testes weight were observed at PND 21, but not at PNW 11. Other observations at this dose include: increased percentages of luteinizing hormone (LH) positive cells, decrease in follicle-stimulating hormone (FSH) and prolactin producing cells in the anterior pituitary at PND 21; and
- At the two highest doses (2000 and 10, 000 ppm), adverse effects on the testicular germ cell development were significant only at PNW 11 and appeared to be reversible by PNW 20.

The following effects were observed in females:

- At 20 ppm and above, hyperplasia of the mammary alveolar bud on PND 21 and vacuolar degeneration of mammary gland alveolar cells on PND 11 were observed;

- At 200 ppm and above, atrophy of alveolar cells of the mammary gland and decreased relative pituitary weight at PNW 20 were observed;
- At 2000 and 10,000 ppm, slight delay in puberty onset, increased percentages of LH-positive cells, decrease in FSH and prolactin-producing cells, increase in incidence of females with extended dioestrus were observed; and
- The proportion of FSH positive cells in the pituitary in DBP-treated females, compared with controls, fluctuated depending on the age of examination. It decreased at PND 21 (200 ppm) and increased at PNW 11 (10,000 ppm).

The study authors concluded that exposure to DBP during development affected female postnatal sexual development of the pituitary function up to maturity (males were also affected), while the testicular toxicity of DBP in males was mostly reversible, unlike the mammary gland toxicity in females, which was persistent at a dose level as low as 20 ppm. The toxicological significance of this effect for male fertility is not known and there was no clear dose response. However, the authors considered 20 ppm as the lowest observed LOAEL and could not establish a NOAEL based on the irreversibility of this effect. As noted above, at the same dose, low severity adverse effects on testicular germ cells were also observed, but these seem to be reversible later in development.

Based on the significant severe reduction in testicular spermatocyte development, aggregations of Leydig cells and decreased epididymal duct cross-section on PND 21 at 2000 ppm, the NICNAS Hazard Assessment (NICNAS 2008b) established a NOAEL of 200 ppm (14–29 mg/kg bw/d) for this study. However, there is significant uncertainty associated with this NOAEL for developmental toxicity, as statistically significant spermatocyte development reduction was observed at PND 21 at all doses, including the lowest tested dose (20 ppm). Although the severity of the reduction of spermatocyte development at 20 and 200 ppm was graded as minimal to slight, it is the same type of effect and, therefore, clearly related to the significantly more severe reduction observed at the two higher doses. On the other hand, even the severe effects observed at the higher doses appear to be reversible by PNW 20 at 2000 ppm (10,000 ppm not examined at PNW 20).

For females, the developmental NOAEL can be established at 20 ppm, based on significant decrease of relative pituitary weight at 200 ppm and above on PNW 20. However, this is also associated with significant uncertainty as no histopathological changes are correlated with the decrease in weight. The changes in the percentage of follicle-stimulating hormone (FSH), luteinizing hormone (LH) or prolactin (PRL) producing cells in the anterior pituitary at PND 21 and PNW 11 did not show a consistent trend.

Zhang et al. (2004) administered DBP (0, 50, 250 or 500 mg/kg bw/d) by gavage to pregnant SD rats from GD 1 to PND 21 (weaning). F1 pups were examined on PND 70. There was a dose-related decrease in birth weight at 250 and 500 mg/kg bw/d and the number of live pups in each litter was significantly decreased at 500 mg/kg bw/d.

In the F1 generation, the following effects were seen:
- at 250 mg/kg bw/d and above:
  - a significant reduction of anogenital distance;
  - increased frequency of testicular atrophy;
  - underdeveloped/absent epididymis and cryptorchidism; and
  - decreased epididymis weight and epididymal sperm motility.
- Total sperm heads per gram of testis was also decreased at 250 mg/kg bw/d and above, while sperm number was decreased at 500 mg/kg bw/d only;
- Histopathology revealed a mild degeneration of seminiferous epithelium at 250 mg/kg bw/d and this was more severe at 500 mg/kg bw/d; and
- The NOAEL was established at 50 mg/kg bw/d and the LOAEL at 250 mg/kg bw/d based on decreased pup weight and male reproductive tract malformations.

Xiao-feng et al. (2009 ND) gavaged five-week-old prepubertal SD rats with 250, 500, 1000 and 2000 mg DBP/kg bw/d for 30 days. Effects included:

- the relative testes and epididymis weight, compared with the control, were significantly decreased at 500 and 1000 mg/kg bw/d, respectively. These effects peristed in the recovery group (dosing regimen switched to vehicle treatment for additional 15 days) at 1000 mg/kg bw/d;
- histopathological changes in the testes were observed at 500 mg/kg bw/d and did not significantly improve in the recovery group;
- serum testosterone (T) was significantly decreased at 500 mg/kg bw/d and above, while glucocorticoid hormone (GC) was increased at 1000 mg/kg bw/d and above;
- hormone levels were comparable to controls at the end of the recovery period; testicular testosterone levels or synthesis were not measured;
- testicular protein and mRNA levels for glucocorticoid receptor (GR) and 11β-HSD1 enzyme, involved in GC synthesis and GR activation, were significantly increased at 1000 mg/kg bw/d and above in the treatment but not in the recovery groups; and
- testicular mRNA and protein levels of steroidogenic acute regulatory protein (StAR) were significantly decreased at 1000 mg/kg bw/d in the treatment but not in the recovery group.

The authors concluded that the decrease of serum levels of T in DBP treated animals is a result of a GC-GR-mediated mechanism that affects LH receptor signal transduction in the Leydig cells. Glucocorticoids are known to affect steroidogenesis and it has been shown that high levels of glucocorticoids suppress basal and LH stimulated Leydig cell steroidogenesis in vitro without affecting LH activation (Sankar et al. 2000). Although the evidence for the hypothesis is consistent, the data in the study by Xiao-feng et al. (2009 **ND**) are not sufficient to establish a direct causal relationship between the DBP effect on T levels in vivo and the changes in GR observed in the Leydig cells in vitro. A NOAEL of 250 mg/kg bw/d for postnatal developmental toxicity of DBP is established based on the decrease of T serum levels correlated with non-reversible histomorphological perturbations in the testes at 500 mg/kg bw and above.

Alam et al. (2010 **ND)** treated prepubertal (3-week-old) male SD rats with 250, 500 or 1000 mg DBP/kg bw/d by gavage for seven days. Significant testicular toxicity was observed. Statistically significant and dose-dependent reduction in testis weight was observed starting from the lowest dose. Histopathological examination showed seminiferous tubule lesions starting from mid dose. Lesions included a decrease in tubular size, depletion of spermatogenic cells, wider tubular lumen and ultimately seminiferous tubules with a thin layer of epithelia and wide lumen. Cellular TUNEL microscopy assay (based on terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labelling—TUNEL—of cells in situ and light microscopy detection) showed significant apoptotic spermatogenic cells at 500 mg/kg bw/day, with only few apoptotic cells found at 1000 mg/kg bw/day due to a complete loss of spermatocytes.

Intratesticular testosterone (ITT) level was measured only in the testes of animals treated with 500 mg/kg bw/day for seven days in a separate experiment and was comparable to controls. Real time-polymerase chain reaction (RT-PCR) also showed no statistically significant effect on mRNA levels for testicular steroidogenic enzymes in the testes of these animals. In contrast, single short three, six or 24-hour treatment with 500 mg DBP/kg bw/d was associated with significantly lower ITT levels, which correlated with reduced mRNA levels for Cyp11a1, Cyp17a1 and Hsd3b steroidogenic enzymes. The same treatment also appeared to increase the number of apoptotic cells in the testes. The type of cells affected is not very clear as no cell-specific markers were used. However, based on combined light and TUNEL microscopy, the authors concluded that these are apoptotic spermatocytes and spermatogonia. Serum FSH levels were decreased in animals treated with a single dose of 500 mg/kg bw and assayed after six hours.

Based on the decreased testes weight at the lowest dose tested, a NOAEL cannot be determined in this study (Alam et al. 2010 **ND)**.

In a study by Clewell et al. (2013 ND), male sexual development was investigated in the male offspring of pregnant rats treated with 0, 50, 250 and 750 mg/kg bw/d DINP and 500 mg/kg bw/day DBP (used as a positive control) in the diet from GD 12 to PND 14. On PND 2, AGD and scaled AGD (adjusted for body weight) were significantly decreased in pups from dams treated with DBP. Testis weight in pups was significantly reduced. The histopathology of the testis revealed increased incidence of MNG and severity of large Leydig cell aggregates in the high dose DINP group and in the DBP-treated group. The DBP group also had segmental dilation of the ductus, which is associated with thinning of the epithelial lining. On PND 14, absolute AGD and scaled AGD were again reduced in the DBP and high-dose DINP groups. There was also a statistically significant increase in nipple or areola development in the DBP-treated goup only. On PND 49, the DBP-treated group showed statistically significant decreases in the weights of:

- bulbocavernosus muscle ;
- ventral prostate;
- seminal vesicles; and
- kidneys.

Reproductive malformations, including flaccid (abnormally soft with a fatty appearance) epididymis, incomplete (hypoplastic) epididymis, undescended testes, atrophic testis, and hypospadias were also evident in a number offspring from DBP-treated animals, and in some offspring from high-dose DINP animals. Histopathology of the atrophic testis/epididymis confirmed extensive necrosis, inflammation and mineralisation of the testis and epididymal tissue. Significant increases in the number of nipples present and delayed preputial separation were reported for DBP, but not with DINP.

The reproductive and developmental effects in male mice (Pzh:Sfis outbred) and their offspring were investigated (Dobrzynska et al. 2011 ND). Three groups of male mice were treated with 0, 500 and 2000 mg/kg bw/day DBP by gavage, three times a week for eight weeks. Following exposure, treated male mice were caged with virgin female mice for one week. At eight weeks of age, F1 male weanlings from the treated and control groups were mated with females from the same group, but with different litters for one week. Females were sacrificed one day before expected parturition and the foetuses were examined for the prenatal developmental effect of DBP of the F2 generation.

At 2000 mg/kg bw/day, male fertility, percentage of successful pregnancies, mean number of implantations, and living foetuses per pregnant female were reduced compared with the control group (not statistically significant). The percentage of skeletal abnormalities was also increased. Effects of DBP on the F1 generation included reduction of the mean body weight, growth retardation, and defects in sperm DNA or chromosomes. There were approximately twice the number of males compared with female offspring in the treated group. Females demonstrated a delay in eye opening and vaginal opening, which suggests abnormality in the germ cells following paternal exposure to DBP. The percentage of abnormal sperm of offspring from the highest dose males was increased. There were no changes in the fertility of the F1 animals and viability of F2 foetuses.

Multigenerational reproductive toxicity studies

In a GLP-compliant study (IRDC 1984*), adult male or female Charles River COBS CD rats were treated at 6 and 14 days, respectively before mating, with a diet containing DBP to a final dose of 0, 5, 50 or 500 mg/kg bw/d. Treatment continued through mating, gestation and lactation. F1 weanlings were given either control diets or diets equivalent to that of their mothers, during a seven-week post-weaning period.

No effect on clinical signs, haematology or fertility was seen in treated females. A reduction in growth was seen throughout the treatment with 500 mg/kg bw/d (statistically significant at weeks 7, 9 and 11). Statistically significant increases were seen in kidney weights in treated females at 500 mg/kg bw/d, but without histopathological changes. Decrease in pup weight and pup growth through lactation were seen at 500 mg/kg bw/d. Offspring body weights were reduced at all dose levels (occasionally significant but not dose-related) during the 7-week post-weaning period.

No effect on clinical signs, haematology or fertility was observed in treated males. Pathology revealed significant increases in absolute and relative liver and kidney weights at 500 mg/kg bw/d. Minor increases in relative kidney weights were also seen at 50 and 5 mg/kg bw/d, but these were not dose-dependent. Histopathology of the kidneys was normal. No abnormalities were seen in reproductive performance, parturition, neonatal viability, newborn growth, organ weights or histopathology in weanlings.

After the 7-week post-weaning period, there were slight decreases in testicular weights in weanlings in the 500 mg DBP/kg bw/d group. Histopathology revealed testicular lesions in six out of 10 weanlings in this dose group and in two of nine weanlings from the litters of mothers in the 500 mg/kg bw/d group that were given the control diet in the post-weaning period. The NOAEL for maternal toxicity and developmental toxicity was 50 mg/kg bw/d.

In a multigenerational dietary study with SD rats (NTP 1995*; Wine et al. 1997) animals (20/sex/group; 40/sex for controls) received DBP at 0 %, 0.1 %, 0.5 % and 1.0 % in the diet during a 7-day premating period and a 112-day continuous breeding period (pairwise mating). Exposure doses were 0, 52-80, 256-385 and 509-794 mg/kg bw/d for males and females, respectively. Final litters delivered during this phase were maintained for a minimum of 21 days. Thereafter, treatment of F1 animals was initiated at the same concentration as their parents. At the end of the continuous breeding period, a week-long crossover mating trial was performed between control F0 animals and 1 % dose groups (both male and female) of F1.

The effects seen at 1 % DBP in F0 animals included increased relative liver and kidney in both sexes, and decreased body weight in females only. There was no effect on sperm parameters in males. The total number of live pups/litter was significantly decreased in a dose-dependent manner from the lowest dose.

The following effects were seen in the F1 generation males:

- at 0.5 %, increased kidney weights and increased testicular atrophy in one out of 20 animals;
- at both 0.1 % and 0.5 % DBP:
  - ~ poor epididymal development in one of 20 animals; and
  - ~ histomorphological examination revealed seminiferous tubule degeneration in three of 10 animals.
- at 1.0 %:
  - ~ reduced body weight and relative weights of all reproductive organs;
  - ~ significantly increased relative liver and kidney weights;
  - ~ significantly decreased epididymal sperm count and testicular spermatid head count;
  - ~ poor epididymal development in 12 of 20 animals;
  - ~ testicular atrophy in four of 20 animals;
  - ~ cryptorchidism in three of 20 animals;
  - ~ impaired seminal vesicle development in four of 20 animals; and
  - ~ underdevelopment of prepuce or penis in four of 20 animals.
- at 1 %, histomorphological examinations showed:
  - ~ seminiferous tubule degeneration in eight of 10 males;
  - ~ testicular interstitial cell hyperplasia in seven of 10 animals; and

~ vesiculitis with inspissated secretion.

In F1 generation females, significantly reduced body weights and absolute ovary, liver and kidney weights at 1 % DBP were observed. Oestrous cyclicity or oestrous cycle length was unaffected at any dose.

Mating, pregnancy and fertility indices were all significantly reduced (30 %, 5 % and 17 %, respectively) for F1 breeding pairs at 1 % DBP in the diet. Live pup weights were significantly reduced at 0.5 % and above in the F1 generation, and at all dose levels in the F2 generation.

In the crossover mating trial, no effect on mating, pregnancy or fertility indices were seen, but pup weight was significantly decreased when treated dams were mated with control males. General toxicity, exhibited as increases of liver and kidney weights, was independent of sex.

The NOAEL for maternal toxicity was established at 0.5 % (385 mg/kg bw/d) based on the reduction in growth and decreased body weight. The NOAEL for fertility and embryotoxicity was 52–80 mg/kg bw/d (males–females) based on the decreased total number of live pups in each litter in F1, and adverse effects on reproductive development in males (see also the description of this study in the fertility studies section).

The NOAEL for developmental toxicity in F1 was at 0.1 % DBP in the diet (52 mg/kg bw) based on the significant increase of kidney weight in F1 males at 0.5 % DBP. In F2 males, significant testicular atrophy and seminiferous tubule degeneration was observed at the dose of 0.5 % DBP in the diet (256 mg/kg bw/d). However, no information about this endpoint was reported for the 0.1 % dose and therefore NOAEL could not be established (NTP 1995; Wine et al. 1997).

The results suggest that the F1 generation exposed to DBP during development and in adulthood is more sensitive to reproductive toxicity of DBP compared to the F0 generation that was exposed during adulthood only.

Wolf et al. (1999) gavaged LE hooded rats (10–12/sex/dose) with 0, 250 or 500 mg DBP/kg bw/d from weaning through puberty, young adulthood, mating and lactation in the P0 generation. F1 pups were untreated. Another group of males received 1000 mg DBP/kg bw/d. Treated P0 animals were also mated with untreated controls. Sixteen F1 animals/sex/group were chosen for fertility assessment for continuous mating over 11 breeding cycles.

In the P0 generation, delayed puberty (preputial separation) occurred in males at all dose levels. DBP treatment did not accelerate the age at which vaginal opening occurred, or cause persistent vaginal cornification (effects indicative of subchronic oestrogen exposure). Decreased fertility was observed in both sexes (crossover mating to untreated animals) at 500 mg/kg bw/d, and in males at 1000 mg/kg bw/d. Male infertility was attributed to testicular atrophy and decreased spermatogenesis. Females in the 500 mg/kg bw/d which mated successfully had abortions at mid-gestation.

In the F1 generation (animals exposed indirectly in utero and through lactation) the following effects were seen at 250 and 500 mg DBP/kg bw/d:

- anophthalmia;
- urogenital malformations (epididymal agenesis, hypospadias, ectopic testis, renal agenesis and uterine malformations); and
- decreased cauda epididymal sperm counts.

A dose-related decrease in fecundity was seen in the F1 offspring (significantly fewer F2 pups/litter) in similarly treated pairs under continuous breeding conditions.

The LOAEL for fertility was 250 mg/kg bw/d based on reduced fertility in both sexes in the crossover study with P0 and the decreased epididymal sperm counts in F1. The developmental

LOAEL was 250 mg/kg bw/d, based on increased frequency of delayed puberty in P0 and increased malformations in F1 males.

In a study by Salazar et al. (2004), female Long Evans rats were fed chow containing 0.6 g/kg or 2.5 g/kg of DBP (authors estimate 0, 12 and 50 mg/kg bw/d) for two months before mating, throughout pregnancy and weaning. Pups were necropsied on PND 14 and PNW 12. At 12 mg/kg bw/d, decreased pup survival and significantly decreased pup weights were observed ($p < 0.01$). At 50 mg/kg bw/d, pronounced decreases in the percent of pregnancies and significant decreases in pup weight ($p < 0.001$) were observed. Decreased relative thymus and testes weights (at PND 14) and delayed vaginal opening and onset of first oestrous cycle in pups were observed at both treatment levels. Preputial separation was significantly delayed in the high dose group. A developmental NOAEL could not be established due to the adverse effects in both sexes observed at the lowest dose tested (12 mg/kg bw/d). However, there is some doubt over the calculated dose in this study. An adult rat typically consumes 20g feed/day. This would be equivalent to 12 mg or 50 mg DBP/day, not 12 mg and 50 mg DBP/kg bw/d as stated in the paper. Assuming a 300 g rat, the estimated doses would be 40 and 166 mg/kg bw/d respectively.

Co-administration studies

Howdeshell et al (2007 **ND)** investigated the effect of co-administration of DBP and DEHP. Pregnant SD dams were gavaged from GD 14–18 with 500 mg/kg bw/d of each phthalate individually, or co-administered (500 mg/kg bw of each chemical). Reproductive malformations were monitored in adult offspring; the levels of testosterone production and insulin-like 3 peptide (insl3) mRNA were measured in ex vivo foetal testicular cultures.

Compared with vehicle controls, maternal body weight gain was not affected by the DBP treatment, but it was reduced when DBP was co-administered with DEHP. The DEHP treatment alone also resulted in reduction of body-weight gain. DBP and DEHP co-administration also significantly reduced litter size and increased foetal and neonatal mortality, while each phthalate administered alone did not have a significant effect on these parameters. At 500 mg/kg bw/d, DBP co-administered with DEHP significantly increased the percentage of male offspring with various reproductive malformations (significant reduction of anogenital distance and increased nipple retention). The effect on AGD was also significant when DBP was administered alone, but severity increased after co-administration with DEHP. The effect on areola and nipple retention, although detected, was not statistically significant for DBP alone. A synergistic effect of DBP and DEHP was indicated in the case of seminal vesicle agenesis, where co-treatment was associated with an incidence of 63.1 % ($p < 0.001$) for this malformation, while no malformations were found after treatment with DBP alone, and only 11.1 % incidence (not statistically significant) after DEHP treatment alone.

DBP alone and co-administered with DEHP significantly reduced ex vivo testosterone production and mRNA levels for the steroidogenic acute regulatory protein, StAR, but the effect was significantly more pronounced upon coadministration of the two phthalates. The level of insl3 mRNA was also significantly reduced in the ex vivo testicular cultures following co-administration treatment, but only slightly (not statistically significantly) by the DBP treatment alone. DEHP alone significantly reduced insl3 mRNA in the ex vivo testicular cultures.

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 with controls.

In another study, the same authors (Howdeshell et al. 2008 **ND**) examined the effect of DBP, DEHP, BBP and DIBP, alone and co-administered, on ex vivo testosterone production in the foetal testes of SD rats treated in utero during GD 8–18. DBP alone, at doses of 33, 50 and 100 mg/kg bw/day had no effect, but doses of 300 and 600 mg/kg bw/day significantly decreased ex vivo testosterone production in the foetal testes. A mixture of the five phthalates containing 300 mg/kg bw/day of DBP and equivalent LOAEL doses for the other four phthalates (100 %

mixture) was tested in serial dilutions up to 5 % (corresponding to 15 mg/kg bw/day of DBP). Treatment of dams with a 20 % phthalate mixture (containing 60 mg/kg bw/day of DBP and equivalent no-effect doses of the other four phthalates) significantly reduced foetal testicular production, consistent with the dose-additive effect of the five phthalates.

Mode of action studies

Oestrogenic activity assays in vitro

DBP was shown to have extremely weak oestrogenic activity in a recombinant yeast assay (Harris et al. 1997).

DBP was a weak competitive agonist at the oestrogen receptor in an in vitro competitive ligand-binding assay and weakly induced oestrogen receptor-mediated gene expression in the human breast cancer cell line MCF-7 (Zacharewski et al. 1998).

DBP was shown to bind to human oestrogen receptor (ER) in a competitive displacement assay in vitro (Nakai et al. 1999) but was negative for oestrogenic activity in a yeast two-hybrid assay (Nishihara et al. 2000).

DBP demonstrated oestrogenic and antiandrogenic activity in CHO-K1 cells transfected with expression vectors for human ERα, ERβ and AR (Takeuchi et al. 2005). However, DBP had no binding affinity for ERα or ERβ in vitro (up to 10^{-5}M) (Toda et al. 2004).

Other assays based on ER activation give contradictory results. DBP increased proliferation of human breast cancer MCF-7 cells in one assay (Hong et al. 2005) but not another (Okubo et al. 2003).

