

**Priority Existing Chemical
Assessment Report No. 35**



Australian Government
Department of Health and Ageing
NICNAS

Diisononyl Phthalate

SEPTEMBER 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME
GPO Box 58, Sydney NSW 2001 AUSTRALIA www.nicnas.gov.au

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Preface

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

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

NICNAS assessments are carried out in conjunction with the Australian Government Department of Sustainability, Environment, Water, Population and Communities (SEWPaC), which carries out the environmental assessment for NICNAS.

NICNAS has two major assessment programs: one focusing on 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 and/or environmental effects.

There is an established mechanism within NICNAS for prioritising and assessing the many thousands of existing chemicals in use in Australia. Chemicals selected for assessment are referred to as priority existing chemicals.

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

In accordance with the Act, publication of 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.

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
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Free call: 1800 638 528

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

- the 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 website:

<http://www.nicnas.gov.au>

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

<http://www.safeworkaustralia.gov.au>

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Overview

Background and scope of the assessment

Diisononyl phthalate (DINP) (CAS No. 68515-48-0 and 28553-12-0) was one of the nine phthalates declared as a priority existing chemical (PEC) for public health risk assessment for use in toys, child-care articles and cosmetics under the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act) on 7 March 2006. The decision for declaration was based on:

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

The purpose and scope of this PEC assessment is to determine the health risks to adults and children from the use of DINP 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 DINP suggest that the total volume of DINP imported for industrial uses was in the range of 1,000 to 9,999 tonnes in 2002 and approximately 600 tonnes in 2004. DINP is imported as a raw material or mixtures for local formulation and in finished (ready-to-use) products. Manufacture of DINP as a raw material in Australia was not reported. The current market consumption volume of DINP in Australia is between 1,600 and 2,000 tonnes per annum.

Uses

The information collected by NICNAS indicated that in Australia DINP is used mainly as a plasticiser (plastic softener) for polyvinyl chloride (PVC) products but also in other applications such as adhesives, laminations, resins, surfactants and screen printing inks, with a small proportion in children's toys. DINP is present in imported PVC toys at a concentration range of 0.005% to 35%.

International sources report that DINP is used as a plasticiser for PVC applications, such as in the manufacture of toys and construction materials. DINP is also used in non-PVC applications, such as rubbers, paints, sealants, lacquer and lubricants.

The information on the use of DINP provided by Australian industry did not include any indication that it is used in cosmetic and personal care products. Furthermore, the available information on the use of DINP in cosmetics overseas indicates that it is not used. There is also no information that supports the substitutability of high molecular weight phthalates, such as DINP, for low and mid molecular weight phthalates commonly used in cosmetics.

Therefore, risk characterisation for adults using cosmetics containing DINP is not discussed in this report.

Restrictions (either interim or permanent) on the use of DINP in toys and child-care articles that can be placed in the mouth by children have been implemented in the European Union (EU), the United States of America (US) and Canada. There are currently no restrictions on the use of DINP in toys and child-care articles in Australia.

Health effects

Orally administered DINP is rapidly absorbed based on animal and human data. The oral bioavailability of DINP is considered to be 100% for both adults and children. In contrast, bioavailability via dermal absorption is expected to be not greater than 4%. The available data suggest that dermal absorption of DINP through human skin may be significantly less than that of rat skin. Tissue distribution of DINP is widespread but there is no evidence of accumulation.

DINP is rapidly metabolised to the monoester MINP, which is further oxidatively metabolised to form additional metabolites (mainly carboxy-MINP, hydroxy-MINP and oxo-MINP), or hydrolysed to phthalic acid. These metabolites are rapidly excreted, mostly in urine.

DINP has low acute toxicity via oral, dermal and inhalation routes of exposure and is a slight skin and eye irritant. DINP shows minimal skin sensitisation potential.

DINP is not mutagenic in *in vitro* bacterial, mammalian or cytogenetic mutation assays and is not clastogenic in an *in vivo* bone marrow assay.

Incidences of mononuclear cell leukaemia (MCL) and kidney and liver neoplasia were observed in *in vivo* rodent carcinogenicity studies. These effects are regarded to be species specific and not relevant to humans.

The main target organs in several species following repeated oral exposure to DINP were the liver and kidney. In rats, liver and kidney toxicity were manifested as increased organ weights, liver biochemical changes and histopathological findings. These effects did not appear directly related to peroxisome proliferation. In rabbits, following repeated dermal exposures, slight or moderate erythema and desquamation were observed at high doses (2,500 mg/kg). No systemic effects were reported.

Overall, a no observed adverse effect level (NOAEL) of 88 mg/kg bw/d was determined for liver and kidney effects.

DINP has no effects on mating, fertility, fecundity, gestational length or index in rat studies. However, reduced testis weights (without histopathological changes) from 742 mg/kg bw/d and epididymis weights from 2,600 mg/kg bw/d were reported in repeated dose studies in mice but not in rats. In rats, DINP was shown to reduce testicular testosterone content and/or production (*ex vivo*) by male foetuses (gestation day (GD) 21) after gavage exposure during GD 7–21 (at 750 mg/kg bw/d) and GD 14–18 (at ≥ 500 mg/kg bw/d) in a similar pattern as observed with DEHP. Foetal expression of genes involved in androgen synthesis was also reduced at ≥ 500 mg/kg bw/d. In another study in rats, there were no testosterone production decreases in male foetuses (GD 19) at 750 mg/kg bw/d after GD 13–17 exposure, although changes in gene expression levels were seen. In a recent study, testicular pathology (increased number of mononucleated gonocytes) was reported and foetal testicular testosterone was statistically significantly reduced (50% reduction) at ≥ 250 mg/kg bw/d on GD 19.

DINP caused nipple retention at doses ≥ 600 mg/kg bw/d and decreased anogenital distance (AGD) and/or anogenital index (AGI) at ≥ 900 mg/kg bw/d in male offspring. Histopathological changes such as degeneration of meiotic spermatocytes and Sertoli cells at $\geq 1,000$ mg/kg bw/d, increased dysgenesis or agenesis/atrophy of testes and epididymis, increased size of Leydig cell aggregates and enlarged seminiferous tubule at ≥ 750 mg/kg bw/d were also reported. DINP at ≥ 900 mg/kg bw/d also affected spatial learning and increased masculinisation of behaviour in female offspring. An overall NOAEL for fertility-related (or sexual developmental) effects was determined to be 50 mg/kg bw/d based on the collective study results and weight of evidence evaluation.

Changes in pup weight were observed in both sexes, in both one and two generations of rats exposed to DINP and at a much lower dose of approximately 100 mg/kg bw/d. In addition, there was no overt maternal toxicity at this dose level where reduced pup weights were observed. The pup weight reduction was also sustained after birth and continued to post-natal day (PND) 21. In a recent study, pup weights were also reduced at ≥ 250 mg/kg bw/d on PND 14. Taking all together,

the reduced pup weight is considered the most sensitive DINP-related adverse effect on offspring growth and development and hence, for the purposes of this review, the developmental NOAEL is established as 50 mg/kg bw/d based on reduced pup weights at 100 mg/kg bw/d and above.

Overall, although the available human data are limited and do not provide sufficient evidence for a causal relationship between exposure to DINP and possible adverse health effects, elements of a plausible mode of action for the effects of DINP on the male reproductive system (reduced testicular testosterone), offspring growth (decreased pup weight) and sexual differentiation (decreased AGD/AGI and increased nipple retention) are considered parallel in rats and humans if the exposure to DINP is high and within a critical window of development. Therefore, the effects observed in animal studies are regarded as relevant to humans for risk characterisation.

Public exposure and health risk

Public health risks from DINP exposure were assessed using a margin of exposure (MOE) approach for use of toys and child-care articles by children. As it was found that there is no evidence of use of DINP in cosmetics in Australia or overseas, risk characterisation was not carried out for the general population using cosmetics containing DINP.