Taken together, the results suggest that the oestrogenic activity of DBP in these in vitro assays is not likely to be mediated through direct receptor binding.

Testicular cell function assays in vitro

In vitro MBP induced the detachment of germ cells from a Sertoli cell monolayer, but it was 100-fold less potent than MEHP, the DEHP monoester metabolite (Gray & Gangoli 1986).

A more recent study examined the effect of MBP on Sertoli cells in polarised monolayer cultures derived from normal 18-day-old SD rats (Zhang et al. 2008 **ND**). Test concentrations (10, 30, 150 and 600 μM) were chosen to correspond to the range of DBP concentrations detected in 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 MBP or MEHP resulted in Sertoli cell vacuolisation and irregular intercellular membrane structures in the culture monolayers. Treatment of established Sertoli cell monolayers (5-day culture) with MBP or MEHP for 24 hours reduced transepithelial electrical resistance (TEER) in a dose-dependent manner. Semi-quantitative RT-PCR indicated that mRNA expression for the tight junction protein occludin was downregulated after treatment with both of the monophthalates. For MBP, the effect was statistically significant only at 150 μM, although a decrease was also observed at 30 μM. Unlike MEHP, MBP treatment did not affect the distribution of F actin and the tight junction protein ZO-1 in the established cell monolayers.

Overall, the results indicate that disruption of Sertoli cell tight junctions might be an aspect of the mechanism underlying the DBP- and DEHP-induced reproductive toxicity in male rodents.

The effect of phthalates in steroidogenesis was examined by Mitchell et al. (2012 **ND**) using human foetal testis. Human foetal testis (14–20 week gestation) was xenografted into castrated male nude mice treated with 500 mg/kg bw/day DBP or 500 MBP for 4–21 days. All mice were treated with hCG to mimic normal human pregnancy. Rat foetal testis xenografts treated with DBP served as a positive control. Testosterone production was assessed by measuring host serum testosterone and seminal vesicle (SV) weights at termination. Serum testosterone and SV weights of the human foetal testes xenografts were similar to the control group. However,

changes to germ cells (aggregation) in the human foetal testes xenografts were reported. Exposure to MBP gave similar results to DBP. Rat testes xenografts resulted in reduced testosterone production, SV weights, reduced testis Cyp11a1 and StAR expression, confirming that DBP exposure can inhibit steroidogenesis in xenografts.

The association between foetal testis steroidogenesis and Leydig cell perturbation was also evaluated in rats by examining the inhibition of sterol regulatory element-binding protein (SREBP)-dependent lipid metabolism gene expression. Rats exposed to 500 mg/kg bw/d DBP showed significantly reduced SREBP2 expression in foetal rat testis cells; the reduction was greatest in Leydig cells. Taken together, the data suggest that inhibition of foetal testis steroidogenesis following exposure to DBP is associated with reduced lipid metabolism pathways and SREBP2-dependent cholesterologenesis in Leydig cells (Johnson et al. 2011 **ND**).

In vivo studies in rats

In vivo, DBP treatment did not increase expression of CaBP-9k mRNA (a gene highly regulated by 17β-oestradiol) in seven-day-old female SD rats receiving oral treatment of 600 mg/kg bw/d DBP for three days (Hong et al. 2005).

DBP did not induce oestrogenic responses in vivo in uterotrophic and vaginal cornification assays using immature and mature ovariectomised rats (Zacharewski et al. 1998).

In utero treatment with DBP (0; 0.1; 1; 10; 50 and 500 mg/kg bw/d) from GD 12 to GD 19 in rats was associated with a dose-related reduction of the expression of vital genes and proteins involved in cholesterol transport and steroidogenesis evaluated at GD 19. Foetal testicular testosterone levels were reduced at 50 mg/kg bw/d and above. At 500 mg/kg bw/d (the only dose examined for this endpoint) there was an increase of mRNA levels of different members of the insulin-like growth factor family associated with morphogenesis of Wolffian ducts. There was also a decrease in androgen receptor protein in ductal epithelial cells in some animals at this dose (Lehmann et al. 2004; Bowman et al. 2005).

Testosterone production ex vivo and insl3 gene expression in foetal rat testes was significantly reduced following oral administration of 1000 mg DBP /kg bw/d to pregnant rat dams on GD 14–18 (Wilson et al. 2004). These effects are likely to result in gubernacular malformations and cryptorchidism in rats (Wilson et al. 2004). In another study, similar treatment with a lower dose of DBP (500 mg/kg bw/d) also significantly reduced ex vivo testosterone production and mRNA levels for the steroidogenic acute regulatory protein, StAR (Howdeshell et al. 2007 **ND;** described in detail in the previous section). At this dose, the level of insl3 mRNA was only slightly (statistically not significantly) reduced. Testosterone production ex vivo was also reduced after treatment with 300 mg/kg bw/d, but not with 100 mg/kg bw/d (Howdeshell et al. 2008 **ND**).

Clewell et al. (2009 **ND**) examined the dose dependence and time course of the effect of DBP on foetal steroidogenesis in vivo by measuring testosterone levels in foetal testes homogenates. Pregnant SD rats were treated with a single dose of 500 mg/kg DBP on GD 19, or daily doses of 50, 100, and 500 mg/kg day on GD 12–19 by gavage. Testosterone levels in foetal testes were decreased 24 hours after the single dose of 500 mg DBP/kg bw/d on GD 19, but recovered after 48 hours. Mean changes in testosterone levels were less pronounced after a single dose, compared with repeated dosing with 500 mg/kg bw/day. Reduction was to 40 % of the control 24 hours after the single dose and 19 % 24 hours after the last dose in the repeated-dose protocol. Similarly, 48 hours post-dosing, testosterone levels were reduced to 76 % of the control for the single dose and to 55 % of the control in the repeated-dose protocol. Time course analysis for each repeated DBP treatment dose showed characteristic decrease of the testosterone level followed by recovery. At the low dose (50 mg/kg bw/d) testosterone levels decreased 12 hours after the final dose, while in the two higher dose groups (100 and 500 mg/kg bw/d) it was observed as early as 0.5 hours post dosing. In the low dose group, recovery was faster and reached levels comparable to the control 24 hours after the final dose, while in the

highest dose group, recovery occurred after 48 hours. At the mid dose of 100 mg/kg bw/d, recovery was seen after 24 hours (levels are not reported for 12 hours post dose), while 48 hours post dosing T levels in the foetal testes were statistically significantly higher than the controls. This study did not address the underlying mechanisms leading to decreased testosterone levels, but demonstrates that testosterone levels in the foetal testes is altered in a dose-dependent manner by DBP that exhibit developmental toxicity (see Developmental studies), and that the magnitude of the effect is significantly dependent on the duration of dosing.

Decreases of intratesticular testosterone and mRNA levels for Cyp11a1, Cyp17a1 and Hsd3b steroidogenic enzymes are also reported under particular exposure conditions in other studies with a focus on the developmental effects of DBP (see Mylchreest et al. 2002; Jiang et al. 2007 **ND**; Mahood et al. 2007 **ND**; Xiao-feng et al. 2009 **ND**; Alam et al. 2010 **ND** in Developmental studies**)**.

In vivo studies in primates

The effect of DBP on steroidogenesis was examined in marmosets by Hallmark et al, (2007 **ND**). Vehicle or 500 mg/kg bw/d MBP (the main DBP metabolite) was orally administered to co-twin animals for 14 days starting at postnatal day four. The level of plasma testosterone was not changed at the end of the treatment period. However, a single dose of 500 mg/kg MBP significantly ($p = 0.019$) reduced blood testosterone levels in newborn (day 2–7) male marmosets five hours after administration (1.36 + 0.23 compared with 2.75 + 0.55 in the controls). The authors interpreted this as consistent with initial MBP-induced inhibition of steroidogenesis followed by compensatory Leydig cell hyperplasia/hypertrophy mediated through increased luteinizing hormone (LH) levels. LH levels were not measured, but histological analysis showed increased Leydig cell volume per testes in the sub-chronically treated animals. Overall, the study indicates that DBP/MBP treatment might initially suppress steroidoigenesis which could be compensated at later stages by mechanisms affecting Leydig cell function and morphology. The effect of Leydig cell hypertrophy on reproductive parameters is not addressed by this study.

6.3 Effects observed in humans

6.3.1 Acute poisoning

Cagianut (1954*) reported the following symptoms in a man following accidental ingestion of 10 g DBP: nausea, vomiting, dizziness, lacrimation, photophobia and eye pain. Keratitis erosiva of the cornea was noticed. Urinalysis revealed pathological leucocyte counts, oxalate crystals and microhaematuria. A 14-day mydriatic and antibiotic course resulted in recovery.

6.3.2 Irritation and sensitisation

Oliewiecki et al. (1991*) reported 71-year-old woman suffered recurrent ear infections from a hearing aid. Dermatitis resulted from areas of contact with spectacle frames (behind the ears and on the temples). Patch tests with 5 % DBP, 5 % DMP and 5 % DEP in petrolatum solvent gave positive results in each case. Less positive reactions were seen with scrapings from the spectacle frame or hearing aid.

Calnan (1975*) and Sneddon (1972*) reported two women who suffered dermatitis of the axillae after they used an antiperspirant spray containing DBP. Both women gave positive responses to DBP in a patch test, but not to other constituents of the spray.

Schulsinger and Mollgaard (1980*) found one out of 1532 had a positive reaction after a routine patch test with a phthalate ester mixture (2 % DMP, 2 % DEP and 2 % DBP in petrolatum).

Patch tests were done with cosmetics (nail polish with 6 % or 9 % DBP, or deodorant with 4.5 % DBP) or with 5 % DBP in petrolatum, on 13 to 159 people in 11 different studies, including 48 hours closed patch tests, modified maximisation tests, modified repeated insult

patch tests, 21 day cumulative irritancy tests, prophetic patch tests and controlled use studies (2 day or four week long). In 9/11 studies, no irritation, contact sensitisation or photosensitisation were seen. In 2/11 studies with 9 % DBP in nail polish and 4.5 % DBP in deodorant, with 13 and 12 persons respectively, minor irritation was observed. In these studies, the subjects received twenty-one patches for 23-24 hours on the same side of the back (no further data available) (Cosmetic Ingredient Review Panel, 1985* cited in ECB 2004).

6.3.3 Epidemiology studies

Polyneuropathy

Workers (147) involved in manufacturing artificial leather and chronically exposed to phthalates (mostly DBP and higher molecular weight phthalates but also traces of adipates, sebacates and tricresylphosphate), were investigated for toxicity in a study by Milkov et al. (1973*). Forty-seven workers experienced polyneuritis (frequency increasing within the period of exposure) and 22 had neurofunctional disturbance. Vestibular and olfactory receptor excitability and cutaneous sensitivity were found to be reduced. Ambient vapour or aerosol levels of the plasticisers at the workplace were 1.7–60 mg/m^3. No control group was available.

Male workers involved in producing phthalate esters, including DBP, were investigated for neurological symptoms in a cross sectional study. Twenty three workers were exposed to phthalates, six to phthalic anhydride and nine to alcohols. Mean phthalate concentration varied from 1–5 mg/m^3 with a maximum of 61 mg/m^3. Polyneuropathy was observed in 12 out of 23 subjects exposed to phthalates—bilateral painful decreased sensitivity of skin or senses of the hands and feet were observed in seven of these 23 subjects (three showed reduced sensitivity to vibration). In the alcohol-exposed group, two out of nine showed sensory neuropathy, and one out of six showed hyporeflexia in the anhydride exposed group (Gilioli, Bulgherain Terrana et al. 1978*).

Fertility

DBP was reported to induce hormonal changes leading to decreased fertility and menstrual disruptions in a cross-sectional study in women (189) working in conditions involving DBP exposure. However, quantitative data were unavailable and the women were also exposed to other unknown compounds (Aldyreva et al. 1975*).

In vitro incubation of human sperm suspensions with DBP (0, 0.4, 4, 40 mM) for up to 18 hours showed a dose-dependent decrease in the mean motility and straight-line motion at doses higher than 0.4 mM (60 % motility at 4 mM compared with control) (Fredricsson et al. 1993).

There is limited evidence in humans associating MBP with effects on sperm motility in vitro and in vivo. Duty et al. (2003) examined the association between the levels of phthalate monoesters in urine, and in semen quality. In this study 168 males, aged 20 to 54 years, were recruited from sub-fertile couples. The comparison group comprised men with all three semen parameters above the WHO reference values (value above the reference value indicates normal sperm parameters). Eight urinary phthalate monoesters were measured in a single spot urine sample collected on the same day as the semen sample. A wide distribution of the levels of phthalate monoester metabolites was found in semen and therefore, for the correlation analysis, the levels were dichotomised using the median for each metabolite. Analysis showed that 40 % of men in the comparison group had MBP levels above the median level, compared with 60 % of men in the low-motility group. Age and abstinence time were taken into account in the covariate analysis. Furthermore, MBP levels above the median were found to be associated with lower values for semen parameters (below the WHO reference values) with odds ratios (OR) and 95 % confidence interval (in brackets) of 2.4 (1.1–5.0) for sperm motility, 2.4 (0.80–7.2) for sperm concentration, and 1.7 (0.8–3.9) for sperm morphology. Following categorisation of the phthalate metabolite levels into tertiles, a direct dose-response relationship was found between MBP levels, below reference level sperm concentration (OR per tertile 1.0, 1.4 and 3.3; P for

trend 0.07) and for sperm motility (OR per tertile 1.0, 1.8 and 3.0; P for trend 0.02). A non-significant inverse association between urine MBP levels and sperm velocity was observed (Duty et al. 2004).

Similar results were obtained by the same group with an extended sample of 463 males, including the sample form the previous study (Hauser et al. 2006 **ND**). In this study the odds ratio per quartile of MBP level for sperm concentration was 1.0, 3.1, 2.5, 3.3 (P for trend 0.04) and for sperm motility or per quartile was 1.0, 1.5, 1.5, 1.8 (P for trend 0.04). Neither study found a significant association with metabolites of any of the other phthalates. Given that the studies were performed with subjects that presented to the collection centre for reasons of suspected infertility, the results may not be representative of the general population.

However, in another study with 234 military recruits, Jonsson et al. (2005) found no significant associations between highest versus lowest urinary MBP quartile and any of the semen parameter variables.

Semen samples from volunteers categorised as fertile (100 men) and infertile (200 men) were collected to investigate the effects of phthalates including DBP (Pant et al. 2008 **ND**). Phthalate levels (DEP, DBP and DEHP) were significantly higher in infertile than in fertile men. The DBP levels were 0.18 and 1.65 µg/ml in fertile and infertile men, respectively. A positive correlation of DEHP and DBP in semen with abnormal sperm or DNA fragmentation index was reported. The percentage of sperm cells with depolarised mitochondrial membrane was significantly higher in the infertile (maximum 63 %) compared with fertile (17 %) group. There was a negative association of DEHP and DBP in semen with sperm motility. There was an inverse correlation between DBP and sperm concentration.

In a more recent study by Pant et al. (2011 **ND**), the effects of DBP in human male volunteers were investigated in vivo and in vitro. A total of 180 men were selected for the in vivo study (65 oligoaesthenospermic—decreased number of sperm, 65 aesthenospermic—decreased sperm motility, and 50 fertile men). For the in vitro study, semen samples from 12 volunteers were incubated with the highest concentration (13.47 µg/mL) of DBP found in the semen samples (DBP1), together with five times higher (DBP2) and 10 times higher (DBP3) concentrations. Sperm viability and motility were assessed for 96 hours.

In vivo, the maximum DBP concentrations were found to be 13.47 µg/mL (oligoaesthenospermic), 4.11 µg/mL (aesthenospermic) and 0.80 µg/mL (fertile), respectively. A significant negative association between sperm motility and DBP levels was also reported. In vitro, DBP caused duration-dependent decrease in sperm viability. At 24, 48, 72 and 96 hours, sperm viability varied from 80 % to 90 %, 75 % to 89 %, 52 % to 72 % and 42 % to 65 %, respectively). Concentration-dependent decrease in sperm motility was reported. At low, mid and high doses, declines of 20 %, 33 % and 60 % were recorded after 18 hours' exposure, respectively. At 13.47 µg/mL (maximum measured DBP level in vivo), 58 % inhibition of sperm motility was observed after 48 hours treatment.

Endometriosis

Reddy et al. (2006 **ND**) conducted an analysis of plasma levels of several phthalates (not metabolites) in 85 infertile women with endometriosis, compared with 135 age-matched fertile control women undergoing laparoscopic sterilisation in the same hospital. Mean plasma DBP (and DEHP) levels in women with endometriosis were at least three times higher than levels in controls. Differences were statistically significant. The correlation between the concentrations of DBP (and DEHP) and the severity of endometriosis was also strong and statistically significant.

Huang et al. (2010 **ND**) examined the correlation between levels of urinary monoester metabolites of phthalates, including DBP, and the occurrence of endometriosis, adenomyosis and leiomyoma. A total of 109 women were examined in four groups: three groups with specific

diagnosis and a control group that underwent laparotomy for unspecified clinical reasons. None of the women were found to have any of the three diagnoses. The median level (creatinine adjusted) of MBP and one DEHP metabolite were significantly higher in the group with endometriosis compared with the control group, while MEP levels were similar between the groups. The correlation with the other two diagnoses was not significant.

In contrast, a study by Itoh et al (2009 **ND**) found no correlation between urinary levels of DBP (and DEHP) metabolites and endometriosis. The study included 137 women, 50 cases and 80 controls, where the control group included women with negative diagnosis as well as stage I of endometriosis.

In all of these studies the sample size was quite small, with subjects from a single sampling centre. In addition, occupational exposure was rarely considered and the measurements of phthalates done at the time of diagnosis may not reflect historical habitual exposure.

Wauve et al. (2010 **ND**) conducted an analysis of the urinary metabolite levels of several phthalates in relation to a self-reported history of endometriosis and uterine leiomyomata among 1,227 women (20–54 years of age) from the National Health and Nutrition Examination Survey (NHANES) 1999–2004, a cross-sectional national survey designed to collect data on the health and nutritional status of the civilian, non-institutionalised U.S. population. Only primary metabolites of DBP, DEP and BBP (MBP, MEP and MBzP) were analysed, while for DEHP, the primary metabolite MEHP and two oxidative metabolites analysed were also considered.

The odds ratios comparing the highest versus lowest three quartiles of urinary MBP were 1.36 (95 % CI, 0.77–2.41) for endometriosis, 1.56 (95 % CI, 0.93–2.61) for leiomyomata, and 1.71 (95 % CI, 1.07–2.75) for both conditions combined. In contrast, inverse association (ORs <1) were observed for MEHP, MEHHP and MEOHP for both conditions. No significant associations were observed for MEP and monobenzyl phthalate (MBzP).

While more comprehensive, this study is not sufficient to establish a causative relationship between MBP (or by inference DBP exposure) and endometriosis or leiomyomata in the general population, for which further investigation in prospective studies is needed.

Gonadal and thyroid hormones

Pan et al. (2006) measured the gonadotrophins 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 vs 129.6 μg/g; MEHP 565.7 μg/g vs 5.7 μg/g). Circulating testosterone was significantly lower in exposed workers (8.4 μg/g) compared with control workers (9.7 μg/g) and was negatively correlated with MBP and MEHP levels.

Meeker et al. (2007 **ND**) examined the relationship between the urinary levels of MBP (and also the primary metabolites of DEP, BBP and DEHP) and blood levels of free thyroxine (FT4), total triiodothyronine (T3) and thyroid stimulating hormone (TSH) in 408 men of subfertile couples seeking evaluation in one fertility centre in the USA. No significant direct or inverse correlation was found for MBP and the primary metabolites of the other phthalates except for DEHP (detils described in NICNAS 2010), where inverse correlation was apparent between urinary MEHP levels and blood levels of FT4 and total T3, but not TSH. However, correlation was much weaker when oxidative metabolites of DEHP (MEHHP and MEOHP) were considered.

Meeker and Ferguson (2011 **ND**) used the data from a representative sample of U.S. adults (n = 1346, aged >20 years) and adolescents (329, aged 12–19) from the NHANES 2007–08 survey (NCHS 2010), a cross-sectional study/survey designed to collect representative data on dietary intake and disease of the civilian non-institutionalised population in the U.S. The analysis examined the relationship between urinary concentrations of metabolites of DBP and DEHP with a panel of serum thyroid measures in the sampled population (Meeker and Ferguson

2011 **ND**). There was no significant association between DBP metabolites and any thyroid measures considered in the survey. For DEHP metabolites, association was reported for mono(3-carboxypropyl) phthalate (MCPP) with several thyroid measures in both age groups, however, MCPP is also a metabolite of di-n-octyl phthalate (DNOP), and the significance of this association is not clear even for DEHP.

Huang et al. (2007 ND) examined the association between urinary levels of MBP (and the primary metabolites of four other phthalates including DEP and DEHP) and serum levels of TSH, T3, T4 and free T4 (FT4) in pregnant women. The study cohort consisted of 76 pregnant women that came to hospital to undergo recommended amniocentesis due to advanced age or abnormal levels of alpha foetal protein and beta-human chorionic gonadotropin (free β-hCG). Analysis showed that out of the five analysed phthalate metabolites, MBP, MEP and MEHP were the highest in the urinary samples. After adjusting for age, BMI and duration of gestation, significant mild negative correlations were found only between urinary MBP levels and serum T4 and FT4. No measurements were performed on the offspring of this cohort. However authors found significant negative correlation between amnionic MBP levels and anogenital index (AGD normalised to newborn's body weight and length) in female but not in male newborns (Huang et al. 2009 **ND**). There was a positive correlation between the serum and amnionic MBP levels. The significance of these findings is unclear particularly in the absence of analysis of the hormonal status in cord blood or serum of newborns.

Overall, these studies do not identify significant associations between MBP and parameters of thyroid function.

Reproductive system development

Main et al. (2006) reported phthalate concentrations in pooled human breast 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. The phthalate monoesters MBP, MEHP, mono-methyl phthalate (MMP), monoethyl phthalate (MEP), monobenzyl phthalate (MBzP) and mono-isononyl phthalate (MINP) were measured in breast milk and gonadotrophins, sex-hormone binding globulin (SHBG), testosterone, and inhibin B in the serum of boys that were breast-milk fed. Cryptorchidism was identified in 62 of the 130 children. However, there was no significant association between phthalate concentrations in human breast milk and cryptorchidism. MBP levels in breast milk showed direct correlations with levels of SHBG and LH/free testosterone ratio; however, there was inverse correlation of MBP levels in breast milk with free testosterone.