For the toy and child-care articles exposure scenario, two routes of exposure of children to DINP were considered: dermal exposure during normal handling of toys and child-care articles; and oral exposure during mouthing, sucking and chewing of these products. Migration rates were determined under chewing condition for DINP in overseas *in vivo* and *in vitro* studies.

Studies conducted overseas indicated that children's mouthing behaviour, and therefore the potential for oral exposure, is maximal in the period between six months and 12 months of age. Based on these studies, for children aged six months to 12 months, a reasonable worst-case exposure scenario considered a maximal mouthing time of 2.2 h/d and a typical exposure scenario considered a mean daily mouthing time of 0.8 h/d.

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

Health risks for children were estimated for both systemic (liver and kidney) toxicity and reproductive/developmental effects, both of which are potentially associated with repeated handling and mouthing of toys containing DINP. The risk estimates for systemic (liver and kidney) toxicity for the typical and worst-case scenarios of toy use by children give MOEs of 2,895 and 497 respectively. The MOE for both fertility-related and developmental effects for the typical scenario was 1,645 and, for the worst-case scenario, 283. In the three cases, the MOEs were above 100 for both the worst-case and typical exposure scenarios of toy use by children. Therefore, an adequate safety margin exists for DINP-induced adverse effects from the use of toys and child-care articles by children.

Overall, the risk estimates for systemic toxicity and fertility-related and developmental effects indicate low concern for children at the current reported levels of DINP in toys and child-care articles.

The effect of cumulative exposures to phthalates can arise from the effects of several phthalates in toys and child-care articles and from the combined exposure to a range of products containing phthalates. While the risks of cumulative exposures to DINP from multiple sources are addressed under Secondary Notification, the determination of risk from cumulative exposures to multiple phthalates will take into account any risk mitigation measures recommended in each PEC assessment. Risks from cumulative exposure of children to DINP in toys and child-care articles with or without DEHP at maximum 1%, together with co-exposure to another phthalate—DEP in cosmetics at maximum 0.5% in body lotions—are considered low, as cumulative MOEs for the three critical health effects identified are all above 100. Risks from cumulative exposure to DINP and other phthalates will be considered on completion of other phthalate PEC assessments and if required, further risk mitigation measures will be recommended.

Conclusion

The current PEC assessment has evaluated the human health risk from the uses of DINP in children's toys and child-care articles. Current risk estimates do not indicate a health concern from exposure of children to DINP in toys and child-care articles even at the highest (reasonable worst-case) exposure scenario considered.

The risks from cumulative exposure of children to DINP in toys and child-care articles with or without DEHP at maximum 1% together with co-exposure to DEP in cosmetics at maximum 0.5% in body lotions have been considered and found to be acceptable based on current public health risk management measures.

No additional recommendations to the existing controls in place for the public health risk management for the use of DINP in toys and child-care articles are required based on the findings of this assessment.

Secondary Notification

Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth), 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 DINP, specific circumstances include the following:

- additional information becoming available on the adverse health effects of DINP;
- DINP being used in cosmetic products;
- additional sources of public exposure to DINP other than toys and child-care articles and cosmetics being identified; or
- 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.

Shortened forms and Glossary

Shortened forms

AGD	anogenital distance
AGI	anogenital index
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BBP	butylbenzyl phthalate
bw	bodyweight
CAS	Chemical Abstracts Service
CD4+	cluster of differentiation 4+
CDC	Centers for Disease Control and Prevention
CERHR	Centre for the Evaluation of Risks to Human Reproduction (US)
CHAP	Chronic Hazard Advisory Panel (US)
CHO	Chinese hamster ovary
CIUCUS	Compilation of Ingredients Used in Cosmetics in the US (CIUCUS)
CPSC	Consumer Products Safety Commission (US)
CSTEE	Scientific Committee on Toxicity Ecotoxicity and the Environment (EU)
d	day
DBP	di-n-butyl phthalate
DEHP	diethylhexyl phthalate
DEP	diethyl phthalate
DEHP	diethylhexyl phthalate
DHeP	diheptyl phthalate
DHP	dihexyl phthalate
DIBP	diisobutyl phthalate
DIDP	diisodecyl phthalate
DIOP	diisooctyl phthalate
DINP	diisononyl phthalate
DMP	dimethyl phthalate
DNA	deoxyribonucleic acid
DnOP	di-n-octyl phthalate
DnNP	di-n-nonyl phthalate
DPeP	dipentyl phthalate
EC	European Commission
ECB	European Chemicals Bureau
ECHA	European Chemicals Agency

EFSA	European Food Safety Authority
EU	European Union
ESIS	European Chemical Substances Information System
EURAR	European Union Risk Assessment Report
FDA	Food and Drug Administration (US)
g	gram
GD	gestation day
GI	gastro-intestinal
GJIC	gap junctional intercellular communication
GLP	good laboratory practice
h	hour
hER	human oestrogen receptor
HMW	high molecular weight
HPVC	high production volume chemical
HVCL	High Volume Industrial Chemical List
ICIDH	International Cosmetic Ingredient Dictionary and Handbook
IgE	immunoglobulin E
IgG	immunoglobulin G
IL	interleukin
IFCS	Intergovernmental Forum for Chemical Safety
kg	kilogram
L	litre
LC50	median lethal concentration
LD50	median lethal dose
LH	luteinising hormone
LMW	low molecular weight
LOAEL	lowest observed adverse effect level
LOD	limit of detection
m ³	cubic metre
MCINP	mono(carboxyisononyl) phthalate
MCIOP	mono(carboxyisooctyl) phthalate
MCL	mononuclear cell leukaemia
MEHP	monoethylhexyl phthalate
mg	milligram
µg	microgram
MHINP	mono(hydroxyisononyl) phthalate
MIDP	monoisodecyl phthalate

MINP	monoisononyl phthalate
mL	millilitre
MOE	margin of exposure
MOINP	mono(oxoisononyl) phthalate
mRNA	messenger ribonucleic acid
ND	new data
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PA	phthalic acid
PEC	priority existing chemical
PND	post-natal day
PPAR	peroxisome proliferator activated receptor
ppm	parts per million
PVC	polyvinyl chloride
SD	standard deviation or Sprague-Dawley (rats), as indicated in the text
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons (formerly known as Standard for the Uniform Scheduling of Drugs and Poisons—SUSDP)
TSCA	<i>Toxic Substances Control Act 1976</i> (US)
US	United States of America
US EPA	United States Environmental Protection Agency
wt	weight
w/w	weight/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>

1. Introduction

1.1 Declaration

Diisononyl phthalate (DINP) (CAS No. 68515-48-0 and 28553-12-0) was one of nine phthalate chemicals declared as a priority existing chemical (PEC) under the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act) on 7 March 2006 for assessment of the public health risk from use of DINP in children's toys, child-care articles and cosmetics. The basis for the declaration was the actual and potential use of DINP in children's toys, child-care articles and cosmetics. The declaration notice is available on the NICNAS website at: http://www.nicnas.gov.au/Industry/Existing_Chemicals/PEC_Declarations.asp

1.2 Objectives

The objectives of this assessment were to:

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

These consumer applications are as defined below:

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

1.3 Sources of information

Information for this assessment was obtained from various sources including Australian industry and government, overseas regulatory agencies 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 DINP, as PECs, importers and manufacturers of DINP as a raw material for use in children's toys, child-care articles and cosmetics, and importers of cosmetics containing DINP, were required to apply for assessment and

supply information on the use of DINP. Unpublished information on health effects of phthalates, including DINP, was also sought.

This call for information was followed in July 2006 by a voluntary call to importers and manufacturers of toys and child-care articles for similar information on phthalates, including DINP, 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, reports from the European Chemicals Bureau (ECB, 2003), the Centre for the Evaluation of Risks to Human Reproduction (CERHR, 2003), the US Consumer Products Safety Commission (CPSC, 1998; 2010), the US Chronic Hazard Advisory Panel (CHAP, 2001) and the European Chemicals Agency (ECHA, 2010) were consulted. Information from these documents was supplemented with new relevant data identified from thorough literature searches on Toxnet, PubMed, ScienceDirect, SciFinder, Embase, CCOH's OSH References and the search engine Google Scholar. The last searches were conducted in June 2012.