Association between 11 maternal urinary phthalate monoesters and genital parameters such as anogenital index (AGI), i.e. anogenital distance (AGD) normalised for body weight, and testicular descent in children, was investigated in 85 mother-son pairs (Swan et al. 2005). There was inverse correlation between AGI and the urinary concentrations of MBP, also observed for three other phthalate metabolites, (MEP, MBzP and monoisobutyl phthalate—MiBP) but not for MEHP. 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 normalised for urine volume.

The reliability of the measurement of AGD in humans has not been verified. A study assessing the correlation of AGD with body weight in 87 male neonates found that body length might be a slightly better predictor for AGD than weight (Salazar-Martinez et al. 2004).

The results from the study by Swan et al. (2005) were later subjected to a new statistical analysis including new mother-son pairs, a total of 106 pairs, and results from AGD measurements over two visits for 68 of the original pairs (second visit 12.8 months post delivery) (Swan 2008 **ND**). In the new analysis, AGI 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 individual weight and, when included as a covariate in the analysis, it eliminates the confounding influence of weight. The new AGI was correlated with the prenatal exposure to phthalates estimated through the maternal urine metabolite as in the previous study. It was found that there was a statistically significant inverse correlation of the AGI and MBP as well as the three DEHP metabolites measured.

For further analysis, the infants' cohort was divided into three groups based on the difference between the AGD expected for the particular age/weight, and measured AGD. The resulting groups: longer, intermediate and shorter AGD, were then analysed in relation to the corresponding maternal metabolite levels. It was found that the levels of the three DEHP metabolites in the longer AGD group were several times greater than those in the shorter group. The significance of this finding is not clear, and no such relationship was applicable in the case of the DBP metabolite, MBP.

Behavioural and cognitive parameters

Recent studies have examined the relationship between estimated phthalate exposure in utero or during the latter stages of children's development, and complex multifactorial behavioural and cognitive parameters such as IQ.

In a pilot study by Swan et al. (2009 ND) 74 male and 71 female five-year-olds were followed-up to examine the relationship between gender-associated play behaviour and phthalate metabolite concentration in the urine samples of their mothers during mid-pregnancy. Play behaviour was assessed and scored based on a Preschool Activities Inventory (PSAI), a standardised questionnaire completed by the mothers. A higher PSAI score is postulated to imply more male-typical play behaviour; a lower score implies more-female typical play behaviour. Covariates such as a child's age, the mother's age and education and parental attitude towards atypical play choices (assessed through a separate, non-standardised questionnaire) were included in the multivariate regression analysis. Resulting regression coefficients indicated an inverse association between maternal urinary MBP (and the sum of MBP and MiBP) concentration and the score on the composite PSAI questionnaire, indicating a less masculine play in boys. Similar coefficients were calculated for boys for the oxidative metabolites of DEHP, MEHHP, MEOHP, but not for MEHP and the metabolites of other examined phthalates including MEP and MBzP. Weak and statistically non-significant variations in composite PSAI scores were also calculated for females. The authors concluded that the findings suggest prenatal exposure to phthalates could be associated with less male-typical play behaviour in boys through the phthalate potential to alter androgen-responsive brain development in humans. The hormonal status of the subjects was not examined at the stage of behavioural assessment or during development. Although sex hormones are significant in foetal sexual differentiation, it is unclear to what extent they can influence this type of behaviour later in life. More extensive studies are needed to further examine the association between phthalate exposure and complex behaviours.

A study by Cho et al. (2010 **ND**) examined urine concentrations of phthalates (in 667 children at nine elementary schools in five South Korean cities) and their association with scores on neuropsychological tests. This cross sectional study indicated no significant relationship between MBP and the full scale IQ and verbal IQ scores after adjusting for demographic and developmental covariates.

Kim et al. (2011) investigated the neurodevelopment of infants from 460 mother–infant pairs in three major cities in South Korea. Two metabolites of DEHP (MEHHP and MEOHP) and one metabolite of DBP (MBP) were measured from urine samples collected from each mother during the first trimester of pregnancy (range of gestational age at urine collection: 35.7–41.7 weeks). Mental and psychomotor indices (MCI and PDI) of infants were measured by the Korean Bayley Scales of Infant Development (BSID-II) at six months. Developmental indices

were made up of composite scores that compare developmental performance of a child with the norms taken from typically developing Korean children of the same age. In this study, prenatal exposure to DBP and DEHP were inversely associated with MCI and PDI of male infants at six months.

7. 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 NOAELs, and discusses the relevance of the effects observed in animal studies to humans.

Given that there is limited information available from human studies on the potential health effects associated with exposure to DBP, the hazard profile is based principally on animal data. In addition, for those toxicological end points where the data are incomplete or unavailable, information from structurally similar phthalates was used to examine the potential toxicity. This information was obtained from other NICNAS assessment reports for relevant phthalates. The NICNAS Phthalates Hazard Compendium (NICNAS 2008b) contains a comparative analysis of toxicity end points across 24 ortho-phthalates, including DBP. DBP has a predominantly 4- to 6-carbon backbone and belongs to a mid molecular weight phthalate group, also known as transitional phthalates (The Phthalate Esters Panel of the American Chemistry Council 2001 & 2006 revised; OECD 2004).

7.1 Toxicokinetics

In laboratory animals (rats and hamsters), DBP is rapidly absorbed and excreted after oral administration, with ≥90 % excreted in the urine within 24–48 hours. Faecal excretion is low (1.0–8.2 %). DBP is also excreted in the bile and consequently enters the enterohepatic circulation (Williams & Blanchfield 1975*; Tanaka et al. 1978*; Foster et al. 1983*). Limited data in humans also indicate that DBP is absorbed after oral exposure (Tanaka et al. 1978*). Therefore, the bioavailability of DBP via the oral route in humans is considered to be 100 % for both adults and children.

Absorption via the dermal route in rats was estimated at around 10–12 % per day over seven days (Elsisi et al. 1989). In vitro, absorption of DBP through epidermal rat skin preparations is about 40 times greater than through human skin preparations (Scott et al. 1987). These data indicate that dermal absorption of DBP in humans is not likely to exceed 2 %. However, this might vary depending on the conditions and formulation in which DBP is applied to the skin. Recent human studies with dermal application of cosmetic lotion formulations containing DBP are consistent with the estimated 2 % dermal absorption of DBP via the human skin. However, significant interindividual and daily variations were observed, with a maximum dermal absorption in volunteers corresponding to approximately 6 % of the applied DBP dose (Janjua et al. 2007, 2008). Based on all data available for DBP, a 5 % bioavailability for DBP is estimated for humans through dermal exposure.

There are limited data regarding DBP absorption via the inhalation route. One inhalation study suggests some accumulation in tissues following inhalation exposure in rats, indicating that DBP might be absorbed via the inhalation route (Kawano et al 1980a*). However, in this study only DBP (and not metabolites) was measured. In the absence of sufficient data, a default of 100 % absorption via the inhalation route is considered appropriate for DBP for risk characterisation.

No significant accumulation of DBP in tissues was seen in laboratory animals after oral and dermal exposure.

DBP is mostly hydrolysed to MBP before absorption by the small intestines. DBP hydrolysis can also occur in liver and kidneys. Metabolites in urine include MBP, MBP-glucuronide, various ω- and ω-1-oxidation products of MBP (more polar ketones and carboxylates) and a small amount of phthalic acid. No data on biotransformation after dermal or inhalation exposure are available.

Placental transfer studies revealed that DBP and its metabolites, MBP and MBP-glucuronide, are rapidly transferred to embryonic tissues without significant accumulation in the placenta or foetal tissues (Saillenfait et al. 1998*; Clewell et al. 2009).

7.2 Acute toxicity

DBP has low acute oral, dermal and inhalation toxicity in rodents with LD_{50}s above 4000 mg/kg bw for oral and above 20,000 mg/kg bw for dermal exposure. LC_{50} for inhalation exposure in rats is ≥15.68 mg/L/4h. Intravenous and intraperitoneal administration of DBP results in higher acute toxicity than oral or dermal administration. The lowest LD_{50} for intravenous exposure in mice is 720 mg/kg/bw. Significant acute toxicity studies are tabulated in Table 6.1 in Section 6.2.1.

One case report of acute poisoning in humans, due to accidental ingestion of 10 g DBP, showed that the symptoms of the poisoning were completely reversible by 14 days.

Overall, DBP is not expected to have significant acute toxicity in humans.

7.3 Eye and skin irritation and sensitisation

DBP shows only minimal skin and eye irritation potential in studies conducted according to OECD guidelines with rabbits. Respiratory irritation was also minimal in rats exposed to DBP.

DBP did not show skin sensitising properties in animal studies including two maximisation tests in guinea pigs. No data are available for respiratory sensitisation.

Available human data are limited and ambiguous. Case studies where patients reported dermatitis of skin areas in contact with plastic medical devices (hearing aid) or cosmetic products (antiperspirant spray) showed positive results in patch tests to DBP, but also to other phthalates (Sneddon 1972*; Calnan 1975*; Oliewiecki et al. 1991*). However, an analysis of a number of other studies with higher numbers of subjects and using cosmetic products (nail polish or deodorant) containing up to 9 % DBP, reported minor skin irritation and the absence of contact sensitisation or photosensitisation. Evaluation was done using 21-day cumulative irritancy tests, prophetic patch tests and controlled use studies (Cosmetic Ingredient Review Panel 1985* cited in ECB 2004).

Overall, DBP is not expected to have eye or skin irritation, or skin sensitising potential, in humans.

7.4 Repeated dose toxicity

Most studies examining subchronic and chronic toxicity of DBP are performed in rodents and involve dosing via the oral route. There are no reliable studies that involve repeated dermal exposure to DBP. In an inhalation study (Gamer et al. 2000*) performed according to OECD Guidelines 412 and 407 (for clinical and neurofunctional examinations and pathology) with Wistar rats, no systemic effects (including neurotoxicity) were seen at up to and including the highest dose of 509 mg/m^3. Dose-dependent changes were localised in the nasal cavity and were considered to be adaptive. The systemic NOAEC was established as 509 mg/m^3. The LOAEC for local adaptive effects in upper respiratory tract was 1.18 mg/m^3.

The major adverse effects of DBP in the repeated oral dosing studies are related to liver and testicular toxicity. Increased kidney weight is also reported in some studies for both sexes or only in females, but not consistently through all studies and generally without any related histopathological changes.

7.4.1 Liver effects

Liver toxicity observed in rodents ranges from adverse effect on organ weight to histopathological changes such as degeneration of liver parenchyma, and biochemical changes

including activation of fatty acid metabolising enzymes, alterations in fatty acids associated with activation of peroxisome proliferator-activated receptor α (PPARα), and increased peroxisome proliferation (summarised in Table 6.2).

The lowest NOAEL of 19.9 mg/kg bw/d was identified in a subchronic, two-week dietary study in rats, based on the activation of enzymes associated with peroxisome proliferation (LAH-11 and LAH-12 (11- and 12- lauric acid hydroxylase, respectively) at 60.6 mg/kg bw/d. In this study PCoA activity was also increased at higher doses (Jansen et al. 1993*).

Studies with phthalates using PPARα-null mice showed that the liver effects induced by DBP, and the structurally similar phthalate DEHP, in rodents (hepatomegaly, peroxisome proliferation) are largely mediated through activation of PPARα (Ward et al. 1998; Lapinskas et al. 2005).

Available studies with humans do not indicate any association between DBP exposure and liver toxicity.

Generally, studies with other hypolipidaemic agents in humans provide 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 is thought to be due to the decreased tissue levels of PPARα, genotypic variations rendering the primate liver receptor less active compared with rodents, and species differences in phthalate hydrolysis and production of active phthalate metabolites (Tugwood et al. 1996; Palmer et al. 1998; Woodyatt et al. 1999).

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

7.4.2 Testicular effects

Several studies specifically examined testicular effects of DBP in rodents.

At doses of 500 mg/kg bw/d and higher, decreased weight of testes and accessory organs associated with spermatocyte depletion, seminiferous tubule degeneration, decrease in testicular zinc and serum testosterone levels, increase in testicular testosterone levels and urinary zinc excretion are reported (Cater et al. 1977*; Oishi & Hiraga, 1980a*; Gray et al. 1982*, Gray, Laskey, Ostby et al. 1983*; Srivastava et al. 1990*). These effects show a high degree of reproducibility across a range of studies and are also observed in the reproductive toxicity studies described in Section 7.6.

The lowest LOAEL of 250 mg DBP/kg bw/d is identified in a 15-day dietary study with young male rats, based on a significant decrease in testes weight at 500 and 1000 mg/kg bw/d that was associated with marked degeneration of seminiferous tubules. No NOAEL could be established in this study as significant alterations were observed in the activity of a number of testicular enzymes associated with specific stages of the spermatogenesis (Srivastava et al. 1990*).

Guinea pigs appear to be less sensitive than rats as testicular effects are observed at much higher doses. However even short-term, 7-day treatment with 2000 mg/kg bw DBP by gavage was associated with severe testicular effects, including severe tubular atrophy with loss of spermatids and a reduction in primary spermatocytes and spermatogonia (Gray et al. 1982*). Similar treatments did not cause significant effects in mice and hamsters. However, longer dosing (55 days) in hamsters with 500 mg/kg bw/d and 1000 mg/kg bw/d was associated with a marked effect on testes size and viability and, at the higher dose, growth of offspring (Gray, Laskey, Ostby et al. 1983*).

The species-specific differences in testicular toxicity have been partly attributed to differences in the ratio of free, unconjugated primary metabolites of DBP, MBP (considered to be the active

component for toxicity) to MBP-glucuronide in rats and hamsters (Tanaka et al. 1978*; Oishi & Hiraga 1980c*; Foster et al. 1981*, 1983*; Zhou et al. 1990*).

Studies in humans do not indicate significant association of DBP exposure with testicular toxicity. However, some indicate possible association of DBP exposure and alterations in gonadotrophin hormones or indicators of reproductive system development. These studies are discussed in more detail in the following sections, together with the plausible mode of action for testicular toxicity in animals and relevance for humans.

7.5 Genotoxicity and carcinogenicity

DBP is considered to be non-genotoxic based on the weight-of-evidence showing no genotoxicity for DBP in most in vitro and all in vivo tests performed to standard testing guidelines (IPCS 1997; Kleinsasser et al. 2000; ECB 2004). The in vivo tests include sex-linked recessive lethal test in Drosophila and micronucleus assay (according to OECD 474 and comparable standards) in NMRI and B6C3F1 mice.

No adequate long-term carcinogenicity studies with DBP in laboratory animals are available.

Based on the information available for genotoxicity, DBP is not genotoxic and is not likely to be a genotoxic carcinogen. Moreover, in several in vitro cell transformation assays, DBP did not induce cell transformation (Nuodex 1982; Litton Bionetics 1985*; Barber et al. 2000).

However sub-chronic exposure to DBP in rodents (Section 6.2.4) is associated with the activation of fatty acid metabolising enzymes leading to activation of peroxisome proliferator-activated receptor α (PPARα), a non-genotoxic effect associated with liver carcinogenicity in rodents. Moreover, it has been shown that peroxisome proliferation induced by DBP (and DEHP) in mice is dependent on PPARα (Lapinskas et al. 2005). As discussed in the previous section, activation of PPARα is known to be associated with induction of hepatocellular tumours by certain non-genotoxic hypolipidaemic substances in rodents but not in humans (Lee et al. 1995; Peters et al. 1997).

DBP is not considered to be carcinogenic to humans when taking into account that the mechanisms by which DBP and hypolipidaemic substances induce peroxisome proliferation in rodents are not considered relevant to humans, and the absence of evidence associating DBP exposure to carcinogenic effects in humans.

7.6 Reproductive toxicity

As described at the beginning of section 6.2.7, the reproductive toxicity studies are organised on the basis of test procedure, mainly timing of exposure (adult, gestation or early postnatal). The effects on fertility (as adults) and development (as foetuses or early in postnatal development) are then discussed separately, to the extent possible. Fertility is tested by exposing sexually mature adults to a chemical and examining the effects on reproductive capacity. Developmental toxicity is studied by exposing pregnant dams and looking for effects on the foetuses. Chemicals like DBP that affect the developing reproductive system following prenatal exposure, also affect sexual maturation or produce functional reproductive disorders that might only be apparent at maturity. Developmental toxicity can therefore lead to effects on fertility and the two end points are clearly interdependent.

The reproductive and developmental toxicity of DBP in rodents was exhibited as perturbations in testicular structure and function, altered steroidogenesis and developmental malformations of the urogenital tract. Decreased anogenital distance (AGD) and nipple retention were also observed in male animal rodents. Overall, rats are the most sensitive to DBP toxicity, followed by mice and hamsters. Reproductive effects in mammals have not been examined extensively and limited studies with marmosets, mainly sexually mature animals, are not sufficient for robust assessments of DBP reproductive toxicity in mammals as compared to rodents.

7.6.1 Fertility

In adult rodents, repeated exposure to DBP doses of 500 mg/kg bw/d and higher is consistently associated with distinct testicular changes, including decreased organ weight and histopathological perturbations in the testes, indicative of testicular degeneration (Section 6.2.4 and Table 6.2) likely to lead to adverse effects on fertility.

Fertility in rodents is also adversely affected by DBP through toxicity in females, although often in the absence of overt histopathological changes in the reproductive tract (Wolf et al. 1999; Gray et al. 2006).

Continuous exposure of LE hooded female rats to DBP, starting from weaning continuing through adulthood and during crossmating with untreated males, showed clear effects on reproductive parameters (reduction of the percentage of females delivering live pups and reduction of live pups per litter) at 500 mg/kg bw/d. Ex vivo progesterone and oestradiol production by the ovarian cultures in these females was also altered at the same dose. The NOAEL for fertility in female rats in this study is 250 mg/kg bw/d and the LOAEL is 500 mg/kg bw/d, based on decreased fertility in the P0 generation (Gray et al. 2006). F1 animals were not examined in this study.

In the study by Wolf et al (1999) with LE hooded rats, fertility was monitored for both females and males (crossover mating to untreated animals). Fertility was decreased at 500 mg/kg bw/d for both sexes. The LOAEL for fertility in this multigenerational study is established at 250 mg/kg bw/d, based on reduced fertility in both sexes in the P0 generation and also decreased epididymal sperm counts in F1 observed at the same, lowest tested dose.

Fertility of males exposed to DBP only through gestation (GD13.5–21.5) and mated to untreated females was also affected (Mahood et al. 2007). In this study, primarily aimed at assessing developmental toxicity of DBP, adverse effects on fertility of the males (F1) treated during gestation was observed at 500 mg/kg bw/d. At the same dose, the testis weight in the F1 adults was also significantly decreased. However, a NOAEL for male fertility can not be established with certainty in this study because a statistically non-significant, but notable, decrease of fertility was also observed at lower doses and it correlated with significant testicular toxicity in the adult F1 animals (increase of dysgenic areas at 100 and 500 mg/kg/d).

In a continuous breeding dietary study with both male and female SD rats exposed to DBP (NTP 1995*; Wine et al. 1997), the LOAEL for fertility and embryotoxicity was at the lowest tested dose of 52–80 mg/kg bw/d (males-females), based on the decreased total number of live pups per litter following breeding of the F0 generation. This adverse effect was observed in the absence of maternal toxicity (observed only at the highest dose). In addition, no histopathological changes in the reproductive tract in F0 animals (males or females) or change in the average number of litters per pair were observed at any dose.

In this study, fertility indices (percentage of females with plug, pregnant and fertile) were significantly decreased at the highest dose (509–794 mg/kg bw/d for males–females) for the F1 but not for the F0 generation, suggesting greater sensitivity of the generation dosed from gestation, compared with F0, dosed seven days premating at adulthood (NTP 1995*; Wine et al. 1997).

Mice appear to be less sensitive than rats. The NOAEL for fertility, parental and embryotoxicity was 420 mg/kg bw/d. and the LOAEL was 1410 mg/kg bw/d in a 14-week dietary study with CD-1 mice (Lamb et al. 1987*; Morrissey et al. 1989*).

Studies in humans examining association of DBP exposure and effects on fertility are limited in number and quality, and without consistent results.

For males, the end points most examined to assess effects on fertility are related to sperm parameters such as sperm motility, morphology and velocity. Some studies involve in vitro

exposure of sperm suspensions with DBP. In one such study (Fredricsson et al. 1993), a dose-dependent decrease in the mean motility and straight-line motion was observed at concentrations of 4 mM and above. However, it is unclear what the relationship of this treatment concentration is to the in vivo exposure dose, and the mechanism of action for such an effect is not addressed. In the two studies by Pant et al. (2008, 2011), there were concentration-dependent decreases in sperm motility, duration-dependent decreases in sperm viability, and positive correlations of DBP in semen with abnormal sperm or DNA fragmentation index. These results, together with in vivo studies, suggest that DBP can act directly on sperm.

There is limited evidence in humans associating MBP with effects on sperm motility. Duty et al. (2003, 2004) found that lower values for semen parameters (below the WHO reference values) were associated with higher urinary levels of MBP in a study group of 168 males. Similar results were obtained by the same group with an extended sample of 463 males (Hauser et al. 2006). In both studies, no significant association was found with metabolites of any of the other phthalates. Given that the studies were performed with subjects who presented to the collection centre for reasons of suspected infertility, the results may not be representative of the general population. However, because they are indicative of negative association between MBP and sperm quality, further studies and closer examination of the confounding factors are needed.

In contrast to the studies above, Jonsson et al. (2005) found no significant associations between highest versus lowest urinary MBP quartile values and any of the semen parameters studied in group of 234 military recruits.

Effects of DBP exposure on testosterone levels in adult males have also been examined. Pan et al. (2006) found that urinary MBP and MEHP levels (normalised to creatine) in 74 male workers in a PVC factory were significantly higher compared with 63 controls, and that serum testosterone was significantly lower in exposed workers compared with the controls.

Studies examining the relationship between DBP exposure and fertility in women focus on end points such as hormonal status, vaginal cycle disruptions, and occurrence of uterine conditions associated with decreased fertility, such as endometriosis.

One cross-sectional study, with 189 women working in conditions involving DBP exposure, concluded that DBP induces hormonal changes leading to menstrual cycle disruptions and decreased fertility. However, quantitative data were unavailable and the women were also exposed to other unknown compounds (Aldyreva et al. 1975*).

Two recent studies report significantly higher plasma levels of DBP and DEHP (Reddy et al. 2006) or urinary levels of their metabolites (Huang et al. 2010) in women diagnosed with endometriosis compared with controls. No significant association was found in women diagnosed with two other related conditions: adenomyosis and leiomyoma (Huang et al. 2010).