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 DINP Hazard Assessment (NICNAS, 2008a) and the Phthalate Hazard Compendium (NICNAS, 2008b), which provides a comparative analysis of key toxicity endpoints for 24 ortho-phthalates.

1.4 Peer review

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

1.5 Applicants

Following the declaration of DINP as a PEC, one company and two organisations applied for assessment of this chemical.

In accordance with the Act, 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:

NSW Government Office of Environment & Heritage
(formerly Department of Environment and Conservation)
59–61 Goulburn St, Sydney NSW 2000

Sigma Aldrich Pty Ltd
12 Anella Ave, Castle Hill NSW 2154

The Vinyl Council of Australia
65 Leakes Road, Laverton North VIC 3026

2. Background

2.1. International perspective

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

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

On the basis of the ester side-chain length, DINP belongs to the HMW phthalates group.

DINP is used in a diverse range of industrial products such as electrical wire and cables, flexible PVC sheet, coated fabrics, automotive parts (synthetic leather for car interiors, car underbody coatings, cables), building and construction (waterproofing), vinyl flooring, footwear, sealings, lamination film and PVC-containing school supplies (scented erasers, pencil cases).

DINP can be blended into a paste (plastisol) for coating (tarpaulins, synthetic leather and wall covering) and rotomoulding (toys, play and exercise balls, hoppers) applications. In addition, DINP is also used in applications such as adhesives, paints, surfactants and printing inks for T-shirts. DINP can also be found in plasticine, in several categories of toys (plastic books, balls, dolls and cartoon characters) and in baby products (changing mats/cushions) that could be placed in the mouth, although this was not the purpose for which they were designed. DINP was also found in other articles for / in contact with children (clothes, mittens, coverage of pacifiers, PVC-containing soap packaging and shower mats).

As a plasticiser, DINP can be present in high concentrations (up to approximately 50%) in polymer materials. DINP was found in baby changing mats / cushions at concentrations of 15%, in plasticine at 10%, in mittens at 8.6%, in soap packaging at 8.8%, in the cover of pacifiers at 0.1% and in shower mats at 14.6% (Danish EPA, 2009*; ECHA, 2010). DINP was also found in toy erasers at 70% and in PVC pencil cases at trace levels (Force Technology, 2007*; ECHA, 2010).

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

In the EU, permanent restrictions on the use of DINP as plasticisers in toys and child-care articles came into effect on 17 January 2007. The legislation was previously agreed by the EU in 2005 (Directive 2005/84/EC) and sets a content limit of 0.1% weight/weight (w/w) of the plasticised material for DINP and another two phthalates, diisodecyl phthalate (DIDP) and di-n-octyl phthalate (DnOP), for toys and child-care articles that can be placed in the mouth by children under three years of age.

The restriction was a precautionary response to uncertainties in the evaluation of exposure to DINP, such as mouthing times and exposure to emissions from other sources. The European Commission was to evaluate the restrictions in the light of new scientific information by 16 January 2010 and, if justified, these restrictions could be modified accordingly. The ECHA report concluded that the available new information does not warrant re-examination of the current restrictions on DINP (ECHA, 2010).

DINP has been pre-registered with ECHA under the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) systems.

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

- DINP is not listed in Annex 1 of Directive 67//548/EEC relating to the classification, labelling and packaging of substances and mixtures;
- DINP is not listed in the Candidate List of Substances of Very High Concern (SVHC);
- DINP is not listed in the Cosmetic Ingredient database (CosIng), a database on cosmetic ingredients contained in the Cosmetic Directive 76/786/EEC, Inventory of Cosmetic Ingredient (as amended) and Opinions on Cosmetic Ingredients of the Scientific Committee on Consumer Safety (SCCS);
- DINP has been reported as a High Production Volume Chemical (HPVC); and
- DINP is listed under Council Regulation 793/93/EEC on the evaluation and control of the risks of existing substances.

Regulatory information on DINP was also available from the US.

In February 2007, the state of California in the US proposed a law to ban toys and baby products with more than a trace amount of phthalates. Subsequently, since January 2009, a ban on six phthalates at more than 0.1% w/w in children's products has been in force.

For DINP, the law in California prescribes that DINP at concentrations exceeding 0.1% cannot be used in any toy or child-care article intended for use by a child under three years of age if that product can be placed in the child's mouth.

In August 2008, the US Congress passed the *Consumer Product Safety Improvement Act* (2008) to restrict certain substances in children's products. The law enacts a permanent restriction on three phthalates and a temporary restriction on DINP and two other phthalates comprising more than 0.1% w/w of any children's product for ages 12 and under. The Consumer Product Safety Commission (CPSC) will review the interim restrictions for DINP and two other phthalates to determine if permanent restrictions are necessary.

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

The US EPA stated that they intended to initiate rulemaking in 2010 to include these phthalates to the Concern List under the US *Toxic Substances Control Act 1976* (TSCA) Section 5(b)(4) as chemicals that present, or may present, an unreasonable risk of injury to health or the environment.

The US EPA also intended 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 disproportionate impact on children and other sub-populations is to be evaluated.

It is envisaged that any rulemaking from these assessments will be initiated in 2012. To date, there is no information available and/or no updates have been reported on the Phthalates Action Plan.

In 2010, CPSC compiled a report on DINP, which contains hazard identification and dose response assessment. The information in this report will contribute to a cumulative risk assessment of exposure to multiple phthalates to be performed by the Chronic Hazard Advisory Panel (CHAP) on phthalates.

DINP is not listed in the Compilation of Ingredients Used in Cosmetics in the US (CIUCUS) (Personal Care Products Council, 2011). The CIUCUS provides a compilation of ingredients that have documented use in cosmetics by the FDA. It is also not listed in the International Cosmetic

Ingredient Dictionary and Handbook (ICIDH). The ICIDH is compiled by the Personal Care Products Council (2011), which provides a comprehensive international reference of descriptive and technical information about substances that have been identified as potential cosmetic ingredients. .

In Canada, a regulatory impact analysis statement proposing new phthalate regulations covering the use of six phthalates in children's toys and child-care articles was published by Health Canada in June 2009.

For DINP, the concentration will be restricted to no more than 1,000 mg/kg (equivalent to 0.1% w/w) in vinyl of children's toys and child-care articles where the vinyl can, in a reasonably foreseeable manner, be placed in the mouth of a child under four years of age. Pursuant to Section 5 of the *Hazardous Products Act*, this restriction on DINP came into force six months after the registration of phthalates regulations in December 2010.

Beyond the recent actions in the EU, the US and Canada, there are no regulatory restrictions on the use of DINP in consumer applications such as children's toys and child-care articles in Australia, Asia and other non-EU countries. This raises the possibility of import into Australia of children's products containing DINP manufactured in 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 nomination of phthalates to the NICNAS Candidate List from which chemicals are selected for assessment.

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

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

DINP is NOT currently listed in the following:

- the Safe Work Australia List of Designated Hazardous Substances contained in the Hazardous Substances Information System (HSIS, <http://hsis.ascc.gov.au>);
- the Standard for Uniform Scheduling of Medicines and Poisons (SUSMP, 2010); and
- the Australian Dangerous Goods Code (National Transport Commission, 2007).

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

2.3 Assessments by international bodies

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

- a European Union Risk Assessment Report (EURAR) on DINP (ECB, 2003);
- the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE, 2001);

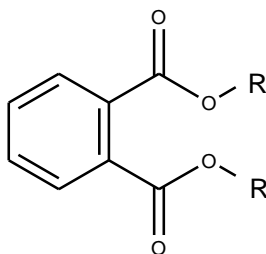
- a monograph on the potential human reproductive and developmental effects of DINP published by the Centre for the Evaluation of Risks to Human Reproduction (CERHR) (NTP–CERHR, 2003);
- risk assessment reports for DINP for use in consumer products (including toys) by the US Consumer Products Safety Commission (CPSC, 1998; 2010) and CSPC Chronic Hazard Advisory Panel (2001); and
- evaluation of new scientific evidence concerning the DINP restrictions contained in Annex XVII to Regulation EC No. 1907/2006 (REACH) by the ECHA (ECHA, 2010).

3. Identity and properties

Diisononyl phthalate (DINP) is listed on the Australian Inventory of Chemical Substances (AICS) as 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich and 1,2-benzenedicarboxylic acid, diisononyl ester.

3.1 Chemical identity

Chemical name:	1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich (68515-48-0) 1,2-benzenedicarboxylic acid, diisononyl ester (28553-12-0)
CAS No.:	68515-48-0; 28553-12-0
EINECS No.:	271-090-9; 249-079-5
Synonyms:	diisononyl phthalate (DINP) Esters, 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl, C9-rich 1,2-benzenedicarboxylic acid, 1,2-diisononyl ester
Molecular formula:	C ₂₆ H ₄₂ O ₄ [average]
Molecular weight:	420.6 [average]
Purity:	> 99.5%
Structural formula:	



CAS No: 68515-48-0

R = an undefined branched alkyl chain comprising eight to 10 carbon atoms, predominantly nine carbon atoms

CAS No: 28553-12-0

R = an undefined branched alkyl chain comprising nine carbon atoms

Note: DINP is not a single compound but a complex mixture containing mainly C9-branched isomers. The composition of CAS No. 68515-48-0 is represented as mixed phthalates with side-chains made up of 5–10% methyl ethyl hexanols, 45–55% dimethyl heptanols, 5–20% methyl octanols, 0–1% n-nonanol, and 15–25% isodecanol; and the composition of CAS No. 28553-12-0 is represented as mixed phthalates with side-chains made up of 5–10% methyl ethyl hexanols, 40–45% dimethyl heptanols, 35–40% methyl octanols, and 0–10% n-nonanol. Thus, diisononyl phthalate [side-chains of dimethyl heptanols (i.e. iso-nonanol)] makes up about 50% of the two ‘DINP’ mixtures that appear to be available on the market.

3.2 Physical and chemical properties

DINP is an oily, viscous liquid at standard temperature and pressure.

Table 3.1—Summary of physicochemical properties
(adapted from CERHR, 2003; ECB, 2003)

Property	Value
Physical state	Liquid
Melting point	ca. -50 °C
Boiling point	> 400 °C
Density	ca. 975 kg/m ³ (20 °C)
Vapour pressure	6 x 10 ⁻⁸ kPa (20 °C)
Water solubility	6 x 10 ⁻⁵ g/L (20 °C)
Partition co-efficient n-octanol/water (log K _{ow})	8.8
Henry's law constant	41.4 Pa·m ³ /mol
Flash point	> 200 °C

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

Conversion factors: 1 ppm = 17.24 mg/m³
 1 mg/m³ = 0.058 ppm

4. Manufacture, importation and use

4.1 Manufacture and importation

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

According to the NICNAS 2002 High Volume Industrial Chemical List (HVICL), the total import volume of DINP was in the range of 1,000 to 9,999 tonnes annually. DINP was not listed on the NICNAS 2006 HVICL. The total volume of DINP imported to Australia for industrial uses, according to responses to a call for information in 2004 on phthalates, was approximately 600 tonnes.

The Vinyl Council of Australia informed NICNAS that the current market consumption volume of DINP in Australia is between 1,600 and 2,000 tonnes per annum.

4.2 Uses of DINP

4.2.1 Uses in Australia

According to information collected by NICNAS through calls for information from introducers of DINP in 2004 and 2006, this chemical is used industrially in Australia for the manufacture of cable insulation, adhesives, laminations, PVC automotive products, sheets, films, surfactants, vinyl flooring, flexible PVC products (including gaskets and gumboots) and interface-backing for products such as carpets. It is also used in inks for screen printing (primarily for printed T-shirts).

During the calls for information on phthalates in 2004 and for this PEC declaration in 2006, the information obtained from Australian industry indicated that DINP was not used in cosmetics. There is no further information to indicate the likely use of DINP in cosmetic products in Australia. However, information did indicate other phthalates, such as DEP, were present in a variety of cosmetic types in the form of liquids, foams, creams, gels, aerosol sprays and bars/sticks. Most of the DINP imported to Australia is used in industrial applications and DINP is also present in imported articles, including PVC toys. Data from the 2006 voluntary call for information on phthalates in articles indicate that DINP is present in imported toys at a concentration range of 0.005% to 35%.

4.2.2 Uses overseas

The estimated consumption volume of DINP in Western Europe in 1994 was 107,000 tonnes per annum (ECB, 2003). The use of DINP has been reported to have constantly increased since 1994, but the precise total usage volume is difficult to ascertain from available information. DINP was pre-registered for all of the different tonnage bands under REACH regulation in February 2010 with a minimum estimated volume of 84,000 tonnes per year (ECHA, 2010). The assessment undertaken by the European Chemicals Bureau (ECB, 2003) and the DINP Information Centre (<http://www.dinp-facts.com/>) indicates that 95% of DINP is used as a plasticiser in PVC applications. The remaining 5% is used in non-PVC applications. More than half of the DINP used in non-PVC applications involves polymer-related uses (e.g. rubbers) and the remainder is used in inks and pigments, adhesives, sealants, paints and lacquers and lubricants. In a report on the use of phthalates in perfumes, a trace amount of DINP (up to 26 mg/kg or 0.0026%) was found in one of 36 perfumery products tested in the EU (Peters, 2005). A subsequent report on phthalates in consumer products suggested that this trace amount of DINP could be due to leaching during early stages of formulation from plastic manufacturing equipment (containers, pipes, pumps) or from plastic tubing during product packaging (SCCP, 2007). DINP is not listed in the Cosmetic Ingredient database (CosIng), a database on cosmetic ingredients contained in the EU Cosmetic Directive (EEC) No. 76/786/, Inventory of Cosmetic Ingredients (as amended) and Opinions on Cosmetic Ingredients of the Scientific Committee on Consumer Safety.

Consumption of DINP in the US was estimated to be 178,000 tonnes in 1998, and DINP production currently exceeds that of DEHP (CPSC, 2010). In the US, DINP is used as a general-purpose plasticiser with a broad range of applications. It is used in toy, construction and general

consumer markets. The range of end-use products containing DINP includes stationery, wood veneers, pool liners, tiles, sheets, artificial leather, coated fabrics, tarpaulins, conveyor belts, gloves, toys, traffic cones, tubing, garden hoses, wire and cables, shoes/shoe soles, underbody coatings, and sealants (carpet backing) (CERHR, 2003). The use of DINP in toys represents < % of total DINP consumption. Most of the toys imported into the US are manufactured by Asian companies (CPSC, 2010). DINP is not listed in the CIUCUS (Personal Care Products Council, 2011). The CIUCUS provides a compilation of ingredients that have documented use in cosmetics by the FDA. It is not also listed in ICIDH. The ICIDH is compiled by the Personal Care Products Council (2012), which provides a comprehensive international reference of descriptive and technical information about substances that have been identified as potential cosmetic ingredients.

4.2.3 Uses of phthalates and possibilities for substitution

Phthalates can be substituted for each other in certain applications. However, given the range of phthalate chemicals that exist, there are likely to be limits to substitutability for any particular application. Information on the use patterns of phthalates indicate generally that lower molecular weight phthalates are used as solvents whilst 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 physicochemical 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 increase in the octanol-water partition coefficient (K_{ow}) and a 10-order of magnitude decrease in vapour pressure. Water solubility is also inversely related to molecular weight and side-chain length (NICNAS, 2008b). Viscosity varies from 9 mPa.s for DEP to 52 mPa.s for DINP and up to 190 mPa.s for ditridecyl phthalate (Eastman, 2002).