In contrast, a study by Itoh et al. (2009) with 137 women (50 with endometriosis and 80 controls), found no correlation between urinary levels of DBP (and DEHP) metabolites and endometriosis.

In all studies, the sample size was quite small and from a single sampling centre. In addition, occupational exposure was rarely considered and the measurements of phthalates done at the time of diagnosis may not reflect historical habitual exposure. Therefore, additional prospective studies are warranted.

In a more comprehensive cross-sectional study on 1227 women from the National Health and Nutrition Examination Survey (Wauve et al. 2010), a positive correlation was found between the urinary MBP metabolite and self-reported history of endometriosis and uterine leiomyoma. In contrast, an inverse association was observed for MEHP, MEHHP and MEOHP for both conditions. No significant associations were observed for MEP and MBzP. While more comprehensive, this study is not sufficient to establish a positive causal relationship between

MBP (or by inference DBP exposure) and endometriosis or leiomyoma in the general population, for which further investigation in prospective studies is needed.

In summary, evidence in rodents constantly shows that DBP adversely affects fertility. The mechanism of toxicity involves overt effects on the reproductive tract organs in males. However, in female rodents, fertility is decreased even in the absence of obvious genital organ toxicity although the potential for progesterone and oestradiol production was shown to be altered in the ovarian cultures of the affected females in one study where this endpoint was measured. Testosterone synthesis is also affected by DBP in male rodents and this is particularly demonstrated in multigenerational and developmental studies (see below). Studies with humans are limited and often contradictory. They do not directly assess fertility but evaluate associations between indicators of DBP exposure, such as DBP or MBP levels in serum or urine, and parameters linked with (in)fertility such as sperm quality, testosterone levels and endometriosis.

Overall, the toxicity of DBP on fertility in rodents is similar to the related phthalate DEHP (NICNAS 2010), as it is mediated through similar adverse effects to the reproductive tract organs and perturbations in oestrogen and androgen synthesis, a mechanism of reproductive toxicity also relevant for humans.

7.6.2 Developmental toxicity

Prenatal

Numerous studies with rats exposed to DBP during gestation show toxic effects on foetal and postnatal development, particularly on male reproductive organs (see Appendix 1).

One of the lowest NOAELs for developmental toxicity following prenatal exposure is 20 mg DBP/kg bw/d, based on reduced testosterone levels correlated with significant testes dysgenesis at the LOAEL dose of 100 mg/kg bw/d (Mahood et al. 2007**).** In the other studies a NOAEL could not be determined as the LOAEL was at the lowest tested dose, except in the study by Mylchreest et al. 2000, where NOAEL was 50 mg/kg bw/d, based on increased seminiferous tubule atrophy and retained nipples at 100 mg/kg bw/d.

Higher doses of DBP given to rats during gestation in these studies also increase the incidence of cryptorchidism and hypospadias in the male offspring.

In a study with SD rats focused on details of histopathological effects (Mylchreest et al. 2002), exposure to 500 mg DBP /kg bw/d during gestation (GD 12–21) was associated with Leydig cell hyperplasia and an increased number of proliferating cell nuclear antigen (PCNA) positive Leydig cells in the foetal testes. The treatment was also associated with a decrease in testicular testosterone. At GD 21, testis atrophy was apparent, seminiferous cords were enlarged and contained PCNA-positive multinucleated gonocytes.

Persistent decrease in androgen concentration is consistent with the occurrence of reproductive tract malformations, as observed in a number of studies where hormone status was not examined. Sertoli cell dysfunction is also indicated by the alterations in gonocyte development (multinuclearity and hyperproliferation).

Studies that focused on examining the critical window of exposure significant for toxicity show that even a short, two-day, exposure to DBP during a critical window of development is sufficient to induce permanent developmental abnormalities. In SD rats, exposure to 500 mg DBP/kg bw/d on GD 15–16 and GD 18–19 was associated with decreased AGD, while retention of areolar nipples was observed in male offspring following exposure on GD 16–17. For increased epididymal malformations and incidences of small testes, two-day exposure on GD 17–18 was sufficient (Carruthers and Foster 2005).

In Wistar rats, DBP treatment from e13.5–e19.5, but not during late development (e19.5–21.5), critically affects development of rat testes. Toxicity exhibited as occurrences of Sertoli cells

outside the normal seminiferous tubules and intermingled with Leydig and interstitial cells, and significant reduction of Sertoli cell numbers at foetal testes at e21. However the latter effect appeared reversible by postnatal day 25 in scrotal testes (Hutchison et al. 2008a, b).

Similarly, in Wistar rats, 500 mg DBP /kg bw/d treatment from e13.5 to e21.5 was associated with a significant decrease in the number of gonocytes in the early postnatal testes, with recovery by adulthood (PND 90). Short-term DBP treatment during late gestation (e19.5–e20.5) had no effect on gonocyte numbers at PND 4. However it induced MNGs at e21.5 with a frequency similar to that induced by daily DBP treatment from e13.5. (Ferrara et al. 2006).

In a more recent study, DBP exposure immediately following testis differentiation in rats (e13.5) caused a major reduction in foetal germ cell numbers. Foetal DBP exposure delayed postnatal resumption of germ cell proliferation, which led to more reductions in germ cell numbers and delayed re-expresssion of DMRT1 and germ cell migration. In contrast, late gestation effects included germ cell aggregation when germ cells are quiescent and had switched off OCT4 (Jobling et al, 2011).

Mice appear to be less sensitive to DBP than rats. In mice exposed to DBP during gestation, malformations of foetuses were observed at 100 mg/kg bw/d in the presence of maternal toxicity and exhibited as a significant increase in incidence of non-closing eyelids, encephalocoele, cleft palates and spina bifida, and increased incidence of skeletal abnormalities (Hamano et al. 1977*). In another study, the NOAEL for foetal toxicity was 350 mg/kg bw/d based on decreased pup weight at 660 mg/kg bw/d without maternal toxicity (Shiota et al. 1980).

Marmosets also appear to show low sensitivity to DBP toxicity. Exposure to 500 mg MBP/kg bw/day during gestation from week seven to 15 is not associated with adverse developmental or reproductive effects in the male offspring studied at birth (1–5 days) or in adulthood (18–21 months of age) (McKinnell et al. 2009). However, reliability of this study is limited considering that only one treatment dose was used, together with a small number of animals (maximum 6) for which significant individual variations were reported in some of the measured end points.

Postnatal and multigenerational studies

Early postnatal treatment, even a short, 7-day exposure to DBP is also associated with toxicity to the development of the reproductive system in rats. However, most studies that aim to evaluate end points relating to postnatal development include prenatal and postnatal treatment, the latter often only indirect through lactation, and can therefore be considered trans-generational studies.

Exposure of prepubertal (3-week-old) SD rats to 250 mg DBP/kg bw/d for seven days, was associated with decreased testes weight, while treatment at higher doses (500 mg/kg/bw/d and above) resulted in histopathological alterations, such as seminiferous tubule lesions, decreased tubular size, depletion of spermatogenic cells, wider tubular lumen and thin layer of seminiferous tubules (Alam et al. 2010**)**. Thirty-day exposure at prepubertal age with 500 mg/kg bw/d, but not 200 mg/kg bw/d, was associated with decreased testostosterone levels in serum and non-reversible histomorphological alterations in testes (Xiao-feng et al. 2009).

Reproductive tract malformations were also observed in adolescent SD male rats (7-week-old) that were exposed to DBP during development (PND 1–21) and only indirectly through lactation (Zhang et al. 2004). The NOAEL for postnatal development in this study was 50 mg/kg bw/d (maternal dose) and the LOAEL at 250 mg/kg bw/d, based on decreased pup weight and male reproductive tract malformations including significant reduction of anogenital distance; increased frequency of testicular atrophy; under developed/absent epididymis; cryptorchidism; decreased epididymis weight and epididymal sperm motility; and decreased sperm heads per gram of testis. Mild degeneration of seminiferous epithelium was also observed at 250 mg/kg bw/d and was more severe at 500 mg/kg bw/d.

The lowest NOAEL for postnatal male developmental toxicity was established at 14 mg DBP/kg bw/day (NICNAS 2008b) in a dietary study with SD rats following gestational (from GD 15) and early indirect exposure (lactation) to DBP (Lee et al. 2004). This NOAEL is based on significant severity and incidence of adverse testicular effects (reduction in the spermatocyte development) at 148 mg/kg bw/d observed at PND21. However, considering that the incidence of the effect was also statistically significant at the lower doses (1.5 and 14 mg DBP/kg bw/day) even though the degree of severity was graded as minimal or slight, a lower LOAEL could be considered more appropriate. On the other hand, even the severe effects observed at the at 148 mg/kg bw/d appear to be reversible by PNW 20 (the highest dose of 1712 mg/kg bw/d was not examined at PNW 20).

In this study (Lee et al. 2004), the developmental NOAEL for females can be established at 3 mg DBP/kg bw/day, based on the significant decrease of relative pituitary weight at 29 mg/kg bw/d and above on PNW 20. However, this is also associated with significant uncertainty as no consistent histopathological changes were correlated with the decrease in weight.

Only a few studies are available where exposure continued during mating and lactation for both sexes. In only one study, with SD rats (NTP 1995*; Wine et al. 1997), treatment of F1 and F2 generations continued postweaning at the same dose level as their parents.

The NOAEL for developmental toxicity in F1 was 52 mg/kg bw/d, based on significant increase of kidney weight in F1 males at 256 mg DBP /kg bw/d. In F2 males, significant testicular atrophy and seminiferous tubule degeneration was observed at the same dose. However, no data were reported for the lowest tested dose and therefore a NOAEL could not be established for the F2 generation. (NTP 1995; Wine et al. 1997).

A similar NOAEL was established in an early study with Charles River COBS CD rats (IRDC 1984*) where both male and female rats were treated with DBP in the diet before mating (60 and 14 days, respectively), through mating, gestation and lactation. F1 weanlings were given a control diet (recovery group) or equivalent to that of their mothers, during a 7-week post-weaning period. The NOAEL for developmental toxicity was 50 mg/kg bw/d, based on slight decreases in testicular weights in weanlings in the 500 mg DBP/kg bw/d group. The decrease in weight correlated with testicular histopathological lesions in six of 10 weanlings continuously treated with the same dose, and in two out of nine weanlings in the corresponding recovery group.

Studies in humans mostly examine correlations between maternal MBP levels (in the urine or milk) and developmental parameters such as gonadotrophins and gonadal hormones, cryptorchidism or anogenital index. Behavioural and neuropsychological parameters have also been analysed. No significant association is reported for cryptorchidism but MBP levels in breast milk showed positive correlations with sex-hormone binding globulin and LH/free testosterone ratio, whereas the correlation with free testosterone was negative (Main et al. 2006). Studies that focused on measuring the anogenital distance in newborns found an inverse correlation between AGI and the maternal urinary concentrations of MBP, but not MEHP, using one statistical methodology (Swan et al. 2005). Another methodology calculated an inverse correlation for both metabolites (Swan et al. 2008).

Overall, the reliability of these studies for determining the effect of DBP in humans is limited because of the inconsistent results, most likely due to the low power of studies (small sample size, unrepresentative sample usually one study centre) and also uncertainties about the significance of the measured end points, for example AGD, as an indicator of developmental toxicity in humans.

7.6.3 Mode of action

In vitro studies examining DBP binding to the ER receptor and its oestrogenic activity in transcriptional assays indicate some oestrogenic activity for DBP without direct binding to the ER receptor.

Studies with testicular cell cultures indicate that DBP might affect morphophysiology of Sertoli cells as MBP-induced detachment of germ cells from a Sertoli cell monolayer in vitro (Gray & Gangoli 1986). MBP also altered transepithelial electrical resistance (TEER) of Sertoli cell monolayers in a dose-dependent manner. The change correlated with the downregulation of cytoskeletal proteins essential for Sertoli cell function (Zhang et al. 2008). These in vitro studies indicate that disruption of Sertoli cell tight junctions might be an aspect of the mechanism underlying the DBP-induced reproductive toxicity in male rodents, similar to the related phthalate DEHP (NICNAS 2010).

As discussed above, studies in rats where biochemical parameters have been monitored consistently show that DBP exposure is associated with decreased testosterone levels. Testicular and serum testosterone levels are affected following gestational and prepubertal exposure in rats. In some studies, testicular testosterone levels recovered after a period without dosing. Other studies showed that DBP doses associated with a decrease of testicular testosterone levels in the foetal tissues, are also associated with histomorphological perturbations in the foetal and adult testes that included adverse effects on Leydig, Setroli and germ cells. Consistent with these results are the findings that DBP exposure in rodents also affects expression of the genes involved in cholesterol transport and steroidogenesis such as steroidogenic acute regulatory protein (StAR), Cyp11a1, Cyp17a1 and Hsd3b steroidogenic enzymes (Howdeshell et al. 2007; Xiao-feng et al. 2009; Alam et al. 2010). Expression of the insl3 gene that is essential for regulating male reproductive system development, is also shown to be affected by DBP exposure in vivo (Wilson et al. 2004; Howdeshell et al, 2007, 2008), consistent with the findings of cryptorchidism and Leydig cell toxicity.

A study in marmosets indicates that a single exposure of newborn animals to 500 mg MBP/kg bw also caused a rapid decrease of blood testosterone levels (Hallmark et al. 2007 **ND**). However, 14-day dosing was not associated with any changes in the testosterone levels at the end of the dosing period, indicating that feedback regulatory loops could be activated in response to the initial effect of MBP. As described above, gestational exposure to the same dose of MBP does not appear to be associated with adverse developmental or reproductive effects in marmosets (McKinnell et al. 2009). However, these studies with very limited numbers of animals (maximum 6 animals) have not been corroborated and the histomorphological changes in the testes observed in the study by Hallmark et al. (increased Leydig cell volume per testes in the sub-chronically treated animals) that were interpreted to be consistent with activation of compensatory mechanisms, need further investigation. A particular duration and timing of the exposure could be critical for DBP toxicity in marmosets as in rodents, where histomorphological perturbations in the testes, likely to result from the decrease in testosterone levels during a critical developmental window, are irreversible, despite the clearly reversible decrease of testosterone levels (Xiao-feng et al. 2009).

Overall, the exact mechanism(s) underlying reproductive toxicity of DBP have yet to be fully elucidated. However, the reproductive tract malformations observed in rodents following DBP exposure (decreased AGD, number of spermatocytes, weight of testes, cryptorchidism, nipple retention, hypospadias, delayed preputial separation and ocurrence of Leydig cell hypertrophia and hyperplasia) are consistently associated with a decrease in androgen concentration that is either persistent or occurs in a critical window during development. This strongly suggests endocrine disruption, and in particular testosterone synthesis deregulation, as a major component of the mode of action (MOA) for DBP toxicity. The perturbations in the morphology of gonocytes described in some studies are indicative of underlying Sertoli cell dysfunction.

This is also supported by the in vitro studies using polarised cell cultures (Gray & Gangoli 1986; Zhang et al. 2008). Perturbation in the expression of insl3 gene, which is essential for proper testicular development and consequently male fertility, is also a plausible aspect of the mechanism(s) underlaying DBP reproductive toxicity in rodents.

A recent analysis of the toxicogenomic data set for DBP that focused on male reproductive developmental effects and was performed as part of a larger case study to test an approach for incorporating genomic data in risk assessment (Euling et al. 2011), also supports a mechanism of action for DBP that includes perturbations of steroidogenesis and insl3 expression as main MOA components. This analysis also indicates other MOAs that include pathways of cell signaling, metabolism, and cell adhesion. The study concludes that the putative new pathways could be associated with DBP effects on the testes that are currently unexplained by the two main MOAs discussed above. These include:

- the occurrence of multinucleated gonocytes (MNGs) in the testes (Mahood et al. 2007; Hutchison et al. 2008a; Ferrara et al. 2006),
- altered proliferation of Sertoli and peritubular cells (Mahood et al. 2007; Hutchison et al. 2008b), gonocyte apoptosis (Alam et al. 2010),
- abnormal Sertoli cell–gonocyte interaction (Mylchreest et al. 2002),
- a decreased number of spermatocytes or cauda epididymal sperm concentration (Wolf et al. 1999),
- decreased numbers or degeneration of seminiferous cords/tubules, altered morphology, degeneration of the epithelium, and enlarged cords/tubules (Mylchreest et al. 2002; Zhang et al. 2004).

Considering that the main components of the postulated modes of action in rodents, perturbations of steroidogenesis and insl3 expression, are applicable to humans, the reproductive toxicity of DBP observed in rodents is regarded as relevant for humans.

7.7 Summary

DBP is rapidly absorbed and excreted after oral administration. The bioavailability via the oral route in humans is assessed to be 100 %. Bioavailability via the dermal route is lower; assessed at 5 %.

There are limited data regarding DBP absorption via the inhalation route. A default of 100 % absorption via this route is considered appropriate for risk characterisation.

DBP shows low acute toxicity in animals and is not expected to have significant acute toxicity in humans. Also, DBP is not expected to have eye or skin irritation, or skin sensitising potential in humans.

DBP is considered to be non-genotoxic based on the weight-of-evidence, which shows no genotoxicity for DBP in most in vitro and all in vivo tests performed according to standard testing guidelines. No adequate long-term carcinogenicity studies with DBP in laboratory animals are available. However, based on the information available for genotoxicity, DBP is not likely to be a genotoxic carcinogen.

Toxic effects related to repeated DBP exposure and target organs include the liver and reproductive system, particularly in male rats. Increased kidney weight is also reported in some studies. Similar effects are observed in rodents exposed to the structurally related phthalate DEHP (NICNAS 2010) and, to a lesser extent, in the lower and higher molecular weight phthalates DEP and DINP respectively (NICNAS 2011 and 2012).

Adverse effects of DBP to testes include decreased weight of testes and accessory organs, spermatocyte depletion, seminiferous tubule degeneration and also perturbations in testicular testosterone content/production. Testosterone synthesis is also affected by lower doses of DBP (as low as 50 mg/kg bw/d) in male rodents. This is particularly demonstrated in

multigenerational and developmental studies where males have undergone gestational exposure to DBP. Testicular toxicity was consistently observed at similar doses for the related phthalate DEHP (NICNAS 2010), and at significantly higher doses for DEP and DINP (NICNAS 2011; 2012).

Reproductive toxicity studies in other species, including mice and primates, are limited. Only one study (one dose treatment and a small number of animals) is available in marmosets and indicates possibly lower sensitivity of primates to DBP reproductive/developmental toxicity.

Overall, the studies with humans have limited significance for risk characterisation of DBP exposure, but indicate that more comprehensive and prospective studies are needed. Some of the main limitations specific for these studies are the small number of subjects and the use of single spot measurement of urinary phthalate levels as a surrogate for longer term exposure. In addition, the associations between any chosen endpoint and a particular phthalate are characterised individually, while phthalate (metabolite) measurements show exposure to multiple phthalates, some of which have similar modes of action, such as DBP and DEHP.

The reproductive toxicity of DBP observed in rodents is regarded as the most relevant for humans and is considered in the risk characterisation as discussed in Section 8. The lowest NOAEL based on significant reduction of foetal testicular testosterone levels was established at 10 mg DBP/kg bw/d in a study where DBP treatment was in utero from GD 12 to 19 in SD rats. The LOAEL was at 50 mg DBP/kg bw/d (Lehmann et al. 2004). At 500 mg/kg bw/d (the only dose examined), there was also an increase of the mRNA levels of different members of the insulin-like growth factor family (Bowman et al. 2005).

8. Human health risk characterisation

8.1 Methodology

A margin-of-exposure (MOE) methodology is frequently used 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 with the NOAEL/NOAEC and deriving an 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) with the appropriate/reliable NOAEL to provide an MOE:

   MOE = NOAEL/EHD

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

The MOE provides a measure of the likelihood that a particular adverse health effect will occur under the conditions of exposure. As the MOE increases, the risk of potential adverse effects decreases. To decide whether the MOE is of sufficient magnitude, expert judgement is required. Such judgements 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 DBP exposure through use of:

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

8.2 Critical health effects

Adverse effects of DBP exposure and their relevance to humans are characterised in detail in Sections 6 and 7. In this section, additional analysis is undertaken to identify critical health effects and NOAEL/LOAELs appropriate for risk characterisation considering the particular uncertainties and the overall weight of evidence.

The critical endpoint of DBP toxicity relevant to the human health is reproductive toxicity with effects on fertility and development where the most sensitive target organ/system is the male reproductive tract. The effects are akin to those observed for the structurally similar phthalate DEHP (NICNAS 2010).

Fertility effects exhibit as decreased numbers of litters and viable pups through to complete infertility. Both sexes are affected as shown in crossover studies (mating treated to untreated animals) with rats and mice (Lamb et al. 1987*; Morrissey et al. 1989*; NTP 1995*; Wine et al. 1997). This is particularly notable in animals that have undergone gestational exposure to DBP where fertility indices, such as percentage of females with plug, pregnant and fertile females, appear to be significantly decreased compared with the parental generation, suggesting that the overall reproductive capacity is adversely affected through toxicity to the development, particularly of the reproductive system (NTP 1995*; Wine et al. 1997).

In males, reproductive toxicity by DBP involves overt effects on the reproductive tract, including the testes and the accessory organs, and perturbations in serum and testicular testosterone levels, all of which are related to fertility. In females, however, decreased fertility is evident even in the absence of obvious organ toxicity, although altered progesterone and oestradiol synthesis was observed in the ovarian cultures in one study where this was measured.

Studies with humans are limited, often contradictory (Section 6.3.3) and of limited significance for risk characterisation. Overall, they do not identify significant or convincing associations. However, one study suggests reduction of circulating testosterone levels in male workers exposed to DBP and DEHP (Pan et al. 2006). The studies examining sperm parameters in men are also contradictory, some indicating association of higher urine MBP levels with lower values for semen parameters in subjects with suspected infertility (Duty et al. 2003; Hauser et al. 2006), while a study in healthy military recruits showed no significant association (Jonsson et al. 2005). In a more recent study, DBP levels were highest in male volunteers with decreased number of sperm (oligoaesthenospermic) and a negative association between sperm motility and DBP levels was reported (Pant et al. 2011).