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

Little information is available in open literature on the subject of substitutability of phthalates. A number of phthalates and their functions are listed in the ICIDH (2011), and DMP, DEP, DBP and DEHP all list functions as fragrance ingredient, plasticiser and solvent. However, the SCCP Opinion on phthalates in cosmetic products (SCCP, 2007) concludes that, among the phthalates found in a study of 36 perfumes (Peters, 2005), only DEP (up to 2.3%) and DMP (0.3%) are likely to have been deliberately added, with DCHP, DBP, DEHP, DIDP, DIBP, BBP and DINP (maximum concentration 0.0026%) likely to be present as impurities arising from leaching during manufacture or storage. This information relates to use in a sample of perfumes and there is no information available to extrapolate from perfumes to other cosmetics.

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

Therefore, while it is clear that phthalates can be considered to be substitutable by other phthalates of similar properties, there are likely to be limits on the extent to which dissimilar phthalates can be used. DINP is an HMW phthalate and thus it is not likely to substitute for DEP—an LMW phthalate commonly used in cosmetics. Furthermore, there is no evidence to suggest that the HMW DINP substitutes for the other lower molecular phthalates typically used in cosmetics. In the absence of information of DINP being used in cosmetics either in Australia or internationally, exposure assessment for DINP from the use of cosmetics was not undertaken in this assessment.

5. Public exposure

Public exposure to DINP is estimated only for the following consumer application:

- use in children's toys and child-care articles.

Although DINP was declared as a PEC for its actual and potential applications in children's toys, child-care articles and cosmetics, there is no evidence to suggest that DINP is used in cosmetic products in Australia currently or in the past. While there may be potential for use of DINP in cosmetic products based on the potential for substitution of phthalates, there are uncertainties over the substitutability of HMW phthalates such as DINP for low and mid molecular weight phthalates such as DEP and DBP, used predominantly as cosmetic ingredients. DINP is not listed as a cosmetic ingredient in the ICIDH (Personal Care Products Council, 2011). Cosmetic uses were not identified in the EURAR on DINP (ECB, 2003) or the monograph on the potential human reproductive and developmental effects of DINP (CERHR, 2003). Assessment of exposure to DINP from use in cosmetics will not be considered in this assessment.

Exposure estimates are derived to allow characterisation of the risks associated with the application of DINP in children's toys and child-care articles.

5.1 Methodology for assessing exposure

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

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

Exposure to DINP in children's toys and child-care articles was estimated for children via both the oral and the dermal routes.

Oral exposure was modelled by:

- estimation of highest concentrations of DINP in toys and child-care articles in Australia; and
- estimation of the available fraction of DINP based on the results of overseas studies of children's mouthing behaviour and the extractability of phthalate plasticisers under mouthing conditions.

Dermal exposure was modelled by:

- estimation of highest concentrations of DINP in toys and child-care articles in Australia;
- use of default values for exposed surface area and estimates of dermal contact time with toys; and
- use of the estimate of the migration rate of DINP from PVC matrix through the skin based on experimental data for PVC plasticised with DEHP (Section 6.1).

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

The uncertainties in the exposure assessment are discussed in the context of the risk characterisation in Section 8.3.

5.2 Children's toys and child-care articles

5.2.1 Sources of exposure

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

It should be noted that the overseas data on levels of phthalates in toys pre-date EU Directive 2005/84/EC prohibiting the use of DINP in children's toys that can be placed in the mouth at levels above 0.1%, effective January 2007 (Directive 2005/84/EC) and which is likely to have affected the use of DINP internationally. The limited Australian information obtained through a voluntary call for information in 2006 indicates that the concentration of DINP in toys available in Australia may be up to 35%. However, considering that the Australian information collected covers only a small proportion of available toys, and the current absence of restrictions on DINP content in toys in Australia and many other countries, the available overseas data have also been examined to establish a reasonable worst-case scenario of DINP exposure of children through the use of toys.

Chen (1998) conducted a study to identify phthalate-containing products (a total of 35 samples) that are likely to be mouthed by children in the US and to determine the amount of phthalate migration from these products using *in vitro* and *in vivo* tests. The products include soothers, teethingers, nipples, pacifiers, books, handbags and a variety of toys. *In vitro* tests were conducted by either shaking a PVC sample in a saliva stimulant or subjecting cut samples of PVC to impaction applied by a piston. For *in vivo* tests, human volunteers gently chewed/mouthed a polyethylene disk from a toy duck for four 15-minute intervals and saliva was collected after each chewing period. The study reported DINP to be the predominant phthalate found in children's toys, with content ranging from 15% to 54% by weight. DEHP and other phthalates—diisooctyl phthalate (DIOP) and di-n-nonyl phthalate (DnNP)—were also found.

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

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

DINP was the phthalate most frequently found in the toy samples (64%) and tended to be present at the highest concentration (up to 51% w/w). DEHP was the next most frequently found in the tested toys (up to 48%) with concentrations ranging from 0.008% to 35.5% w/w. However, few of the sampled toys contained DEHP as the dominant phthalate plasticiser (8%, with a variety of countries of origin), with the majority of the remainder having < 1% DEHP in conjunction with higher levels of DINP. Other phthalates found included diethyl phthalate (DEP), di-n-butyl phthalate (DBP), diisobutyl phthalate (DIBP), butylbenzyl phthalate (BBP), di-n-octyl phthalate (DnOP), DIOP, DnNP and di-isodecyl phthalate (DIDP). Variations between batches and the contamination of commercial and industrial mixes with other phthalates or other compounds were noted. Several phthalates were also found in concentrations too low to have a plasticising function. These phthalates may have been present as a constituent or contaminant of other phthalates,

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

The National Environment Research Institute (NERI) in Denmark also investigated the content of phthalates in toys and other articles for children up to three years of age (Rastogi & Worsoe, 2001; Rastogi et al., 2002). The content of DINP in the tested toys was found to range up to 41.9%.

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

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 toys were tested. All of the samples were reported to contain phthalates. The predominant phthalates in the soft toys were DINP and DEHP. DINP was found in 40% (six out of 15) of the soft toys and 44% (four out of nine) of the hard toys. The highest DINP concentration was 16.2% in a soft toy marketed for children aged three months to 18 months (Johnson et al., 2011).

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

The overall findings from the above studies indicated that phthalates were typically present in toys at weight concentrations of approximately 5% to 50%, with the predominant phthalates being DINP and DEHP. The DINP concentration in toys ranged up to 54%. Other phthalates such as DEP, DBP, DIBP, BBP, DnOP, DIOP, DnNP and DIDP were also found in toys.

5.2.2 Routes of exposure

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

When children mouth or chew child-care articles or toys, phthalate plasticisers can migrate into the saliva and be swallowed and absorbed in the gastro-intestinal (GI) tract or can be absorbed directly through the buccal mucosa. The amount of phthalate released from a product when it is mouthed or chewed is determined by the amount of time the product is in the child's mouth and the migration rate of phthalate from the product. The studies used for estimation of mouthing times and migration rates of phthalates from plastic articles under mouthing conditions are mostly performed on PVC that contains DINP, and are summarised in Appendix 2. 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 being the likely dominating factor. The phthalate migration rate from articles appears largely determined by the magnitude of the mechanical force applied to an article and the properties of the PVC grade comprising the article and less by the physicochemical characteristics or concentration of the particular phthalate.

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

Oral exposure of children to DINP from mouthing of toys was estimated from the typical bodyweight of children, estimated mouthing duration and phthalate migration rate from toys. The main estimate is for a six-month-old infant, based on the studies which demonstrate that six-month-old infants are within an age range showing maximum mouthing behaviour and have the lowest bodyweight in this age range (Appendix 2). The following assumptions were also used:

- A child of six months weighs 7.5 kg. The mean bodyweight is based on the 50th percentile weight of six-month-old children (combined sexes) (US EPA, 2006).
- The surface area of a child's open mouth and the typical surface of an article available for mouthing at any one time is approximately 10 cm² (LGC, 1998).
- The reasonable worst-case total time the child spends mouthing toys is 2.2 hours per day and a typical mouthing time is around 0.8 hours per day (Appendix 2).
- Phthalate bioavailability via the oral route is 100% (Section 7.1).

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

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

Where:

$D_{\text{int,oral}}$ = Internal dose via the oral route, µg/kg bw/d

M = Migration rate of DINP from toys, µg/cm²/h

S_{mouth} = Surface area of a child's open mouth, cm²

t = Mouthing time, hours

n = Frequency/day

B_{oral} = Bioavailability via the oral route, %

BW = Child bodyweight, kg

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

Table 5.1—Exposure parameters and estimated daily internal dose from oral exposure to children mouthing toys and child-care articles

	M (µg/cm ² /h)	BW (kg)	S_{mouth} (cm ²)	t · n* (h/d)	$D_{\text{int,oral}}$ (µg/kg bw/d)
Typical exposure scenario	26.03	7.5	10	0.8	27.8
Worst-case exposure scenario	57.93	7.5	10	2.2	169.9

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

The estimate of daily exposure using the worst-case scenario is comparable with the estimate in the EURAR (2003) of 200 µg/kg bw/d for oral exposure to DINP from the use of children's toys and child-care articles.

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

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

Limited quantitative absorption data are available for DINP. However, for the scenario of dermal absorption of DINP directly from plasticised PVC, which also involves the rate of migration of DINP from the PVC to the skin, the results of a study on DEHP plasticised PVC are more relevant. The migration rate of DINP from the plastic matrix to the skin has not been determined, and thus the study on DEHP where the effects of both migration through the plastic film and absorption through the skin are accounted for is likely to give a better estimate of dermal absorption directly from the PVC articles. Deisinger et al. (1998) investigated the skin absorption of DEHP from PVC film in rats. Sheets of PVC film (15 cm²) with ¹⁴C-DEHP (total of 40.4% DEHP w/w) were applied to shaved backs of eight male rats in two separate experiments. The mean dermal absorption of DEHP in rats was determined to be 0.24 µg/cm²/h (Section 6.1).

In *in vitro* tests, rat skin was determined to be four times more permeable to DEHP than human skin (Barber et al., 1992*; Scott et al., 1987; 1989 errata). Equivalent comparative *in vivo* data are not available. The rate of dermal absorption of 0.24 µg /cm²/h for DEHP, determined in the *in vivo* test in rats, is used for the exposure estimates. No information on relative permeability of adult and infant skin to DEHP or DINP under these conditions was available.

For this exposure route, the internal dose is dependent on the time handling the toys and the rate of dermal absorption. Dermal exposure to DINP was calculated based on the area of skin in contact with the toy, the duration of contact and the rate of dermal absorption of DINP through the skin.

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

- a child of six months weighs 7.5 kg (US EPA, 2006);
- the reasonable worst-case total time the child spends handling toys is 2.2 hours per day and a typical contact time is around 0.8 hours per day (Appendix 2); and
- the contact surface area is 100 cm² based on exposure to lips and hands (Exponent, Inc. 2007).

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

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

continued next page

Where:

$D_{\text{int,derm}}$ = Internal dose via the dermal route, µg/kg bw/d

R_{derm} = Dermal absorption rate of DINP in skin, µg/cm²/h

S_{derm} = Surface area of a child's lips and hands, cm²

t = Time of contact, hours

n = Frequency/day

BW = Bodyweight, kg

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

Table 5.2—Exposure parameters and estimated daily internal doses from dermal exposure to children mouthing toys and child-care articles

	R_{derm} (µg/cm ² /h)	BW (kg)	S_{derm} (cm ²)	t · n* (h/d)	D_{int,derm} (µg/kg bw/d)
Typical exposure scenario	0.24	7.5	100	0.8	2.6
Worst-case exposure scenario	0.24	7.5	100	2.2	7.0

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

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

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

Table 5.3—Estimated total internal exposure for children

Route of exposure	Typical D_{int} (µg/kg bw/d)	Worst-case D_{int} (µg/kg bw/d)
Oral	27.8	169.9
Dermal	2.6	7.0
Combined	30.4	176.9

5.3 Biomonitoring data

Biomonitoring data for a particular chemical or its metabolites represent exposure to the chemical from all sources and pathways. The toxicokinetics of DINP demonstrate that DINP 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. For this purpose modelling is most suitable. However, population biomonitoring data are useful in determining whether the exposures calculated through modelling are within the observed range of exposure and comparable with the integrated exposure of the population.

There is limited reliable biomonitoring data available for DINP. This is largely due to early studies looking at the single metabolite, monoisononyl phthalate (MINP). Later studies have demonstrated that MINP is only a minor metabolite of DINP, with a range of other oxidative metabolites dominating. The difficulty of monitoring for DINP is compounded by the fact that there is a range of structures for the isononyl group, such that oxidative metabolites of each of the possible structures will be found (Koch & Angerer, 2007a; Silva et al., 2006a).

Silva et al. (2006b) examined the urinary levels of MINP and three additional oxidative metabolites, described as mono(carboxyisooctyl) phthalate (MCIOP), mono(hydroxyisononyl) phthalate (MHINP) and mono(oxoisononyl) phthalate (MOINP), in 129 subjects from the general US adult population. Levels of MHINP varied from 1.4–202.7 ng/mL urine (median 13.2 ng/mL

urine), with 5% of the samples having > 43.7 ng/mL urine. For MCIOP, the range was less than the limit of detection (LOD) to 310.8 ng/mL urine (median 8.4 ng/mL urine) and for MOINP the range was < LOD to 201.7 ng/mL (median 1.2 ng/mL urine). The wide range between the median and the outliers in this study indicates that some members of the population have been exposed to much higher DINP doses than the population average. A graphical comparison of median levels of DINP metabolites and metabolites of the common phthalate DEHP in the same study population indicates similar levels for some individual metabolites.

However, conclusions could not be drawn about the relative levels of DEHP and DINP exposures in this study due to uncertainty about the range of DINP metabolites that might be present. Koch & Angerer (2007a) concluded that 43.6% of an oral dose of DINP was recovered as MINP or one of three specific oxidative metabolites and that recovery of individual metabolites was lower than recovery of DEHP metabolites. This study confirms that DINP exposure is likely to be underestimated compared with DEHP exposure by urinary biomonitoring.

More recent biomonitoring studies not discussed above are presented in Table 5.4.

Table 5.4—Other biomonitoring studies of DINP metabolites detected in urine (in µg/L)

Study	Population group	Metabolites	Median	Maximum
Becker et al. (2009)	599 children aged 3–14 years in Germany	7OH-MMeOP 7oxo-MMeOP 7cx-MMeHP	11.0 5.4 12.7	198 86.7 195
Boas et al. (2010)	845 Danish children aged 4–9 years	MCIOP MINP	7.2 (boys), 6.5 (girls) 0.6 (boys), 0.5 (girls)	2,063 (boys), 598 (girls) 1,100 (boys), 61 (girls)
Lin et al. (2010)	100 pregnant women, and children aged 2 (n = 30) and 5 (n = 59) years in Taiwan	OH-MINP oxo-MINP cx-MINP	7.94 (5 years), 6.15 (2 years) 4.3 (5 years), 3.84 (2 years) 9.42 (5 years), 9.36 (2 years)	364 (women), 1,188 (5 years), 398.84 (2 years) 288 (women), 352.62 (5 years), 287.46 (2 years) 281 (women), 915.6 (5 years), 932.74 (2 years)
Calafat et al. (2011)	2,548 people aged 6 years and above (2005–2006 US NHANES survey)	MCIOP MINP		4,961 148.1

The urinary metabolite concentrations discussed above showed significant variations owing to the differences in the study design and the metabolites chosen as biomarkers. The range of maximum levels of DINP metabolites detected in urine was from 86.7 µg/L to 4,961 µg/L.