Overall, the evidence shows that the toxicity of DBP on fertility in rodents is mediated through adverse effects on the reproductive tract organs and perturbations in the oestrogen and androgen synthesis, a mode of reproductive toxicity also relevant for humans and similar to the related phthalate DEHP (NICNAS 2010). A reliable NOAEL specifically for fertility effects cannot be established for both sexes and for all generations, as it appears to depend on whether the animal was exposed from adulthood, during gestation or from puberty only. For fertility effects on the parental generation, a NOAEL of 250 mg/kg bw/d was established in Gray et al. (2006), but in a study where only females were treated and mated to untreated males. In the study by Mahood et al. (2007) the NOAEL was established at 100 mg/kg bw/d, based on statistically significant adverse effects on fertility parameters for the gestationally exposed F1 generation. However, a trend of decreased fertility as well as developmental effects in the reproductive tract of the male offspring, likely to be related to the decreased fertility of F1, was reported from the lowest dose. An evaluation of an overall reproductive toxicity NOAEL or LOAEL that includes developmental toxicity effects to the reproductive system will be more appropriate for risk characterisation in scenarios that involve exposure to the general population through consumer products.Developmental toxicity effects of DBP include: delayed preputial separation; decreased AGD; retention of nipples in the male offspring; increase of testicular dysgenesis; increased malformation in foetuses and F1 adults; and decreased testicular testosterone levels in foetuses. The effects reported in a particular study are often dependent on the specific focus of the study but also on timing of exposure during gestation. In addition, some effects observed in foetal testes appear to be reversible later during the postnatal development if exposure had been discontinued after birth or after weaning.

Therefore, the most adequate studies for risk characterisation of reproductive toxicity, and particularly in cases where developmental toxicity includes adverse effects on reproductive organs, are the multigenerational studies all of which have specific limitations and uncertainties. Multigenerational studies are summarised in Appendix 1. However, no overall NOAEL could be established with certainty, even in the most comprehensive three-generation study with SD rats, where exposure started with the adults in the parental generation and continued at the same dose level for the F1 and F2 generations (NTP 1995*; Wine et al. 1997, mentioned above for toxicity to fertility). In this study a NOAEL for developmental toxicity in F1 is 52 mg/kg bw, based on a significant increase of kidney weight at 256 mg DBP /kg bw/d. At this dose, only slight testicular effects are reported for F1 (poor epididymal development in one out of 20 animals, and seminiferous tubule degeneration in three out of 10 animals), but in the F2 generation significant testicular atrophy and seminiferous tubule degeneration were observed. However, because no data were reported for the lowest tested dose for F2, an overall developmental NOAEL could not be established. It should be noted that the F1 developmental NOAEL (56 mg DBP /kg bw/d) is a LOAEL for the parental generation (F0), based on the decreased total number of live pups per litter (see above). Overall, the study suggests increased sensitivity to DBP reproductive toxicity in successive generations and an overall NOAEL for developmental toxicity below 50 mg/kg bw/d. In a later study by Lee et al. (2004) in which dosing started during gestation and was only continued through lactation postnatally, effects were observed in females and, in much lower doses, in males (Table 8.1). In female offspring, a

decrease in the relative pituitary weight was significant from 29 mg DBP /kg bw/d at PNW 20. In males, the lowest dose at which adverse effects were observed was the lowest tested dose of 1.5 mg DBP/kg bw/day. At this dose and above, minimal to slight, but statistically significant, incidence of spermatocyte development reduction was observed at weaning. The effect was significantly more severe from 148 mg DBP/kg bw/day and above, demonstrating a dose-dependent trend in the incidence of the histopathological findings at weaning (PND21). On the other hand, the testicular effects appear to be reversible or significantly less severe by PNW 20, contributing some uncertainty with regard to the adversity of the effects observed at weaning. Additional uncertainty in determining the reversibility of the effect is contributed by the absence of data for the highest dose at PNW 20. Overall, no reliable developmental NOAEL can be established and the information about the LOEL/LOAEL is considered in the context of the overall data including that related to the mode of action for testicular and reproductive toxicity.

The malformations observed in the male reproductive tract of rodents following DBP exposure, such as the decreased number of spermatocytes, weight of testes, cryptorchidism, nipple retention, or Leydig cell hypertrophy and hyperplasia (elaborated in Section 7.6.3) are consistent with endocrine disruption, and, in particular, testosterone synthesis deregulation, an MOA of reproductive toxicity relevant to humans.

Two significant studies in rodents determined a NOAEL based on testicular testosterone levels (Table 8.1). Mahood et al. (2007 ND) identify a NOAEL of 20 mg DBP/kg bw/d, based on reduced testosterone levels (measured only in foetal testes) at the LOAEL dose of 100 mg/kg bw/d, concurrent with significant testicular dysgenesis in foetuses but also for adults at PND 90. However, in this study, an increasing trend of dysgenic areas in the testes of adults was notable from the lowest dose of 4 mg/kg bw/d. Lehmann et al. (2004) identified a NOAEL of 10 mg DBP/kg bw/d, based on significant reduction of foetal testicular testosterone levels at 50 mg DBP/kg bw/d that correlated with reduction in the expression of genes and proteins involved in cholesterol transport and steroidogenesis. Testicular histopathology was not extensively examined in this study.

Considering the overall information, the most appropriate NOAEL for this risk characterisation is that from the study by Lehmann et al. (2004). It is based on the major component of the plausible MOA for DBP toxicity relevant to humans and the decrease in androgen concentration, which is consistent with most of the reproductive tract malformations observed following gestational exposure to DBP. It should be noted that mild testicular dysgenesis is also observed at somewhat higher and lower doses in the multigenerational studies (NTP 1995*; Wine et al. 1997). However, the NOAEL of 10 mg DBP/kg bw/d is consistent with the results of the studies concomitantly testing DBP and DEHP, which show that these two phthalates have similar potency for overall testicular toxicity and particularly for reduced foetal testicular testosterone production (Howdeshell et al. 2007, 2008).

Pup weight decrease was also consistently observed in the developmental toxicity studies for DBP and the other assessed phthalates: DEP and DINP. For DBP the LOAEL for reduced pup weight in developmental studies was determined at 250 mg/kg bw/d. At this dose, male reproductive tract malformations were also observed. The NOAEL was 50 mg/kg bw/d (Zhang et al. 2004), and this NOAEL is used for cumulative risk assessment of the four phthalates in the exposure scenarios discussed in Appendix 3.

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

Toxicity	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Effect at LOAEL	Species and age at treatment	Ref
Fertility/ embryotoxicity	Could not be determined	52m–80f	↓ number of live pups/litter	Rat: adult pre-mating and through mating	NTP, 1995*; Wine et al. 1997
Development/ fertility	(f) 3 **(m)** 14 / lower	**(f)** 29 **(m)** 148 /lower [u] [u] ss but not severe effect notable from the lowest tested dose (1.5 mg/kg bw/d). The severe effects observed at the LOAEL appear reversible by PNW 20 (highest dose not examined)	**(f)** at PNW 20: ↓ relative pituitary weight **(m)** at weaning: severe ↓ testicular spermatocyte development, aggregations of Leydig cells and decreased epididymal duct cross section	Rat:gestational	Lee et al. 2004
Development/ fertility	20 [u]	100 (F1) [u] [u] trend in increasing of dysgenic areas observed from lowest dose of 4 mg/kg bw/d	Foetal F1: ↓ testosterone levels in testes concurrent with ↑ ss dysgenic areas in testes (seminiferous cords with MNGs) Adult F1; ↑ dysgenic areas in testes (Sertoli cell-only seminiferous tubules or areas with irregular staining for specific testes proteins)	Rat:gestational	Mahood et al. (2007 **ND**)
Development/ fertility	10	50	↓ T levels in foetal testes (GD 19)	Rat gestational	Lehmann et al. 2004

LE—Evans; ss—statistically significant; SD—Sprague Dawley; u—uncertainty (specified for each study); m—male; f—female

8.3 Risk estimates

8.3.1 Risk estimate related to use of toys and childcare articles

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

The combined internal dose for children arising from contact with toys and childcare articles is discussed in sections 5.2.4 and 5.2.5, and summarised in Table 8.2. Two 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 2.2 hours/day identified for children aged 6–12 months. The typical scenario considers the mean daily mouthing time of 0.8 hours/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 - 36 months of age. Overall, these studies demonstrate that mouthing times are highest for children aged 6–12 months and they decrease with increasing age. In the absence of Australian information, these mouthing behaviours are assumed applicable to Australian children.

Additional assumptions considered are as follows:

- maximal and typical migration rates for DBP plasticiser from plastic toys into saliva through biting and chewing is similar to that determined for DINP in a study conducted with adult volunteers (Chen 1998);
- the highest migration rate, which is applied to the worst-case exposure scenario, is 58 μg/cm^2/h. The mean migration rate, which is applied to the typical exposure scenario, is 26 μg/cm^2/h (Chen 1998);
- bioavailability of DBP via the oral route is assumed to be 100 %; and
- dermal absorption of DBP 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	0.32	1.97
Dermal	0.03	0.08
Combined	0.35	2.05

Estimation of margin of exposure

Risk estimates take into account the likelihood for reproductive effects at future life stages related to long-term exposure through repeated handling and mouthing of toys. Table 8.3 provides the MOE estimated from the internal DBP dose in children, and the dose at which no adverse effect is observed in the reproductive systems in experimental animals, i.e. the NOAEL.

Table 8.3: Calculated MOE in children for the critical effect of DBP 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	10	28571	4878

The risk estimates for the reproductive toxicity of DBP in both scenarios of toy use by children derive MOEs above 1000 (Table 8.3) and hence indicate a low risk of adverse effects on reproductive development, under the exposure scenario described in Section 5.2.6.

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

Uncertainties in the risk estimate

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

- absence of Australian-specific data on DBP content in toys and childcare articles;
- absence of Australian-specific data on children's mouthing behaviours;
- absence of specific information on migration rate of DBP from plastic matrices through the skin;
- the significance of the observed toxicity in animals, particularly the reproductive effects, to the human population; and
- lack of adequate epidemiological studies for determining the health effects of DBP in children following repeated exposure.

Studies in non-rodent animals are extremely limited. Marmosets appear to show low sensitivity to MBP (and by inference, to DBP) toxicity compared with rodents as indicated in the one available study (McKinnell et al. 2009) using one treatment dose (500 mg/kg/day) and a small number of animals. Epidemiological studies mostly examine correlations between maternal MBP levels (in the urine or milk) and developmental parameters such as gonadotrophins and gonadal hormones, cryptorchidism or anogenital index. Overall, these studies have limited significance for risk characterisation due to the small number of subjects they include and because the associations between any chosen endpoint and a particular phthalate are characterised individually, while phthalate (metabolite) measurements show exposure to multiple phthalates, some of which have similar mode of action, like DBP and DEHP.

Assessment of MOE considering a toy use scenario where DBP is used as a secondary plasticiser at 0.5 %, alone or with 1 % of DEHP (the maximum allowed in Australia), and DINP as primary plasticiser at 42.5 % or 41.5 % (based on the percentage of total phthalate plasticiser DINP at 43 % in the extractability studies) was used to derive the worst case exposure scenario (see Appendix 2), is calculated in Appendix 3, and indicates low risk with MOEs above 100.

Areas of concern

The risk estimates above do not indicate particular areas of concern from exposure of children to DBP via handling/mouthing of toys and childcare articles. Concern would arise if DBP was used as a sole plasticiser in toys under the same conditions as DINP (NICNAS 2012), where the MOE for the worst case exposure scenario (same assumptions as for DEHP—NICNAS 2010—and based on the data for DINP) would be 57, i.e. below 100.

It should be noted that exposure of children to DBP and/or other phthalates with similar modes of action can also occur from applying personal care products. Co-exposure to cosmetics containing DEP is discussed in Appendix 3 and is found not to be of concern. However, exposure to DBP from cosmetics alone is found to be an area of concern (see next section) and appropriate risk management recommendations are made that would preclude co-exposure to DBP from cosmetics.

8.3.2 Risk estimate related to use of cosmetics

The main route of exposure to DBP from cosmetic use 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 phthalates used in products, such as toothpastes, mouthwashes, lipsticks and lip-glosses, being prone to accidental oral ingestion.

Given the low acute toxicity, low skin and eye irritation and skin sensitising potential for DBP, the risk of adverse acute effects for consumers from use of DBP-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 DBP from daily use of various DBP-containing cosmetic products is estimated to be 156.2 µg/kg bw/d (Section 5.3.6) 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 substances and their safety evaluation* (SCCP 2012) and EU TGD (EC 2003). Additional assumptions are as follows:

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

Estimation of margin of exposure

Table 8.4: Calculated MOE for the critical health effect of DBP 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	10	64

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 DBP is high.

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

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

Infant Age	$D_{int,derm}$ (µg/kg bw/day)	MOE
Newborn	61.7	162
6 months	48.2	207
12 months	42.9	233

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, therefore there is no precise exposure assessment for cosmetics. Given the limited available data, conservative plausible assumptions, such as daily use of all cosmetics containing DBP, have been used to determine the risk to consumers.

In addition, Australian-specific data are not available on typical or maximum DBP content in specific types of cosmetic products. Therefore, for this risk characterisation, the DBP content in products is assumed to be similar to that currently reported for DEP across different cosmetic

product types in Australia (see Section 5.3.2 and Table 5.4). However, the extent to which this assumption of substitution overestimates DBP exposure via cosmetics currently, or in the future, is not known.

In the EU in a sample of 36 perfumes, DBP was found in trace amounts of 0.1–14ppm, while DEP and DMP, which are likely to have been deliberately added, were found at concentrations up to 2.3 % and 0.3 %, respectively (Peters 2005; SCCP 2007). The SCCP (2007) concluded that the presence of only trace amounts of DBP is likely to arise from leaching during manufacture or storage, rather than deliberate addition. This is consistent with the ban on DBP use in cosmetic products in the EU. This information relates to use in a sample of perfumes. There is no information available to extrapolate from perfumes to other cosmetics.

Outside the EU, DBP has been detected in 2007 in Canada (Koniecki et al. 2011) in 15 out of 252 tested products that included fragrances, hair care products (hair sprays, mousses, and gels), deodorants (including antiperspirants), nail polishes, lotions (body lotions and body creams), skin cleansers, and baby products (oils, lotions, shampoos and diaper creams). DBP was the second most frequently used phthalate, in 15 out of 252 products, after DEP (103/252) and before DIBP (9/252), DEHP (8/252) and DMP (1/252). The highest concentration of DBP, 2.4 %, was found in nail polish products, while in the other products it was found at 0.00036 % or less. No other data are available on the use or presence of DBP in cosmetic products manufactured in countries where DBP use in cosmetics has not been restricted. However, the possible use of DBP as a fragrance ingredient, solvent and container plasticiser for cosmetic use is indicated in the INCI (International Nomenclature Cosmetic Ingredient) Dictionary.

The exposure and MOE estimates assume a reasonable (but worst-case) scenario, where all possible DBP-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, who may have significantly different use patterns for cosmetic products. Use of several products from one preferred manufacturer with DBP 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 exposure estimates in newborns and infants, as there are very limited data on use of DBP in baby lotions or creams (Koniecki et al. 2011). In addition, information related to use patterns and/or levels of personal care products for babies and children is not available.

The inadequacy of human data on the health effects of DBP 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 DBP use in cosmetics in Australia and other countries, with the exception of the EU and USA (California), the potential for introduction of cosmetic products containing DBP with widespread use and exposure cannot be excluded. Therefore, given the low MOE of 64 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 DBP from use in cosmetics is of concern.

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 might 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 DBP might vary significantly as indicated by the studies in animals suggesting that the F1 generation exposed to DBP during development and

in adulthood is more sensitive to reproductive toxicity of DBP compared with the F0 generation that was exposed during adulthood only (NTP 1995; Wine et al. 1997). This indicates that there are specific concerns for exposure of young children, as exposure at this age can impact later in adulthood.

Determining the level of exposure to DBP for the different subpopulations that might be at highest risk in the cosmetic use scenario is difficult. However, the results of the large biomonitoring studies (Section 5.4), where a substantial difference was detected between the average levels for the population (mean) compared with the outliers, clearly indicate that some members of the population have been exposed to much higher DBP doses than the average population. For example, the maximum calculated exposure from biomonitoring data was 28.0 µg/kg bw/d, for one female participant, compared with a median dose of 8.4 µg/kg bw/d for female adults (Wormuth et al. 2006). This indicates that high exposure scenarios might be applicable to a subset of the population.

The estimates for cosmetic use for a single product such as perfume spray, and even body lotion, are close to the 95th percentile and maximum concentrations measured in the large biomonitoring studies (Section 5.4). This indicates that the worst-case exposure scenarios considered in this assessment are applicable for highly exposed individuals. This also raises concerns that the high exposure scenarios with an MOE below 100 might be applicable to the subpopulation that is most at risk for reproductive developmental effects in their progeny i.e. pregnant and breastfeeding women.

9. Current human health risk management

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

9.1 Current public health risk standards

9.1.1 Toys and childcare articles

In Australia, DBP was identified as being in use, or with the potential for use, in children's toys, some of which are intended for children aged 0–6 years, at a concentration up to 0.5 %.

There are currently no restrictions on the use of DBP in toys and childcare articles in Australia. DBP is not included in the Australian/New Zealand Standard AS/NZS ISO—8124 *Safety of Toys*.

In contrast, current EU, USA and Canadian legislation restricts the use of DBP to less than 0.1 % w/w of the plastic used in any type of toys and childcare articles.

9.1.2 Cosmetics

DBP was identified as being used in finished cosmetics and personal care products such as nail polish; fragrance bases for personal care and cosmetic products. The typical concentration of DBP in personal care products (nail polish) was identified as 7 %.

Currently in Australia there are no restrictions on the use of DBP in consumer products such as cosmetics and personal care products. DBP is not listed in the current Poison Standard, *Standard for the Uniform Scheduling of Medicines and Poisons No3* (SUSMP 2012).

Current EU legislation prohibits the use of DBP in cosmetic products.

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

Appendix 1—Summary of reproductive toxicity studies

Table A. 1: Summary of reproductive toxicity studies

LE—Long-Evans; ss—statistically significant; SD-—Sprague Dawley; u—uncertainty (specified for each study); m—male; f—female; F1—first filial/offspring generation; F2—second filial/offspring generation

Species	Exposure dose mg/kg bw/d	Exposure timing	Effect at LOAEL	LOAEL mg/kg bw/d	NOAEL mg/kg bw/d	Reference
Fertility/development						
LE rats	0, 250, 500, 1000 gavage	Females only. Continuous from weaning, through young adulthood, mating and lactation	↓ decreased fertility (% of females delivering live pups, litter size), ↓ ovarian ex vivo progesterone production ↑ ovarian ex vivo oestradiol production	500 (P0) (for females mated to untreated males)	250	Gray et al. (2006)
Wistar rat	0, 4, 20, 100, 500 gavage	GD 13.5–21.5	**Fertility of gestationally treated males** ss ↓ fertility of F1 males only (treated males crossed to untreated females) ↓ testes weigh in F1 males	[u]500 (F1) [u]statistically nonsignificant trend notable from lower doses	[u]100 (F1)	Mahood et al (2007 **ND**)
		GD 13.5–20.5 (foetal tissue analysis) GD 13.5–21.5 (postnatal tissue analysis at PND 90)	**Development** Foetal F1: ↓ testosterone levels in testes correlated with ↑ ss dysgenic areas in testes (seminiferous cords with MNGs) Adult F1; ↑ dysgenic areas in testes (Sertoli cell-only seminiferous tubules or areas with irregular staining for specific testes proteins)	[u]100(F1) [u]trend in increasing of dysgenic areas observed from lowest dose	[u]20	
SD rat	0, 52–80, 256–385, 509–794 (m–f) Diet: 0, 0.1, 0.5, 1 %	Continuous for F0 from 7 days premating through 112 days pairwise mating.	**Fertility/embryotoxicity** ↓ number of live pups/litter	52m–80f (F0)	Could not be determined	NTP, 1995*; Wine et al. 1997

Species	Exposure dose mg/kg bw/d	Exposure timing	Effect at LOAEL	LOAEL mg/kg bw/d	NOAEL mg/kg bw/d	Reference
			Development ↑ kidney weight in F1 males ↑ testicular atrophy and seminiferous tubule degeneration in F2 males	256 (F1)	[u]52 (F1) [u]no data reported for 0.1 % for F2	
CD-1 mice	0, 40, 420, 1410 Diet:0, 0.03, 0.3, 1.0 %	Adults during 14 weeks mating to weaning	↓ percentage of fertile pairs, number of litters/pair, number of live pups/litter and pups born alive.	1410	420	Lamb et al. (1987*); Morrissey et al. (1989*)
Prenatal development						
ICR-JCL mice	ca. 10, 100, 400 Diet: 0.005, 0.05, 0.5 %	GD 1–18	↓ number of live offspring ↑ incidence of malformations (non-closing eyelids, encephalocoele, cleft palates and spina bifida, and skeletal abnormalities)	[u]400 [u]also maternally toxic dose	100	Hamano et al. (1977*)
ICR-JCL mice	ca. 80, 180, 350, 660 and 2100 mg/kg bw/d) Diet: 0.05, 0.1, 0.2, 0.4, 1.0 %	GD 1–18	↓ decreased foetal weight.	[u]660 [u]also maternally toxic dose	350	Shiota et al. 1980
Wistar rats	0, 120, 600 gavage	GD 0–21	↓ placental weight, number of foetuses, foetal weight ↑ foetal resorptions	600	120	(Nikonorow et al. 1973*).
Wistar rats	0, 500, 630, 750, 100 gavage	GD 7–15	↑ foetal resorptions	[u]630 [u]also maternally toxic dose	500	Ema et al. 1993*
Wistar rats	ca. 0, 331, 555 or 661 Diet: 0, 0.5, 1.0 or 2.0 %	GD 11–21	↑ no. of malformations of urogenital tract in male foetuses	555 [u]also maternally toxic dose	331	Ema et al (1998*)
SD rats	100, 250, 500 gavage	GD 12–21	delayed preputial separation in offspring	100	Could not be determined	Mylchreest et al. (1999)
	0, 0.5, 5, 50, 100, 500 gavage		↑ seminiferous tubule atrophy retained nipples in the male offspring	100	50	Mylchreest et al. 2000)
SD rats	0, 100, 500 gavage	GD 12–21	↓ AGD in male offspring	100	Could not be	Barlow et al.