Wittasek & Angerer (2008) examined non-oxidised and oxidative metabolites of a range of phthalates in 102 subjects aged between six and 80 in Germany and used information on metabolism to calculate intakes of the parent phthalates. Median DINP intake was calculated as 0.6 µg/kg bw/d, with the maximum intake being 36.8 µg/kg bw/d, similar to the maximum calculated DEHP intake in the same population of 42.2 µg/kg bw/d.

Kransler et al. (2012) presented a comprehensive review of DINP internal exposure intakes from calculations of various studies and from urinary metabolite concentrations based on population biomonitoring data. The review concluded that the mean DINP exposure intake based on direct and indirect estimates is within the range of 1 to 2 µg/kg bw/d. The highest calculated 95th percentile intakes were from the Becker et al. (2009) German biomonitoring data, with the following values estimated (in µg/kg bw/d) from this review: 38.85 (3–5 years), 34.69 (6–8 years), 39.62 (9–11 years), and 12.00 (12–14 years).

In a similar study, Frederiksen et al. (2011) analysed phthalate metabolites from urine of 129 Danish children and adolescents, with ages ranging from six to 21 years. The median and maximum DINP intakes were 1.7 µg/kg bw/d and 11.9 µg/kg bw/d, respectively.

The US Centers for Disease Control and Prevention (CDC) presented the ongoing evaluation of the levels of environmental chemicals the US population is exposed to by the use of biomonitoring and divided into age groups (6–11 years, 12–19 years, and 20 years above) (CDC, 2009). All of the median urinary MINP levels for the years 1999–2000, 2001–2002 and 2003–2004 for all age groups were lower than the metabolite levels observed by Wittasek & Angerer (2008) and Frederiksen et al. (2011).

The US CDC analysed phthalate metabolites as part of the 2007–2008 National Health and Nutrition Examination Survey (NHANES) from 2604 individuals aged six years and above. The range of values of urinary metabolite concentrations of MINP and MCOP were 0.8712 µg/L to 214.83 µg/L and 0.49 µg/L to 776.9 µg/L respectively (CDC, 2009). The calculated internal exposures based on urinary metabolite concentrations of the CDC (2009) study were estimated to have mean and 95th percentile values of 1.45 µg/kg bw/d and 11.43 µg/kg bw/d respectively (Kransler et al., 2012).

The calculated reasonable worst-case DINP exposure from toys and child-care articles in this assessment is greater than that from the German biomonitoring data of the DINP metabolites. There is an absence of biomonitoring data for the population expected to have maximum exposure through mouthing toys and child-care articles—infants aged six months. However, the calculated exposure from the typical mouthing scenario is close to the maximum intake calculated by Wittasek & Angerer (2008) for a population where the worst-case exposure pathway is not expected to be relevant.

The lack of biomonitoring data for infants means that it is not possible to compare and validate the worst-case estimate for infant-specific behaviour.

6. Human health hazard assessment

The Existing Chemical Hazard Assessment Report on DINP was published by NICNAS in June 2008 (NICNAS, 2008a) using as data sources the key international reviews prepared by (i) the European Chemicals Bureau (ECB, 2003); and (ii) the Center for the Evaluation of Risks to Human Reproduction (CERHR, 2003). 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 DINP-related literature up to June 2012.

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

6.1 Kinetics and metabolism

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

6.1.1 Absorption

Absorption via the oral route

In an early study, male albino rats were dosed orally with ^{14}C -DINP at approximately 2,500 mg/kg bw/d for 6 days. Within 72 hours, about 85% of the administered dose was excreted in the faeces and 12% in the urine, most within the first 24 hours. Given the high level of radioactivity was recovered in faeces, it was suggested that the absorption was saturated or incomplete at this dose level (Hazleton, 1972*; ECB, 2003).

In another oral kinetic study, Fischer 344 (F344) rats were administered by gavage with a single dose of ^{14}C -DINP at 50 or 500 mg/kg bw (four males and 20 females per dose) or five daily doses of 50, 150 or 500 mg/kg bw (15 males per dose). Excretion after 72 hours was 49% to 39% (at low and high dose respectively) in the urine and 51% (at either dose) in the faeces, with the majority excreted within the first 24 hours and no great differences between genders. At the high dose (500 mg/kg bw), more radioactivity recovered in faeces than in urine indicated that the absorption was also saturated. Following repeated exposures, approximately 66% of the administered dose was excreted in the urine at all doses after 72 hours (McKee et al., 2002).

A male volunteer receiving a single oral dose of 1.27 mg/kg bw d_4 -DINP (deuterium-labelled) showed a renal excretion of 40% and 44% after 24 hours and 48 hours respectively. The authors noted that the total renal excretion of DINP metabolites would be higher, as these quantities were determined only for four main metabolites of DINP (Koch & Angerer, 2007a ND).

In a more recent study using dose levels of 0.013 and 0.121 mg/kg bw d_4 -DINP (co-administered orally with d_4 -DEHP) in 20 healthy volunteers, the cumulative excreted amount of the same four metabolites after 48 hours was 33% and was considered in agreement between these two human studies, with more than 90% of the excretion occurring within the first 24 hours and the remainder in the 24-hour to 48-hour period (Anderson et al., 2011 ND).

In summary, based on the urinary excretion data, the oral absorption of DINP is rapid and may become saturated or incomplete following high single doses and repeated dosing. Information on the total excretion via all routes and/or the extent of faecal excretion, whether as the result of bile elimination or saturated urinary excretion, is lacking. Taking all together, bioavailability of DINP via the oral exposure is assumed to be 100% for both adults and children.

Absorption via the dermal route

DINP was applied to the shaved backs of three groups of male F344 rats. Two groups received a single application of radiolabelled ^{14}C -DINP (six animals at 1.2 mL/kg bw and three animals at 0.6 mL/kg bw), whereas the other group were pre-treated (conditioned) with non-labelled DINP for 3 days prior to the application of labelled DINP (six animals at 1.2 mL/kg bw). All applications were occlusive and remained on the skin for 1, 3 or 7 days prior to sacrifice. The dermal absorption rate was slow as indicated by the total recovery (approximately 0.3% to 0.6% per day) of the applied dose in urine, faeces, GI tract and tissues. There were no major differences in radioactivity in the tissues or excreta in all treated animals (conditioned or not conditioned or at low dose). The total absorption ranged from 2% to 4% of the applied dose over the 7-day period. Most of the radioactivity was recovered from the application sites (93% to 101%) (McKee et al., 2002).

In a study to examine the dermal absorption of various phthalates (but not DINP) with different alkyl side-chain lengths, ^{14}C -labelled phthalates were applied and kept occluded to the back skin of male F344 rats (5–8 mg/cm²). Taking urinary and faecal excretion as an index of dermal absorption, up to 50% of the applied DEP (side-chain length of 2), but approximately only 5% of DEHP (side-chain length of 8) and 0.5% of DIDP (side-chain length of 10) were absorbed after 7 days, suggesting that the absorption decreased as the side-chain length increased or became branched, with a shift noted in the route of excretion from urine to faeces (Elsisi et al., 1989). DINP would be expected to show dermal absorption greater than DIDP but less than DEHP.

In an *in vitro* study, the comparative percutaneous absorption of four phthalates through human and rat skin (epidermal membranes) was evaluated. Results showed that human skin was consistently less permeable than the rat skin and, as the phthalates became more lipophilic and less hydrophilic, the absorption was reduced. For DEHP, the highest molecular weight phthalate tested, the rate of absorption through human skin was approximately four times less than through rat skin (5.6 vs 22.4 µg/cm²/h) (Scott et al., 1987; 1989 errata ND).

A similar *in vitro* percutaneous absorption ratio of 4.2 was also obtained for DEHP using human stratum corneum versus full thickness rat skin (0.10 vs 0.42 µg/cm²/h) (Barber et al., 1992 ND).