Species	Exposure dose mg/kg bw/d	Exposure timing	Effect at LOAEL	LOAEL mg/kg bw/d	NOAEL mg/kg bw/d	Reference
			↑ testicular lesions and malformations of the urogenital tract in male offspring		established	(2004)
SD rats	0, 250, 500, 750, 1000 gastric intubation	GD 14–18	↑ cryptorchidism in offspring ↓ serum testosterone levels in offspring	250	Could not be established	Jiang et al (2007 **ND**)
Postnatal development						
SD rats	0, 250, 500, 750 gavage	GD 3–PND 20 (weaning)	atrophy of seminiferous tubules and hypospadias in the male offspring assessed at adulthood	250	Could not be determined	Mylchreest et al. (1998)
SD rats	ca 0, 1.5-3, 14-29, 148-291, 712-1372 Diet: 0, 20, 200, 2000, 10000 ppm	GD 15–PND 21	(m) ↓ reduction in testicular spermatocyte development, aggregations of Leydig cells and decreased epididymal duct cross section on PND 21 [u]Statistically significant reduction of spermatocyte development notable from at the lowest tested dose (1.5 mg/kg bw/d) on PND 21 but with low severity. The severe effects (observed at the 148 mg/kg bw/d) appear to be reversible by PNW 20 (highest dose not examined at PNW 20). (f) ↓ relative pituitary weight on PNW 20. [u]Not correlated with consistent histopathological changes.	(m) [u]148/1.5 (f) [u]29	(m) 14/lower (f) 3	Lee et al. 2004
SD rats	0, 50, 250, 500 gavage	GD 1–PND 21(weaning)	↓ offspring weight and male reproductive tract malformations at PND 70	250	50	Zhang et al. (2004)
SD rats	0, 250, 500, 1000, 2000 gavage	Prepubertal for 30 days	↓ T serum levels correlated with non-reversible histomorphological perturbations in the testes	500	250	Xiao-feng et al. (2009 **ND**)
SD rats	0, 250, 500, 1000 gavage	Prepubertal for 7 days	↓ testes weight	250	Could not be determined	Alam et al. (2010 **ND)**
Multigenerational						
Charles River COBS CD rats	0, 5, 50 or 500 diet	Continuous staring at adulthood for F0 to 7 weeks postweaning for F1	↓ testes weigh in F1 ↑ testicular lesions F1	[u]500 [u]also maternally toxic dose	50	IRDC, 1984*
LE rats	0, 250, 500 gavage	From weaning, through	↑ frequency of delayed puberty in P0 animals	250	Could not be	Wolf et al.

Species	Exposure dose mg/kg bw/d	Exposure timing	Effect at LOAEL	LOAEL mg/kg bw/d	NOAEL mg/kg bw/d	Reference
		puberty, young adulthood, mating and lactation in the (P0). F1 pups were untreated after weaning.	↑ malformations in F1 males (epididymal agenesis, hypospadias, ectopic testis, renal agenesis and uterine malformations, anophthalmia and decreased cauda epididymal sperm counts ↓ cauda epididymal sperm counts in F1 ↑ uterine malformations in F1 females		determined	1999
LE rats	0, 40, 166 (estimated) 0, 12, 50 (stated by authors) Diet: 0.6 g/kg or 2.5 g/kg	Adults females: starting 2 months premating throughout to weaning.	↓ pup survival ↓ pup weights ↓ relative thymus and testes weights (at PND 14) delayed vaginal opening and onset of first oestrous cycle in offspring	[u]12/40 [u]dose calculation uncertainty	Could not be determined	Salazar et al. (2004),
Mode of action in vivo						
SD rat	0, 0.1, 1, 10, 50, 500	GD 12 to 19	↓ T levels in foetal testes (GD 19)	50	10	Lehmann et al. 2004
SD rat	0, 50, 100, 500 gavage	GD 12-19	↓ T levels in foetal testes (12 hours after final dose on GD 19 and not earlier). Levels recovered at 24 hours post final dose.	50 (LOEL)	-	Clewell et all., (2009 **ND**)

Appendix 2—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 child-care articles.

In the Netherlands, Groot et al. (1998) investigated the mouthing behaviour of 42 children aged between three and 36 months, for five categories of objects: pacifiers, teethers, fingers, toys and non-toys. Parents conducted ten 15-minute observations of mouthing behaviour over two days with a total of 42 children aged between three and six months; six and 12 months; 12 and 18 months; and 18 and 36 months. Of the four age groups observed, children of six to 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 four 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 two hours (range 1–3 hours) per day for a child aged three to 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 US 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 one-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, six minutes; and other objects, two minutes. In a second one-day study, for 110 children between 19 and 36 months of age, the average daily durations of mouthing were: pacifiers, 126 minutes; plastic toys, two minutes; teethers, 0 minutes; and other objects, two minutes. A final study with 168 children aged three to 18 months of mouthing of all objects excluding pacifiers over five non-consecutive observation days revealed an average daily mouthing time of 36 minutes. A small number of children—five out of 168—consistently mouthed objects for more than two hours a 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 US as part of the Consumer Products Safety Commission (CPSC) assessment of children's exposure to DINP. A total of 169 children aged three months to 36 months were studied by trained observers for a total of four hours on at least two different days. Three groups of children were studied: three to 12 months of age, 12 to 24 months of age and 24 to 36 months of age. For all objects except pacifiers, the estimated average daily mouthing times were 70 minutes (95 % confidence interval 60–80 minutes) for children aged three to 12 months; 47 minutes (40–57 minutes) for children aged 12 to 24 months; and 37 minutes (27–49 minutes) for children aged 24 to 36 months.

Greene (2002) conducted further statistical analyses of the data from Kiss's study (2001). The upper 95th percentiles for mouthing times across the three age groups ranged between 122 minutes/day (12–24 months) and 134 minutes/day (3–12 months), whereas the corresponding upper 99th percentiles ranged between 153 minutes (3–12 months) and 180 minutes (12–24 months).

DTI (2002) presented the findings of an investigation into the mouthing behaviour of 236 children aged one month to 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 six months to nine months (at approximately one hour) and decreased as children grow older. The maximum daily mouthing time for toys and

other objects (excluding pacifiers) for children aged six months to nine months was 297 minutes.

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

Table A2.1: Summary of minimum and maximum daily mouthing time from mouthing time studies

Study	No. 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. (2001)	107	0–18	Plastic toys;	17	NR	-
			Teethers;	6		-
			Other objects (excludes pacifiers & fingers)	9		-
	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	NR	-
		12–24	All objects, excluding pacifiers	48		-
		24–36	All objects, excluding pacifiers	37		-
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. Pacifiers were excluded from mouthing time calculation in these studies because the authors did not believe that any pacifiers made with DINP are currently in use (Babich et al. 2002, 2004).

Selection of mouthing time for use in exposure assessment

Table A2.1 reveals substantial variability in mouthing times among children aged three months to 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 six months to 12 months, with a maximum value of approximately three hours per day. The mouthing times then gradually decrease as the age of the child increases. Therefore, the mouthing time for children aged six months to 12 months represents a reasonable 'worst-case' estimate of the maximum mouthing time for use in exposure assessment. The 95th percentile total mouthing time of children aged three months to 12 months from the Greene (2002) study—134 minutes/day (2.2 hours/day)—is taken as the reasonable worst-case total mouthing time.

For the six-month to 12-month age group, a mean daily mouthing time of approximately 49 minutes/day (0.8 hours/day) was calculated by averaging results across the studies that 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.

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 cm2) were cut from each of five 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 µg/cm2/h (range 6.14–57.93 µg/cm2/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 0.1–4.4 µg/cm^2/h) from samples of children's toys or child-care 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 migration rates 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 one 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 one 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 four 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 four 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 A2.2.

Table A2.2: Summary of migration rates for phthalate plasticisers from in vivo testing

Study	PVC product	Phthalate	Wt. %	Test condition	Migration rate (SD) (μg/cm²/h)	
					Mean (SD)	maximum
Chen (1998)	Toy ducks	DINP	15–54	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 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 i.e. 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.

The migration rates determined for DINP under chewing condition can be extrapolated to 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 µg/cm^2/h from articles with up to 54 % DINP content (Chen 1998). This migration rate is therefore applicable for a worst-case exposure assessment for children from the use of DINP in toys. The mean migration rate for DINP in this study was 26.03 µg/cm^2/h (Chen 1998), which is similar to the highest mean migration rate of 32.6 µg/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 in toys.

Appendix 3—Risk estimate from cumulative exposures

Effects due to cumulative exposures can arise from use of cosmetics and/or toys and childcare articles containing multiple phthalates acting on the same biological targets, from exposure to mixed phthalates from a single source or from multiple sources.

The determination of risk from cumulative exposures to multiple phthalates will take into account any risk mitigation measures recommended in the PEC assessment for each phthalate. Risks from cumulative exposure to DBP and other phthalates will be considered on completion of other phthalate PEC assessments, and if required, further mitigation measures will be recommended. Any specific circumstances that will change the risk associated with the use of DBP will be considered under relevant secondary notification assessment requirements.

The calculation of the risk from the cumulative exposures was undertaken according to the WHO/IPCS Framework for risk assessment of combined exposure to multiple chemicals (Meek et al. 2011 ND). The assumption is made that the phthalates operate by a similar mode of action for each of the two end points (fertility related and developmental effects) considered relevant for DBP without antagonising or synergising each other's effects. Accordingly, dose additivity with adjustment for the potency of each of the phthalates (Tier 1 of the Framework) was used. Under Tier 1 of the Framework, the hazard index, which is the ratio of the exposure (EHD) to the toxicity reference value (e.g. NOAEL) for each of the chemicals, can be added and a combined MOE determined. It should be noted that the hazard index for individual chemical calculated in this way is the inverse of the MOE (i.e. HI = 1/MOE). Equations for calculating the combined MOE are provided in the Appendix 4—*Mixture risk assessment methodology—evaluating the health risk due to exposure to mixtures of chemicals* in the Sixth Framework Programme of the Health and Environment Integrated Methodology and Toolbox for Scenario Development (HEIMTSA) (Sarigiannis et al. 2010). This includes a number of different equations for determining cumulative risk and the choice of the most appropriate equation depends on the available input data. For the current calculations, the equation used is:

$$MOE_{cumulative} = (1/(1/MOE_1 + 1/MOE_2 + \cdots + 1/MOE_n)$$

The calculations for combined exposure were undertaken for two scenarios:

- Combined exposure to a mixture of plasticisers in toys and childcare articles consisting of 0.5 % DBP, 41.5 % DINP and 1 % DEHP (Table A3.1); and
- Combined exposure to a mixed DBP/DINP/DEHP plasticiser in toys and childcare articles and DEP in lotions for children (Table A3.2).

A scenario for combined exposure to DBP as only phthalate in toys and childcare articles and DEP or DBP in cosmetics is not relevant as DBP is not used as a primary plasticiser and is not likely to be used alone but only in combination with DINP and DEHP.

An example calculation can be given for combined developmental toxicity (pup weight) and reproductive toxicity (testes-related toxicity) of DINP in toys and childcare articles, and DEP in cosmetics in Appendix 1 of the NICNAS PEC assessment of DINP (NICNAS 2012). The values for DBP are calculated in a similar manner, with adjustment, where necessary for relative concentrations and combinations (Tables A3.1 to A3.2).

Risks from cumulative exposure in both scenarios are considered low as cumulative MOEs for the critical health effects all indicate an adequate safety margin (Table A3.1 and 3.2). These MOEs are specifically calculated for 6-month infants because the mouthing time studies (Appendix 2) indicate that newborn babies are unlikely to use teethers or childcare articles while

MOE estimates for older infants (e.g. 12-month infants) are expected to be higher based on their higher body weights.

Table A3.1: Calculated cumulative MOEs for exposure to a plasticiser mixture containing 41.5 % DINP and 1 %DEHP and 0.5 % DBP used in toys and childcare articles

	DINP		DEHP		DBP		Cumulative
Toxicity	***NOEL[a]***	***MOE[a]***	***NOEL[b]***	***MOE[b#]***	***NOEL[c]***	***MOE[c]***	***MOE[d]***
Reproductive [e]	50	283	4.8	27	10	57	223
Developmental[f]	50	283	46	260	50	283	282

a From Table 8.2 of the DINP risk characterisation (NICNAS 2012)

b From Table 8.3 of the DEHP PEC assessment report (NICNAS 2010) and **b#** from Table 8.3 of the DEHP PEC assessment report (NICNAS 2010) corrected for worst case total mouthing time (2.2 hrs/d)

c MOE for DBP if used as substituted for DINP at 43 %

d Calculated from the formula 1/[(41.5/MOE of DINP + 1/MOE of DEHP + 0.5/MOE of DBP/43]

e Based on NOAELs for testes related toxicity. For DINP: reduced foetal testicular testosterone content &/or production in Boberg et al. 2011; Hannas et al. 2011a (NICNAS 2012, Table 7.1); For DEHP: reduced testes wt, seminiferous tubule atrophy in F1 and F2 in Wolfe & Layton (2003) (NICNAS 2010, Table 8.1); For DBP reduced foetal testes testosterone in Lehman et al (2004), this report.

f Based on NOAELS for decreased pup weight. For DINP: from Waterman et al. 2000; Masutomi et al. 2003 NICNAS 2012); For DEHP: from Wolfe & Layton (2003) (NICNAS 2010); For DBP: from Zhang et al. (2004), this report.

Table A3.2: Calculated cumulative MOEs for combined exposure to a mixed DBP/DINP/DEHP plasticiser in toys and childcare articles and DEP in cosmetics for six month old children

Toxicity	Cumulative MOE[a] 41.5 %DINP/ 1 %DEHP 0.5 %DBP	MOE 0.5 %DEP	Cumulative MOE[b]
Reproductive[c]	223	207	108
Developmental [d]	282	1022	221

a From Table A3.1 above

b Calculated from the formula 1/(1/MOE of mixed DINP/DEHP/DBP (in toys)+ 1/MOE of DEP (in cosmetics)).

c Based on NOAELs for testes related toxicity. See **[e]** above for DINP, DEHP and DBP. For DEP: reduced serum testosterone in F0 & abnormal sperm in F0/F1 (Fujii et al. 2005).

d Based on NOAELS for decreased pup weight. See **[f]** above for DINP, DEHP and DBP. For DEP from (Fujii et al. 2005).

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 characterization. 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 Characterisation 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.

References

ABS (Australian Bureau of Statistics) (2005). *National Health Survey 2004–2005: Summary of results*. Canberra, Australian Government Publishing Service.

Alam MS, Ohsako S, Matsuwaki T, Zhu XB, Tsunekawa N, Kanai Y, Sone H, Tohyama C & Kurohmaru M (2010) Induction of spermatogenic cell apoptosis in prepubertal rat testes irrespective of testicular steroidogenesis: a possible oestrogenic effect of di(n-butyl) phthalate. *Reproduction*, 139:427–437.

Albertini R, Bird M, Doerrer N, Needham L, Robison S, Sheldon L, & Zenick H (2006) The use of biomonitoring data in exposure and human health risk assessments. *Environmental Health Perspectives*, 114:1755–1762.

Albro PW & Moore B (1974) Identification of the metabolites of simple phthalate diesters in rat urine. *Journal of Chromatography*, 94:209–218.

Aldyreva MV, Klimova TS, Izyumova AS, & Timofievskaya LA (1975) Effect of plasticizers on reproductive function. Gig Tr Prof Zabol, 12:25–29. In: IPSC (1997) International Programme on Chemical Safety. *Environmental Health Criteria 189- Di-n-butyl Phthalate*. World Health Organization, Geneva. pp 131–132.

ATSDR (2001) *Toxicological profile for di-n-butyl phthalate*. Agency for Toxic Substances and Disease Registry, Atlanta, Georgia.

Australian Toy Association (2009) Personal communication—Ms Beverly Jenkin.

Bailey JE, ed. (2011) *Compilation of ingredients used in cosmetics in the United States*. First Edition, Personal Care Products Council, Washington DC.

Barber ED, Astill BD, Moran EJ, Schneider BF, Gray TJ, Lake BG, & Evans JG (1987) Peroxisome induction studies on seven phthalate esters. *Toxicology and Industrial Health*, 3:7–24.

Barber ED, Cifone M, Rundell J, Przygoda R, Astill BD, Moran E, Mulholland A, Robinson E & Schneider B (2000) Results of the L5178Y mouse lymphoma assay and the Balb/3T3 cell in vitro transformation assay for eight phthalate esters. *Journal of Applied Toxicology*, 20:69–80.

Barlow NJ, McIntyre BS, & Foster PM (2004) Male reproductive tract lesions at 6, 12 and 18 months of age following in utero exposure to di(n-butyl) phthalate. *Toxicologic Pathology*, 32: 79–90.

BASF (1957) Confidential data. Abteilung Toxikologie, unveroeffentlichte Untersuchung, V/282. Dated 11.04.1957.

BASF (1958) Confidential data. Palatinol C (flüssig) = Phthalsäure-di-butylester uns. Vers. Nummern VIII/117 und VIII/332. Dated 1-12-1958.

BASF (1961) Confidential data. Bericht über die toxikologische Prüfung von Palatinol C, IC, AH, DN und VII/3-6. IX/418. Dated 10-1-1961.

BASF (1990a) Confidential report. *Report on the acute dermal irritation/corrositivity to the intact dorsal skin of dibutylphthalate in white rabbits*. Project No.: 18H0449/892113. Dated 12-2-1990.

BASF (1990b) Confidential report. *Report on the acute irritation to the eye of dibutylphthalate in white rabbits*. Project No.: 11H0449/892114. Dated 12-2-1990.

BASF (1990c) Confidential report. *Report on the Maximization Test for sensitising potential of dibutylphthalate in guinea pigs.* Project No. 30H0449/892115. Dated 1 March 1990.

BASF (1992) *Study of the oral toxicity of dibutyl phthalate in Wistar rats. Administration via the diet over 3 months*. (Confidential Report). BASF Project no. 31S0449/89020. Dated 23-03-1992.

BIBRA (1986) Confidential report to Chemical Manufacturers Association. Project No. 3.0495/3/85. Report No. 0495/3/85. CMA Ref. PE 28.0-BT-BIB. *A 21-day feeding study of di-n-butyl phthalate to rats: Effects on the liver and liver lipids.* British Industrial Biological Research Association. Dated February 1986.

BIBRA (1987) *Toxicity profile on dibutyl phthalate (DBP)*. British Industrial Biological Research Association. Dated March 1987.

BIBRA (1990) Confidential Report. *An investigation of the effect of dibutyl phthalate (DBP) on rat hepatic peroxisomes*. British Industrial Biological Research Association. Project No. 3.0826. Report No. 826/2/90.Dated January 1990.

Bowman CJ, Turner KJ, Sar M, Barlow NJ, Gaido KW, & Foster PM (2005) Altered gene expression during rat Wolffian duct development following di(n-butyl) phthalate exposure. *Toxicological Sciences*, 86:161–74.

Bremmer HJ, Prud'homme de Lodder LCH, & van Engelen JGM (2006) *Cosmetics fact sheet* (RIVM report 320104001/2006). Prepared for the National Institute of Public Health and the Environment (RIVM). Bilthoven, The Netherlands.

BUA (1987) *Dibutylphthalate,* BUA-Report 22.German Chemical Society. GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance.

Cagianut B (1954) *Keratitis erosiva und Nephritis toxica nach Einnahme von Dibutylphthalat*. Schweiz Med Wochenschr, 84:1243–1244.

Calley D, Autian J, & Guess WL (1966) Toxicology of a series of phthalate esters. *Journal of Pharmaceutical Sciences*, 55:158–162.

Calnan CD (1975) Dibutyl phthalate. *Contact Dermatitis*, 1:388.

Carruthers CM & Foster PM (2005) Critical window of male reproductive tract development in rats following gestational exposure to di-n-butyl phthalate. Birth Defects Research Part B *Developmental and Reproductive Toxicology*, 74:277–85.

Cater BR, Cook MW, Gangolli SD, & Grasso P (1977) Studies on dibutyl phthalate-induced testicular atrophy in the rat: effect on zinc metabolism. *Toxicology and Applied Pharmacology*, 41:609–618.

CDC (2009) *Fourth National Report on Human Exposure to Environmental Chemicals*. Department of Health and Human Services, Centers for Disease Control and Prevention. Accessed August 2011, http://www.cdc.gov/exposurereport/pdf/FourthReport.pdf.

CERHR (2000) *Monograph on the potential human reproductive and developmental effects of di-n-butyl phthalate (DBP)*. National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR), US Department of Health and Human Services, accessed 29/10/08 at http://cerhr.niehs.nih.gov/chemicals/phthalates/dbp/DBP_Monograph_Final.pdf

CERHR (2003) *Monograph and Expert Panel Report on Di-n-Butyl Phthalate (DBP)*. National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR), US Department of Health and Human Services.

Chahoud I & Paumgartten FJR (2005) Relationships between foetal body weight of Wistar rats at term and the extent of skeletal ossification. *Brazilian Journal of Medical and Biological Research*, 38: 565–575.

Chanda M & Roy SK (2007) *Plastics technology handbook*. CRC Press, Taylor & Francis Group, Boca Raton, FL,

Chen S-B (1998) Migration of DINP from polyvinyl chloride (PVC) children's products. Appendix A to *The risk of chronic toxicity associated with exposure to diisononyl phthalate (DINP) in children's products* by MA Babich, 1998, US Consumer Product Safety Commission.

Cho SC, Bhang SY, Hong YC, Shin MS, Kim BN, Kim JW, Yoo HJ, Cho IH & Kim HW (2010) Relationship between Environmental Phthalate Exposure and the Intelligence of School-Age Children. *Environmental Health Perspectives*, 118 (7): 1027–1032.

Clark K, David RM, Guinn R, Kramarz KW, Lamp MA & Staples CA (2012) Modelling human exposure to phthalate esters: a comparison of indirect and biomonitoring estimation methods. *Journal of Human Ecology Risk Assessment*, 17 (4):923–965.

Clayton GD & Clayton FE eds (1994) *Patty's Industrial Hygiene and Toxicology*, 4th ed. John Wiley & Sons Inc., vol 2, Part D, New York, pp 3050.

Clewell RA, Kremer JJ, Williams CC, Campbell JL, Sochaski MA, Andersen ME & Borghoff SJ (2009). Kinetics of selected di-n-butyl phthalate metabolites and foetal testosterone following repeated and single administration in pregnant rats. *Toxicology* 255(1–2):80–90.

Clewell RA, Thomas A, Willson G, Creasy DM Andersen ME (2013). A dose-response study to assess effects after dietary administration of diisononyl phthalate (DINP) in gestation and lactation on male rat sexual development. *Reproductive Toxicology* 35:70–80.

Cosmetic Ingredient Review Panel (1985) Final report on the safety assessment of dibutyl phthalate, dimethyl phthalate, and diethyl phthalate. *Journal of the American College of Toxicology*, 4: 267–303.

CSTEE (European Commission. Scientific Committee on Toxicity, Ecotoxicity and the Environment) (1998) *Opinion on phthalate migration from soft PVC toys and child-care articles*—Data made available since the 16th of June 1998, opinion expressed at the 6th CSTEE plenary meeting, Brussels, 26/27 November 1998. Accessed from http://ec.europa.eu/health/ph_risk/committees/sct/docshtml/sct_out19_en.htm

Doan K, Bronaugh RL & Yourick JJ (2010). In vivo and in vitro skin absorption of lipophilic compounds, dibutyl phthalate, farnesol and geraniol in the hairless guinea pig. *Food and Chemical Toxicology* 48: (18–23)

Dobrzynska MM, Tyrkiel EJ & Patchocki KA (2011) Developmental toxicity in mice following paternal exposure to Di-n-butyl Phthalate (DBP). *Biomedical and Environmental Science*, 24 (5): 569–578.

Duty S, Calafat AM, Silva MJ, Brock JW, Ryan L, Chen Z, Overstreet J & Hauser R (2004) The relationship between environmental exposure to phthalates and computer-aided sperm analysis motion parameters. *Journal of Andrology*, 25: 292–302.

Duty S, Silva M, Barr D, Brock J, Ryan L, Chen Z, Herrick R, Christiani D & Hauser R (2003) Phthalate exposure and human semen parameters. *Epidemiology*, 14: 269–277.

Eastman (2002) *Eastman plasticisers: selection chart*. L-174L. Eastman Chemical Company.

Eastman (2011) *Eastman Announces Discontinuation of Manufacture of DEP and DBP Plasticizers* (press release dated March 16, 2011). http://www.eastman.com/Company/News_Center/2011/Pages/Eastman_Announces_Discontinuation_of_Manufacture_of_DEP_and_DBP_Plasticizers.aspx (Accessed July 2011)

EC (2003) *Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances*, Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances, Directive 98/8/EC of the

European Parliament and of the Council concerning the placing of biocidal products on the market. Office for Official Publications of the European Communities, Luxembourg.

EC (2011) ESIS: *European Chemical Substances Information System*. European Commission Joint Research Centre, Institute for Health and Consumer Protection, http://esis.jrc.ec.europa.eu/ (Accessed May 2009).

ECB (2004) *Dibutyl Phthalate Risk Assessment* (with addendum 2004). European Communities, European Chemicals Bureau.

ECETOC (2003) *Derivation of assessment factors for human health risk assessment* (Technical Report No: 86). Brussels, European Center for Ecoroxicology and Toxicology of Chemicals.

ECHA (2009) *Data on manufacture, import, export, uses and releases of dibutyl phthalate (DBP) as well as information on potential alternatives to its use* (Revised 29 January 2009) (ECHA/2008/02/SR5/ECA.227). European Chemicals Agency, accessed January 2010, http://echa.europa.eu/doc/consultations/recommendations/tech_reports/tech_rep_DBP.pdf,.

ECPI (1998) *ECPI Position and response to KemI preliminary draft DEHP risk assessment site specific emission data from production in EU*. November 1998.

Elsisi AE, Carter DE, & Sipes IG (1989) Dermal absorption of phthalate diesters in rats. *Fundamentals of Applied Toxicology*, 12:70–77.

Ema M, Amano H, Itami T & Kawasaki H (1993) Teratogenic evaluation of di-n-butyl phthalate in rats. *Toxicology Letters*, 69:197–203.

Ema M, Amano H, & Ogawa Y (1994) Characterization of the developmental toxicity of di-n-butyl phthalate in rats. *Toxicology*, 86:163–174.

Ema M, Harazona A, Miyawaki E, & Ogawa Y (1997a) Embryolethality following maternal exposure to dibutyl phthalate during early pregnancy in rats. *Bulletin of Environmental Contamination and Toxicology*, 58:636–43.

Ema M, Harazono A, Miyawaki E & Ogawa Y (1997b) Developmental effects of di-n-butyl phthalate after a single administration in rats. *Journal of Applied Toxicology*, 17:223–239.

Ema M, Miyawaki E & Kawashima K (1998) Further evaluation of developmental toxicity of di-n-butyl phthalate following administration during late pregnancy in rats. *Toxicology Letters*, 98:87–93.

enHealth (2003) *Australian exposure assessment handbook*, consultation draft. Environmental Health Council (enHealth), Department of Health and Ageing, Commonwealth of Australia, Canberra.

EPA (1989) *NHATS broad scan analysis: Population estimates from fiscal year 1982 specimens*. US Environmental Protection Agency, Office of Toxic Substances, EPA 569/5-90-001, Washington, DC.

Euling SY, White LD, Kim AS, Sen B, Wilson VS, Keshava C, Keshava N, Hester S, Meric Ovacik A, Ierapetritou MG, Androulakis IP & Gaido KW (2011) Use of genomic data in risk assessment case study: II. Evaluation of the dibutyl phthalate toxicogenomic data set. *Toxicology and Applied Pharmacology*, Accessed July 2011 at http://www.sciencedirect.com/science/journal/0041008X.

EWG (2009) *Environmental Working Group's Skin Deep Cosmetic Safety Database* <http://www.cosmeticsdatabase.com/> Accessed 15 October 2009.

Ferrara D, Hallmark N, Scott H, Brown R, McKinnell C, Mahood IK & Sharpe RM (2006) Acute and long-term effects of in utero exposure of rats to di(n-butyl) phthalate on testicular germ cell development and proliferation. *Endocrinology*, 147(11):5352–5362.

Foster PM, Cook MW, Thomas LV, Walters DG & Gangolli SD (1983) Differences in urinary metabolic profile from di-n-butyl phthalate-treated rats and hamsters. A possible explanation for species differences in susceptibility to testicular atrophy. *Drug Metabolism and Disposition*, 11:59–61.

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

Fredricsson B, Muller L, Pousette A & Westerholm R (1993) Human sperm motility is affected by plasticisers and diesel particle extracts. *Pharmacology and Toxicology*, 72: 128–133.

Frederiksen H, Aksglaede L, Sorensen K, Skakkebaek NE, Juul A & Andersson AM (2011) Urinary excretion of phthalate metabolites in 129 healthy Danish children and adolescents: estimation of daily phthalate intake. *Environmental Research*, 111:656–63.

Gamer AO et al. (2000) *Di-n-butyl Phthalate—Subacute inhalation study in Wistar rats. 20 Exposures as a liquid aerosol*. Confidential report from BASF Aktiengesellschaft, Experimental Toxicology and Ecology, Ludwigshafen/Rhein, Germany. Project No. 4010486/98063, dated February 09, 2000.

Gilioli R, Bulgherain C, Terrana T, Filippini G, Massette N, & Boeri R (1978) Horizontal and longitudinal study of a population employed in the production of phthalates. *La Medicina del Lavoro*, 69:620–631. In: IPCS (1997) *Environmental Health Criteria 189 Di-n-butyl Phthalate*. World Health Organization, International Programme on Chemical Safety, Geneva, pp. 130–131.

Government of Canada (1994) *Dibutyl phthalate* (Priority substances list assessment report). Government of Canada, Environment Canada, Health Canada.

Gray TJ & Gangolli SD (1986) Aspects of the testicular toxicity of phthalate esters. *Environmental Health Perspectives*, 65:229–35.

Gray LE Jr, Laskey J & Ostby J (2006) Chronic di-n-butyl phthalate exposure in rats reduces Fertility and alters ovarian function during pregnancy in female Long Evans hooded rats. *Toxicological Sciences*, 93:189–195.

Gray LE Jr, Laskey JW, Ostby J, & Ferrell J (1983) The effects of dibutyl phthalate on the reproductive tract of the male and female rat and hamster. *Toxicologist*, 3:22 (Abstract No. 87). In: IPSC (1997) International Programme on Chemical Safety. *Environmental Health Criteria 189 Di-n-butyl Phthalate*. World Health Organization, Geneva, pp. 97–105.

Gray TJ, Rowland IR, Foster PM & Gangolli SD (1982) Species differences in the testicular toxicity of phthalate esters. *Toxicology Letters*, 11:141–147.

Greenough RJ et al. (1981) Confidential report from Inveresk Research International to Hüls Ag. Report No. 1956. *Safety tests of Vestinol C Dibutylphthalate*. IRI Project No. 416746. Dated February 1981.

Guerra MT, Scarano WR, de Toledo FC, Franci JAA & Kempinas W de G (2010) Reproductive development and function of female rats exposed to di-η-butyl-phthalate (DBP) in utero and during lactation. *Reproductive Toxicology*, 29(1):99–105.

Guo Y, Alomirah H, Cho HS, Minh TB, Mohd MA, Nakata H & Kannan K (2011) Occurrence of phthalate metabolites in human urine from several Asian countries. *Environmental Science and Technology*, 45:3138–3144.

Hall B, Tozer S, Safford B, Coroama M, Steiling W, Lenevau-Duchemin MC, McNamara C & Gibney M (2007) European consumer exposure to cosmetic products, a framework for conducting population exposure assessments. *Food and Chemical Toxicology*, 45:2097–2108.

Hallmark N, Walker M, McKinnell C, Mahood IK, Scott H, Bayne R, Coutts S, Anderson RA, Greig I, Morris K & Sharpe RM (2007) Effects of Monobutyl and Di(*n*-butyl) Phthalate in vitro on Steroidogenesis and Leydig Cell Aggregation in Foetal Testis Explants from the Rat: Comparison with Effects in vivo in the Foetal Rat and Neonatal Marmoset and in vitro in the Human. *Environmental Health Perspectives*, 115(3).

Hamano YA, Inoue K, Oda Y, Yamamoto H, Mitsuda B & Kunita N (1977) Studies on toxicity of phthalic acid esters—Teratogenic effects in mice administered orally. *Osaka-furitsu Koshu Esei kenkyusho Kenkyu Hokoka Shokukhim Eisei Hen*, 8:29–33. In: IPCS (1997) Environmental Health Criteria 189 Di-n-butyl Phthalate. World Health Organization, International Programme on Chemical Safety, Geneva. p 120.

Harris CA, Henttu P, Parker MG & Sumpter JP (1997) The oestrogenic activity of phthalate esters in vitro. *Environmental Health Perspectives*, 105:802–11.

Hauser R, Meeker JD, Duty S, Silva MJ & Calafat AM (2006) Altered Semen Quality in Relation to Urinary Concent rations of Phthalate Monoester and Oxidative Metabolites. *Epidemiology*, 17 (6): 682–691

Health Canada (1998) *Risk Assessment on diisononyl phthalate (DINP) in vinyl children's products*. Product Safety Bureau, Health Protection Branch, Health Canada.

Health Canada (2009) Phthalate Regulations. *Canada Gazette*. Health Canada, Ottawa, Canada http://www.gazette.gc.ca/rp-pr/p1/2009/2009-06-20/html/reg3-eng.html. Accessed October 2011

Higuchi TT, Palmer JS, Gray LE Jr & Veeramachaneni DN (2003) Effects of dibutyl phthalate in male rabbits following in utero, adolescent, or postpubertal exposure. *Toxicological Sciences*, 72:301–313.

Hong E, Ji YK, Choi KC, Manabe N & Jeung EB (2005) Conflict of oestrogenic activity by various phthalates between in vitro and in vivo models related to the expression of Calbindin-D-9k. *Journal of Reproduction & Development*, 51(2): 253–263.

Howdeshell KL, Furr J, Lambright CR, Rider CV, Wilson VS & Gray LE, Jr (2007) Cumulative effects of dibutyl phthalate and diethylhexyl phthalate on male rat reproductive tract development: altered foetal steroid hormones and genes. *Toxicological Sciences*, 99(1):190–202.

Howdeshell KL, Wilson VS, Furr J, Lambright CR, Rider CV, Blystone CR, Hotchkiss AK, & Gray LE Jr (2008) A mixture of five phthalate esters inhibits foetal testicular testosterone production in the sprague-dawley rat in a cumulative, dose-additive manner. *Toxicological Sciences*, 105(1):153–65.

Hubinger JC (2010) A survey of phthalate esters in consumer cosmetic products. *Journal of Cosmetic Science*, 61:457–465.

Hubinger JC & Havery DC (2006) Analysis of consumer cosmetic products for phthalate esters. *Journal of the Society of Cosmetic Chemists*, 57:127–137.

Huang PC, Kuo PL, Chou YY, Lin SJ & Lee CC (2009) Association between prenatal exposure to phthalates and the health of newborns. *Environment International* 35(1):14–20.

Huang PC, Kuo PL, Guo YL, Liao C, Lee CC (2007) Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. *Human reproduction*, 22 (10) 2715–2722

Huang PC, Tsai EM, Li WF, Liao PC, Chung MC, Wang YH & Wang SL. (2010) Association between phthalate exposure and glutathione S-transferase M1 polymorphism in adenomyosis, leiomyoma and endometriosis. *Human Reproduction* Apr.25(4):986-94. Epub 2010 Feb 10.

Hutchison GR, Scott HM, Walker M, McKinnell C, Ferrara D, Mahood IK, Sharpe RM (2008b) Sertoli cell development and function in an animal model of testicular dysgenesis syndrome. *Biology of Reproduction* 78(2):352–60.

Hutchison GR, Sharpe RM, Mahood IK, Jobling M, Walker M, McKinnell C, Mason JI & Scott HM (2008a) The origins and time of appearance of focal testicular dysgenesis in an animal model of testicular dysgenesis syndrome: evidence for delayed testis development? *International Journal of Andrology*, 31(2):103–111.

IARC (1995) *Peroxisome Proliferation and its Role in Carcinogenesis*. Technical report No: 24. The International Agency for Research in Cancer, Lyon.

IARC (2000) *IARC monographs on the evaluation of carcinogenic risks to humans. Some industrial chemicals*. World Health Organization, International Agency for Research on Cancer.

ICI (1982) *Di(2-ethylhexyl) phthalate: a comparative subacute toxicity study in the rat and marmoset*. ICI Americas Inc.

IFCS (Intergovernmental Forum on Chemical Safety) (2006) *Toys and chemical safety: a thought starter*. Document 03-TS, Agenda Item 10, Forum V—Fifth Session of the Intergovernmental Forum on Chemical Safety, Budapest, Hungary, 15–29 September 2006 [IFCS/FORUM-V/03-TS]. Geneva, World Health Organization. Accessed from http://www.who.int/ifcs/documents/forums/forum5/03_ts_en.pdf (October 2006).

IGHRC (Interdepartmental Group on Health Risks from Chemicals) (2004) *Guidelines for good exposure assessment practice for human health effects of chemicals*. Institute for Environment and Health, Leicester UK.

IPCS (International Programme on Chemical Safety) (1994) *Assessing human health risks of chemicals: Derivation of guidance values for health-based exposure limits*. World Health Organization, International Programme on Chemical Safety, Geneva.

IPCS (International Programme on Chemical Safety) (1997) *Environmental Health Criteria 189: Di-n-butyl phthalate*. Geneva, World Health Organization. Accessed at http://www.inchem.org/documents/ehc/ehc/ehc189.htm

IPCS (2005) *Harmonization project document No. 3—Principles of characterizing and applying human exposure models*. World Health Organization Press, Geneva.

IRDC (1984) Confidential report to Monsanto Chemical Company provided by Huels AG. *Test article: Dibutyl phthalate. Subject: Study of Fertility and General Reproductive Performance in Rats*. International Research and Development Corporation. Dated December 2, 1984.

Itoh H, Iwasaki M, Hanaoka T, Sasaki H, Tanaka T & Tsugane S. (2009) Urinary phthalate monoesters and endometriosis in infertile Japanese women. *Science of the Total Environment*. 408(1):37–42.

Janjua NR, Frederiksen H, Skakkebaek NE, Wulf HC & Andersson AM (2008) Urinary excretion of phthalates and paraben after repeated whole-body topical application in humans. *International Journal of Andrology* 31(2):118–30

Janjua NR, Mortensen GK, Andersson AM, Kongshoj B, Skakkebaek NE & Wulf HC (2007) Systemic uptake of diethyl phthalate, dibutyl phthalate, and butyl paraben following whole-body topical application and reproductive and thyroid hormone levels in humans. *Environmental Science and Technology* 41(15):5564–70.

Jansen EHJM et al. (1993) Confidential Report from the National Institute of Public Health and Environmental Protection (RIVM), the Netherlands to the Dutch Chief Inspectorate of Health Protection. Report nr. 618902013. *Toxicological investigation of dibutylphthalate in rats*. Dated June 1993.

Jiang J, Ma L, Yuan L, Wang X & Zhang W (2007) Study on developmental abnormalities in hypospadiac male rats induced by maternal exposure to di-n-butyl phthalate (DBP). *Toxicology*, 232(3): 286–93.

Jiang J, Sun W, Jing Y, Liu S, Ma, S, Hong Y, Ma L, Qin C, Liu Q, Stratton H & Xia S (2011) Prenatal exposure to di-c-butyl phthalate induces anorectal malformations in male rat offspring. *Toxicology* 290:322–326.

Jobling MS, Hutchison GR, Driesche van den S & Sharpe RM (2011) Effects of di(n-butyl) phthalate exposure on foetal rat germ-cell number and differentiation: identification of age-specific windows of vulnerability. *International Journal of Andrology*, 34:e386–e396.

Johnson KJ, McDowell EN, Viereck MP & Xia JQ (2011) Species-specific dibutyl phthalate foetal testis endoctine disruption correlates with inhibition of SREBP2-dependent gene expression pathways. *Toxicological Sciences*, 120(2):460–474.

Johnson S, Saikia N & Sahu R (2011) Phthalates in toys available in Indian market. *Bulletin of Environmental Contamination and Toxicology* 86(6) 621–626.

Jonsson BA, Ritchoff J, Rylander L, Giwercman A & Hagmar L (2005) Urinary phthalate metabolites and biomarkers of reproductive function in young men. *Epidemiology*, 16(4):487–493.

Kaneshima H, Yamaguchi T, Okui T & Naitoh M (1978) Studies on the effects of phthalate esters on the biological system (part 2)—In vitro metabolism and biliary excretion of phthalate esters in rats. *Bulletin of Environmental Contamination and Toxicology* 19:502–509.

Kaufmann W (1992) Confidential Report from BASF. Department of Toxicology. Pathology Report. *Study on the examination of the influence of dibutyl phthalate on the content of peroxisomes in the liver of Wistar rats after the administration via the diet over 3 months*. Project No. 99S0449/89021. Dated 11 March 1992.

Kawano M (1980a) Toxicological studies on phthalate esters. 1. Inhalation effects of dibutyl phthalate (DBP) on rats. *Nippon Eiseigaku Zasshi* [Jap J Hyg], 35:684–692.

Kawano M (1980b) Toxicological studies on phthalate esters. 2. Metabolism, accumulation and excretion of phthalate esters in rats. *Nippon Eiseigaku Zasshi* [Jap J Hyg], 35:693–701.

Kim Y, Ha E, Kim E, Park H, Ha M, Kim J, Hong Y, Chang N & Kim B (2011) Prenatal exposure to phthalates and infant development at 6 months: Prospective mothers and children's environmental health (MOCEH) study. *Environmental Health Perspectives*, 119 (10):1495–1500.

Kim HS, Kim TS, Shin JH, Moon HJ, Kang IH, Kim IY, Oh JY & Han SY (2004) Neonatal exposure to di(n-butyl) phthalate (DBP) alters male reproductive-tract development. *Journal of Toxicology and Engironmental Health Part A* 67: 2045–2060.

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

Koch HM & Christensen LY (2012) Di-n-butyl phthalate (DnBP) and siisobutyl phthalate (DIBP) metabolism in a human volunteer after single oral doses. *Archives in Toxicology*, 86: 1829–1839.

Koniecki D, Wang R, Moody RP, & Zhu J (2011) Phthalates in cosmetic and personal care products: concentrations and possible dermal exposure. *Environmental Research*, Article in Press.

Koo HJ & Lee BM (2004) Estimated exposure to phthalates in cosmetics and risk assessment. *Journal of Toxicology and Environmental Health Part A*, 67:1901–14.

Kremer JJ, Williams CC, Parkinson HD & Borghoff SJ (2005) Pharmacokinetics of monobutylphthalate, the active metabolite of di-n-butylphthalate, in pregnant rats. *Toxicology Letters*, 159:144–153.

Lake BG, Phillips JC, Linnell JC & Gangolli SD (1977) The in vitro hydrolysis of some phthalate diesters by hepatic and intestinal preparations from various species. *Toxicology and Applied Pharmacology* 39:239–248.

Lamb JC 4th, Chapin RE, Teague J, Lawton AD & Reel JR (1987) Reproductive effects of four phthalic acid esters in the mouse. *Toxicology and Applied Pharmacology,* 88:255–269.

Lapinskas PJ, Brown S, Leesnitzer LM, Blanchard S, Swanson C, Cattley RC & Corton JC (2005) Role of PPAR alpha in mediating the effects of phthalates and metabolites in the liver. *Toxicology*, 207: 149–163.

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

Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, Fernandez-Salguero PM, Westphal H & Gonzalez FJ (1995) Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Molecular and Cellular Biology*, 15:3012–3022

Lee KY, Shibutani M, Takagi H, Kato N, Takigami S, Uneyama C & Hirose M (2004) Diverse developmental toxicity of di-n-butyl phthalate in both sexes of rat offspring after maternal exposure during the period from late gestation through lactation. *Toxicology*, 203: 221–238.

Lehman AJ (1955) Insect repellents. *US Food Drug Official Quarterly Bulletin*, 19: 87–99.

Lehmann KP, Phillips S, Sar M, Foster PM & Gaido KW (2004) Dose-dependant alterations in gene expression and testosterone synthesis in the foetal testes of male rats exposed to di (n-butyl) phthalate. *Toxicological Sciences*, 81: 60–68.

Litton Bionetics (1985). Confidential Report to Chemical Manufacturers Association. *Evaluation of 1C in the in vitro transformation of Balb/3T3 cells assay*. Final Report. LBI Project No.: 20922. Report Date: April l985.

Mahood K, Scott HM, Brown R, Hallmark N, Walker M & Sharpe RM (2007) In utero exposure to di(n-butyl) phthalate and testicular dysgenesis: comparison of foetal and adult end points and their dose sensitivity. *Environmental Health Perspective*s, 115 SUPPLEMENT 1.

Main KM, Mortensen GK, Kaleva MM, Boisen KA, Damgaard IN, Chellakooty M, Schmidt IM, Suomi AM, Virtanen HE, Petersen JH, Andersson AM, Toppari J & Skakkebaek NE (2006) Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. *Environmental Health Perspectives*, 114(2): 270–276.

Marsee K, Woodruff TJ, Axelrad DA, Calafat AM & Swan SH (2006) Estimated daily phthalate exposures in a population of mothers of male infants exhibiting reduced anogenital distance. *Environmental Health Perspectives*, 114: 805–809.

McEwen GJ & Renner G (2006) Validity of anogenital distance as a marker of in utero phthalate exposure. *Environmental Health Perspectives*, 114(1): A19–20.

McKinnell C, Mitchell RT, Walker M, Morris K, Kelnar CJH, Wallace WH & Sharpe RM (2009) Effect of foetal or neonatal exposure to monobutyl phthalate (MBP) on testicular development and function in the marmoset. *Human Reproduction*, 24(9): 2244–2254

Meek ME, Boobis AR, Crofton KM, Heinemeyer G, Raaij MV and Vickers C (2011) Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. *Regulatory Toxicology and Pharmacology*, 60:S1–S14.

Meeker JD, Calafat AM, Hauser R (2007) Di(2-ethylhexyl) phthalate metabolites may alter thyroid hormone levels in men. *Environmental Health Perspectives*, 115(7) 1029–1034.

Meeker JD & Ferguson KK (2011) Relationship between Urinary Phthalate and Bisphenol A Concentrations and Serum Thyroid Measures in U.S. Adults and Adolescents from NHANES 2007–08. *Environmental Health Perspective*, 119(10):1396-402.

Milkov LE, Aldyreva MV, Popova TB, Lopukhova KA, Makarenko YL, Malyar LM & Shakhova TK (1973) Health status of workers exposed to phthalate plasticizers in the manufacture of artificial leather and films based on PVC resins. *Environmental Health Perspectives*, 3:175–8.

Mitani K, Narimatsu S, Izushi F & Kataoka H (2003) Simple and rapid analysis of endocrine disruptors in liquid medicines and intravenous injection solutions by automated in-tube solid-phase microextraction/high performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, 32:469–478.

Mitchell RT, Childs AJ, Anderson RA, Driesche van den S, Saunders TK, McKinell C, Wallace WHB, Kelnar CJH & Sharpe RM (2012) Do phthalates affect steroidogenesis by the human foetal testis? Exposure of human foetal testis xenografts to di-n-butyl phthalate. *Journal of Clinical Endocrinology and Metabolism*, 97(3):E341–E348.

MITI (Ministry of International Trade and Industry Japan) (1992) *Data of Existing Chemicals based on the CSCL Japan*. 3–90.

Morrissey RE, Lamb JC 4th, Morris RW, Chapin RE, Gulati DK & Heindel JJ (1989) Results and evaluations of 48 continuous breeding reproduction studies conducted in mice. *Fundamentals of Applied Toxicology*, 13:747–777.

Murakami K et al. (1986) Toxicity of dubutylphthalate and its metabolites in rats. Jpn J Hyg, 41:775–781. In: IPSC (1997) International Programme on Chemical Safety. *Environmental Health Criteria 189—Di-n-butyl phthalate*. World Health Organization, Geneva, p. 75.

Mylchreest E, Cattley RC & Foster PM (1998) Male reproductive tract malformations in rats following gestational and lactational exposure to Di(n-butyl) phthalate: an antiandrogenic mechanism? *Toxicological Sciences*, 43:47–60.

Mylchreest E, Sar M, Cattley RC & Foster PM (1999) Disruption of androgen-regulated male reproductive development by di(n-butyl) phthalate during late gestation in rats is different from flutamide. *Toxicology and Applied Pharmacology*, 156:81–95.

Mylchreest E, Sar M, Wallace DG & Foster PM (2002) Foetal testosterone insufficiency and abnormal proliferation of Leydig cells and gonocytes in rats exposed to di(n-butyl) phthalate. *Reproductive Toxicology*, 2002:16:19–28.

Mylchreest E, Wallace D, Cattley RC & Foster PMD (2000) Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to di(n-butyl) phthalate during late gestation. *Toxicological Sciences*, 55: 143–151.

Nakai M, Tabira Y, Asai D, Yakabe Y, Shimyozu T, Noguchi M, Takatsuki M & Shimohigashi Y (1999) Binding characteristics of dialkyl phthalates for the oestrogen receptor. *Biochemical and Biophysical Research Communications*, 254: 311–314.

NCHS (National Center for Health and Statistics). 2010. *National Health and Nutrition Examination Survey*. Available: http://www.cdc.gov/nchs/nhanes.htm [Accessed 7 February 2010].

NICNAS (2008a) *Phthalates hazard compendium: A summary of physicochemical and human health hazard data for 24 ortho-phthalate chemicals*. National Industrial Chemicals Notification and Assessment Scheme.

http://www.nicnas.gov.au/Publications/CAR/Other/Phthalate %20Hazard %20Compendium.pdf and http://www.nicnas.gov.au/Publications/CAR/Other/Phthalates.asp. Accessed January 2013.

NICNAS (2008b) Existing Chemical Hazard Assessment Report: *Dibutyl phthalate*. National Industrial Chemicals Notification And Assessment Scheme. http://www.nicnas.gov.au/Publications/CAR/Other/DBP %20hazard %20assessment.pdf. Accessed January 2013.

NICNAS (2010) Priority Existing Chemical Assessment Report No. 32: *Diethylhexyl phthalate (DEHP)*. National Industrial Chemicals Notification and Assessment Scheme. Accessed July 2010, http://www.nicnas.gov.au/Publications/CAR/PEC/PEC32/PEC_32_Full_Report_PDF.pdf.

NICNAS (2011) Priority Existing Chemical Assessment Report No. 32: *Diethyl phthalate (DEP)*. National Industrial Chemicals Notification and Assessment Scheme. Accessed December 2011, http://www.nicnas.gov.au/Publications/CAR/PEC/PEC33/PEC33_DEP_Full_Report_PDF.pdf.

NICNAS (2012) Priority Existing Chemical Assessment Report No. 35: *Diisononyl phthalate (DINP)*. National Industrial Chemicals Notification and Assessment Scheme. Accessed December 2011, http://www.nicnas.gov.au/Publications/CAR/PEC/PEC35/PEC35_DIN_Full_Report_PDF.pdf.

Nikonorow M, Mazur H & Piekacz H (1973) Effect of orally administered plasticizers and polyvinyl chloride stabilizers in the rat. *Toxicology and Applied Pharmacology* 26:253–259.

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

NTP (1995) *Toxicity Report Series Number 30. Technical Report on toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F334/N rats and B6C3F1 mice.* NIH Publication 95-3353. National Toxicology Program, US Department of Health and Human Services, Public Health Service, National Institutes of Health.

Nuodex (1982) *Evaluation of carcinogenic potential of Nuoplaz 6938 employing the C3H/10T1/2 cell transformation system*. OTS 84003A. Doc #878210256.

O'Brien ML, Spear BT & Glauert HP (2005) Role of oxidative stress in peroxisome proliferator-mediated carcinogenesis. *Critical Reviews in Toxicology*, 35 (1):61–88

OECD (2001) *SIDS Initial Assessment Profile (SIAP) of the OECD Screening Information Data Set (SIDS) on dibutylphthalate* [Meeting paper for SIAM (SIDS Initial Assessment Meeting) 12, 27–29 June, Paris, 2001].

OECD (2004) SIDS Initial Assessment Report for SIAM 19. *High molecular weight phthalate esters (HMWPE)*. July 18, 2004.

Oishi S & Hiraga K (1980a) Testicular atrophy induced by phthalic acid esters: effect on testosterone and zinc concentrations. *Toxicology and Applied Pharmacology*, 53:35–41.

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

Oishi S & Hiraga K (1980c) Testicular atrophy induced by phthalic acid monoesters: effects of zinc and testosterone concentrations. *Toxicology*, 15:197–202.

Okubo T, Suzuki T, Yokoyama Y, Kano K, & Kano I (2003) Estimation of oestrogenic and anti-oestrogenic activities of some phthalate diesters and monoesters by MCF-7 cell proliferation assay in vitro. *Biological and Pharmacuetical Bulletin*, 26(8): 1219–1224.

Oliwiecki S, Beck MH & Chalmers RJ (1991) Contact dermatitis from spectacle frames and hearing aid containing diethyl phthalate. *Contact Dermatitis*, 25:264–265.

Ota H, Onda H, Kodama H & Yamada N (1974) Histopathological studies on the effect of phthalic acid esters on the biological system of mice. Nippon Eiseigaku Kaishi, 29:519–524. In: IPSC (1997) International Programme on Chemical Safety. *Environmental Health Criteria 189 Di-n-butyl Phthalate*. World Health Organization, Geneva. pp 80.

Ota H, Takashima K, Takashima Y, Onda H, Kodama H, & Yamada N (1973) Biological effects of phthalate esters. (I) Histopathological findings from experiments in mice. Nippon Byorigakkai Kaishi, 62:119-120 In: IPSC (1997) International Programme on Chemical Safety. *Environmental Health Criteria 189 Di-n-butyl Phthalate*. World Health Organization, Geneva. pp 80.

Palmer C, Hsu M, Griffin K, Raucy J & Johnson E (1998) Peroxisome proliferator activated receptor-alpha expression in human liver. *Molecular Pharmacology*, 53(1): 14–22.

Pan G, Hanaoka T, Yoshimura M, Zhang S, Wang P, Tsukino H, Inoue K, Nakazawa H, Tsugane S & Takahashi K (2006) Decreased serum free testosterone in workers exposed to high levels if di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP): A cross-sectional study in China. *Environmental Health Perspectives*, 114: 1643–1648.

Pant N, Pant AB, Shukla M, Mathur N, Gupta YK, Saxena DK (2011) Environmental and experimental exposure of phthalate esters: The toxicological consequences on human sperm. *Human and Experimental Toxicology*, 30(6):507–514.

Pant N, Shukla M, Patel DK, Shukla Y, Mathur N, Gupta YK Saxena DK (2008) Correlation of phthalate exposures with semen quality. *Toxicology and Applied Pharmacology*, 231:11–-116.

Personal Care Products Council (2012) *International Cosmetic Ingredient Dictionary and Handbook*. 14th Edition, Personal Care Products Council, Washington DC.

Peters RJB (2005) *Phthalates and artificial musks in perfumes*. TNO Report R&I-A R 2005/011. Apeldorn, The Netherlands, Nederlanse Organisatie voor toegepast-natuurwetenschappelijk onderzoek (Netherlands Organisation for Applied Scientific Research). Accessed October 2011, http://www.greenpeace.org/raw/content/international/press/reports/phthalates-and-artificial-musk.pdf

Peters JM, Cattley RC & Gonzalez FJ (1997) Role of PPAR alpha in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643. *Carcinogenesis*, 18: 2029–2033

Phthalate Esters Panel of the American Chemistry Council (2001 and 2006) *High Production Volume (HPV) chemical challenge program test plan for the phthalate esters category*. Accessed December 2006, http://www.epa.gov/hpv/pubs/summaries/benzene/c13467rt3.pdf

Rastogi S (1998) Gas chromatographic analysis of phthalate esters in plastic toys. *Chromatographia*, 47:724–726.

Rastogi S, Jensen G & Worsoe I (2002) *Analytical chemical control of phthalates in toys*: analytical chemical control of chemical substances and products: NERI Technical Report No. 404. National Environmental Research Institute, Ministry of the Environment, Denmark.

Rastogi S, Jensen G & Worsoe I (2003) *Compliance testing of phthalates in toys*: NERI Research Notes No. 185. National Environmental Research Institute, Ministry of the Environment, Denmark.

Rastogi S & Worsoe I (2001) *Analytical chemical control of phthalates in toys*: analytical chemical control of chemical substances and products: NERI Technical Report No. 373. National Environmental Research Institute, Ministry of the Environment, Denmark.

Reddy BS, Rozati R, Reddy S, Kodampur S, Reddy P & Reddy R (2006) High plasma concentrations of polychlorinated biphenyls and phthalate esters in women with endometriosis: a prospective case control study. *Fertility and Sterility*, 85:775–9.

Rowland IR, Cottrell RC & Phillips JC (1977) Hydrolysis of phthalate esters by the gastro-intestinal contents of the rat. *Food and Cosmetics Toxicology*, 15:17–21.

RTECS (1993a) *Registry of Toxic Effects of Chemical Substances*. Update Code 9301 February 1993. Gigiena Truda Professional 'nye Zabolevaniya, 17(8), 1973, 26.

RTECS (1993b) *Registry of Toxic Effects of Chemical Substances*. Update Code 9301 February 1993. Union Carbide Data Sheet 12, 1971, 29.

RTECS (1993c) *Registry of Toxic Effects of Chemical Substances*. Update Code 9301 February 1993. Kanagawa-ken Eisei Kenkyusho kenkyu Hokoku 3, 1973, 19.

RTECS (1993d) *Registry of Toxic Effects of Chemical Substances*. Update Code 9301 February 1993. Science Reports of the Research Institutes, Tokohu University, Serie C: Medicine 36 (1-4), 1989, 10.

Saillenfait AM, Payan JP, Fabry JP, Beydon D, Langonne I, Gallissot F & Sabate JP (1998) Assessment of the developmental toxicity, metabolism, and placental transfer of Di-n-butyl phthalate administered to pregnant rats. *Toxicological Sciences*, 45:212–224.

Salazar V, Castillo C, Ariznavarreta C, Campon R & Tresguerres JA (2004) Effect of oral intake of dibutyl phthalate on reproductive parameters of Long Evans rats and pre-pubertal development of their offspring. *Toxicology*, 205: 131–137.

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

Sankar BR, Maran RR, Sudha S, Govindarajulu P & Balasubramanian K (2000) Chronic corticosterone treatment impairs Leydig cell 11beta-hydroxysteroid dehydrogenase activity and LH-stimulated testosterone production. *Hormone and Metabolic Research*, 32(4):142–6.

Sarigiannis DA, Hansen U & Karakitsios SP (2010). Part A—Methodologies for quantifying health effects of exposure by multiple routes and the effects of mixtures in the light of the case studies. In: Sarigiannis DA, ed. *Methodologies for quantifying health effects of exposure by multiple routes and the effects of mixtures in the light of the case studies, including a report on suitable indices of exposure*, Sixth Framework Programme, Health and Environment Integrated Methodology and Toolbox for Scenario Development (HEIMTSA), pp 5–42.

Sathyanarayana S, Calafat A, Liu F & Swan S (2008b) Maternal and infant urinary phthalate metabolite concentrations: are they related? *Environmental Research*, 108(3):413–418.

Sathyanarayana S, Karr C, Lozano P, Brown E, Calafat A, Liu F & Swan S (2008a) Baby care products: possible sources of infant phthalate exposure. *Pediatrics*, 121:260–8.

SCCP (Scientific Committee on Cosmetic Products) (2007) *Opinion on phthalates in cosmetic products*. Adopted by the SCCP at its 11th plenary meeting of 21 March 2007. Accessed January 2013. http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_106.pdf

SCCS (2012) *Notes of guidance for the testing of cosmetic ingredients and their safety evaluation*, 8th revision, adopted during the 17th plenary meeting of 11 December 2012. The Scientific Committee on Consumer Safety. Accessed January 2012, http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_006.pdf.

Schulsinger C & Mollgaard K (1980) Polyvinyl chloride dermatitis not caused by phthalates. *Contact Dermatitis*, 6:477–680.

Scott RC, Dugard PH, Ramsey JD & Rhodes C (1987) In vitro absorption of some o-phthalate diesters through human and rat skin. *Environmental Health Perspectives*, 74:223–237.

Shiota K, Chou MJ & Nishimura H (1980) Embryotoxic effects of di-2-ethylhexyl phthalate (DEHP) and di-n-buty phthalate (DBP) in mice. *Environmental Research*, 22:245–253.

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

Smith CC (1953) Toxicity of butyl stearate, dibutyl sebacate, dibutyl phthalate, and methoxyethyl oleate. *American Medical Association Archives of Industrial Hygiene and Occupational Medicine*, 7:310–318.

Sneddon IB (1972). Dermatitis from dibutyl phthalate in an aerosol antiperspirant and deodorant. *Contact Dermatitis* newsletter, 12: 308.

Srivastava SP, Srivastava S, Saxena DK, Chandra SV & Seth PK (1990) Testicular effects of di-n-butyl phthalate (DBP): biochemical and histopathological alterations. *Archives of Toxicology*, 64:148–52.

Stringer R, Labunska I, Santillo D, Johnston P, Siddorn J & Stephenson A (2000) Concentrations of phthalate esters and identification of other additives in PVC children's toys. *Environmental Science and Pollution Research*, 7:1–10.

SUSMP (2012) *Standard for the uniform scheduling of medicines and poisons* No.3. Australian Government. Department of Health and Ageing. National Drugs and Poisons Committee.

Swan S (2008) Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. *Environmental Research*, 108: 177–184.

Swan SH, Liu F, Hines M, Kruse RL, Wang C, Redmon JB, Sparks A & Weiss B (2009) Prenatal phthalate exposure and reduced masculine play in boys. *International Journal of Andrology*, 33:259–269.

Swan S, Main K, Liu F, Stewart S, Kruse R, Calafat A, Mao C, Redmon J, Ternand C, Sullivan S, Teague J (2005) Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environmental Health Perspectives*, 113:1056–1061.

Takeuchi S, Iida M, Kobayashi S, Jin K, Matsuda T & Kojima H (2005) Differential effects of phthalate esters on trandscriptional activities via human oestrogen receptors α and β, and androgen receptor. *Toxicology*, 210: 223–233

Tanaka A, Matsumoto A & Yamaha T (1978) Biochemical studies on phthalic esters. III. Metabolism of dibutyl phthalate (DBP) in animals. *Toxicology*, 9:109–123.

Toda C, Okamoto Y, Ueda K, Hashizume K & Itoh K, Kojima N (2004) Unequivocal oestrogen receptor-binding affinity of phthalate esters featured with ring hydroxylation and proper alkyl chain size. *Archives of Biochemistry and Biophysics*, 431:16–21.

Tomita I, Nakamura Y, & Yagi Y (1977) Phthalic acid esters in various foodstuffs and biological materials. *Ecotoxicology and Environmental Safety*, 1:275–287.

Tugwood J, Aldridge T, Lambe K, MacDonald N & Woodyatt N (1996) Peroxisome proliferator-activated receptors: Structures and function. *Annals of the New York Academy of Sciences*, 804: 252–265.

TURI (Toxic Use Reduction Institute) (2010) *Massachussets Chemical Fact Sheet on DBP—Use in Massachussets*

<http://turadata.turi.org/report.php?action=report_chemical_quantity_summary_all_years&cas_number=84742> Accessed 5 February 2010.

US FDA (2008) *Phthalates and cosmetic products*. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Cosmetics and Colors, April 19, 2001; Updated March 31, 2005 and February 7, 2008. http://www.cfsan.fda.gov/~dms/cos-phth.html Accessed June 2008.

Walseth F & Nilsen OG (1984) Phthalate esters. II. Effects of inhaled dibutylphthalate on cytochrome P-450 mediated metabolism in rat liver and lung. *Archives of Toxicology*, 55:132–136.

Walseth F & Nilsen OG (1986) Phthalate esters: effects of orally administered dibutylphthalate on cytochrome P-450 mediated metabolism in rat liver and lung. *Acta Pharmacologica et Toxicologica*, 59:263–239.

Ward J, Peters J, Perella C & Gonzalez F (1998) Receptor and nonreceptor-mediated organ-specific toxicity of di(2-ethylhexyl)phthalate (DEHP) in peroxisome proliferator-activated receptor alpha-null mice. *Toxicologic Pathology*, 26(2): 240–246.

Weuve J, Hauser R, Calafat AM, Missmer SA & Wise LA (2010) Association of Exposure to Phthalates with Endometriosis and Uterine Leiomyomata: Findings from NHANES, 1999–2004. *Environmental Health Perspectives*, 118 (6) 825–832.

White RD, Carter DE, Earnest D, & Mueller J (1980) Absorption and metabolism of three phthalate diesters by the rat small intestine. *Food and Cosmetics Toxicology*, 18:383–386.

WHO (2005) *Principles of characterizing and applying human exposure models*. International Program on Chemical Safety http://www.inchem.org/documents/harmproj/harmproj/harmproj3.pdf. Accessed January 2013.

Wilkinson CF & Lamb JC (1999) The potential health effects of phthalate esters in children's toys: a review and risk assessment. *Regulatory Toxicology and Pharmacology* 30:140–155.

Williams DT & Blanchfield BJ (1975) The retention, distribution, excretion, and metabolism of dibutyl phthalate-7-14 C in the rat. *Journal of Agricultural and Food Chemistry*, 23:854–858.

Wilson VS, Lambright , Furr J, Ostby J, Wood C, Held G & Gray LE (2004) Phthalate ester-induced gubernacular lesions are associated with reduced insl3 gene expression in the foetal testis. *Toxicology Letters*, 146: 207–15.

Wine RN, Li LH, Barnes LH, Gulati DK & Chapin RE (1997) Reproductive toxicity of di-n-butylphthalate in a continuous breeding protocol in Sprague-Dawley rats. *Environmental Health Perspective*, 105:102–107.

Wolf C, Lambright C, Mann P, Price M, Cooper RL, Ostby J & Gray LE Jr (1999) Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, p,p'-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. *Toxicology and Industrial Health*, 15:94–118.

Woodyatt N, Lambe K, Myers K, Tugwood J & Roberts R (1999) The peroxisome proliferator (PP) response element upstream of the human acyl CoA oxidase gene is inactive among a sample human population: significance for species differences in response to PPs. *Carcinogenesis*, 20(3):369–372.

Wormuth M, Scheringer M, Vollenweider M & Hungerbühler K (2006) What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? *Risk Analysis*, 26:803–824.

Wypych G (2003) *Handbook of plasticizers*. Toronto, Ontario. ChemTec Publishing, Canada.

Xiao-feng Z, Nai-qiang Q, Jing Z, Zi L & Yang Z. (2009) Di (n-butyl) phthalate inhibits testosterone synthesis through a glucocorticoid-mediated pathway in rats. *International Journal of Toxicology*, 28(5):448–56.

Zacharewski TR, Meek MD, Clemons JH, Wu ZF, Fielden MR & Matthews JB (1998) Examination of the in vitro and in vivo oestrogenic activities of eight commercial phthalate esters. *Toxicological Sciences*, 46:282–293.

Zhang Y, Jiang X & Chen B (2004) Reproductive and developmental toxicity in F1 Sprague-Dawley male rats exposed to di-n-butyl phthalate in utero and during lactation and determination of its NOAEL. *Reproductive Toxicology*, 18: 669–676.

Zhang Y-H, Lin L, Liu Z-W, Jiang X-Z & Chen B-H (2008) Disruption effects of monophthalate exposures on inter-sertoli tight junction in a two-compartment culture model. *Environmental Toxicology*, 23(3): 302–8

Zhou Y, Fukuoka M & Tanaka A (1990) Mechanism of testicular atrophy induced by di-n-butyl phthalate in rats. Part 3. Changes in the activity of some enzymes in the Sertoli and germ cells, and in the levels of metal ions. *Journal of Applied Toxicology*, 10:447–53.