Deisinger et al. (1998) investigated the *in vivo* percutaneous absorption of DEHP leaching from plastics. Sheets of PVC film (15 cm²) plasticised with ^{14}C -DEHP (40.4% w/w) were applied to shaved backs of 8 male F344 rats in two separate experiments. In Study I, the film was removed at 24 hours, the application site was rewrapped and the excreta were collected daily for 7 days, while Study II terminated at 24 hours, the application site was washed and the excreta were collected. A similar absorption rate of 0.24 µg/cm²/h was calculated from both studies based on the sum of radioactivity at the exposure site and that absorbed systemically and then eliminated. This study examines the combined rate of phthalate migration from PVC and absorption through skin, although the relative rates between the two processes cannot be determined.

In summary, the dermal absorption of DINP is low (2% to 4% over 7 days) in rats. Absorption of DINP through human skin is expected to be lower than rat skin based on *in vitro* studies.

Quantitative dermal absorption data for DINP are limited, thus the mean dermal absorption rate of 0.24 µg/cm²/h for DEHP migrated from the PVC film is considered appropriate to apply to DINP without the need for use of a correction factor for extrapolating from rats to humans.

6.1.2 Distribution

Following oral administration to male albino rats of six daily doses of 2,500 mg/kg bw ^{14}C -DINP, only trace amounts of the radioactivity were found in tissues after 72 hours, with the liver containing the highest level (i.e. 0.01% of the administered dose) (Hazleton, 1972*; ECB, 2003).

In male and female rats given single doses of 50 or 500 mg/kg bw or five daily doses of 50, 150 or 500 mg/kg bw ^{14}C -DINP, radioactivity peaked at 1–4 hours and appeared higher after repeated than single dosing. The levels were greatest in the liver, followed by blood and kidney, and declined to 6% to 24% of peak values by 24 hours. Levels in other tissues such as fat, muscle and testes were much lower and also declined rapidly over time (McKee et al., 2002).

Therefore, there appear to be no gender differences in tissue distribution and no evidence of either persistence or accumulation in any organ.

6.1.3 Metabolism

After oral dosing of ^{14}C -DINP in F344 rats, metabolites excreted in the urine were mainly oxidation products (unidentified) (78–85%) and phthalic acid (9–21%), while in the faeces DINP was the major form recovered (46–67%), with the remainder as MINP (19–21%) and oxidation products (12–31%) and a small amount of phthalic acid (< 1%). Formation of oxidation products appeared to increase following high single doses (i.e. 500 mg/kg bw) and repeated dosing (i.e. five daily doses of 50 or 500 mg/kg bw), while the hydrolysis to phthalic acid decreased (McKee et al., 2002; CERHR, 2003).

In the urine of Sprague-Dawley (SD) rats given a single gavage dose of 300 mg/kg bw ^{13}C -DINP (isomeric mixtures of either CAS No. 68515-48-0 or CAS No. 28553-12-0), oxidative metabolites of DINP identified included carboxy-MINP (mono(carboxyisononyl) phthalate (MCINP), mean 127 $\mu\text{g/mL}$) as the major metabolite, followed by hydroxy-MINP (MHINP, 12 $\mu\text{g/mL}$) and oxo-MINP (MOINP, 5 $\mu\text{g/mL}$). Although most metabolites contained the same alkyl side-chain length of 9 as the parent compound, metabolites with shorter or longer side-chains were also identified at low levels, including metabolites of diisooctyl phthalate (DIOP) and diisodecyl phthalate (DIDP). A very small percentage of the administered DINP was excreted in the urine as the hydrolytic metabolites such as phthalic acid and MINP. It was also shown that metabolism of DINP yielded the same types of metabolites regardless of its isomeric mixtures (Silva et al., 2006a ND).

Silva et al. (2006b ND) also measured MINP and the three oxidative metabolites in single urine samples from 129 adults living in US with no known exposure to DINP. Although MINP was not detectable, the oxidative metabolites were present in all samples, with their concentrations highly correlated with each other, confirming the same parent precursor (DINP). In this human study, the major urinary metabolite was MHINP (median 13.2 ng/mL), followed by MCINP (8.4 ng/mL) and MOINP (1.2 ng/mL). While MHINP was excreted as either a conjugate (glucuronidated) or a free form equally, MCINP was excreted mostly as free form and MOINP mostly glucuronidated.

Forty-eight hours after single oral doses of d_4 -DINP in one or 20 human subjects, MHINP was also the major metabolite recovered in the urine, followed by MCINP, MOINP, and MINP with the ratios of 20:11:11:2 (totalling 44% of the dose) or 12:11:7:3 (totalling 33%) respectively (Koch & Angerer, 2007a ND; Anderson et al., 2011 ND).

However, in 25 and 102 spot urine samples taken from the general German population not occupationally exposed to phthalates, metabolite concentrations were highest for MCINP, then MHINP and MOINP (median 5.0, 2.5, and 1.3 ng/mL and 4.0, 2.0 and 1.3 ng/mL respectively) (Koch et al., 2007b ND; Wittassek & Angerer, 2008 ND). Higher concentrations for MHINP than for MOINP (2.0 vs 1.0 ng/mL) were also found from a retrospective biomonitoring study of 634 German students, age range 20 to 29 years (Wittassek et al., 2007 ND).

In 399 urine samples collected over seven to eight consecutive days from 50 German adults not occupationally exposed to phthalates, the median concentrations of MHINP and MOINP were 5.6 and 3.1 ng/mL respectively. Quantification of other DINP metabolites was not examined in this study. Phthalate metabolite levels were shown to be unaffected by sex or age but varied considerably day by day within individuals, and thereby the authors suggested that exposure assessment should not be based on a single urine measurement (Fromme et al., 2007 ND).

In summary, after exposure DINP is primarily de-esterified to the monoester MINP, which is further metabolised by oxidation to form oxidative metabolites (mainly MCINP, MHINP and MOINP) or by hydrolysis to phthalic acid. MCINP is excreted mostly as free form, MOINP mostly glucuronidated, and MHINP equally in either form. This metabolic profile of DINP is considered similar to those of DEHP and other HMW phthalates, with the monoester being only a minor urinary metabolite. In addition, although the ratios between DINP metabolites differed between US

and German populations or after exposure of rats to different isomeric mixtures, the same types of metabolites were observed and highly correlated with each other, confirming a common precursor.

6.1.4 Elimination and excretion

In rats, orally administered ^{14}C -DINP (2,500 mg/kg bw/d for 6 days) was rapidly excreted with 85% in faeces and 12% in the urine (Hazleton, 1972*; ECB, 2003). In another study, excretion of radioactivity at 72 hours after low doses (50 mg/kg bw) was about in equal amounts by either route, but more was excreted in faeces than in urine after high doses (500 mg/kg bw). Following repeated oral exposures, radioactivity recovered was higher in urine than in faeces at all doses (50, 150 or 500 mg/kg bw) with the majority excreted within the first 24 hours, similarly to single exposures. Excretion after a single dermal application of ^{14}C -DINP was higher in urine than in faeces. Faecal excretion of radioactivity could result from bile elimination and saturated GI absorption (i.e. excretion of unabsorbed DINP) (McKee et al., 2002).

Based on the urinary toxicokinetics in rats dosed with 300 mg/kg bw of either DINP isomeric mixtures, excretion was biphasic and relatively fast during the first 24 hours, with the half-lives for elimination of MCINP, MOINP and MHINP being 7.6, 8.3 and 8.6 hours respectively (Silva et al., 2006a ND).

In a human volunteer, elimination of DINP metabolites also followed a biphasic pattern after single oral doses of d₄-DINP. For the first phase (8–24 hours post dosing), half-lives were estimated as 3 hours for the monoester MINP and 5 hours for the oxidative metabolites. In the second phase (beginning 24 hours post dosing), estimated half-lives were 5 hours for MINP, 12 hours for MHINP and MOINP and 18 hours for MCINP (Koch & Angerer, 2007a ND). In another human volunteer study with 20 subjects (Anderson et al., 2011 ND), more than 90% of the four main metabolites were collected in the urine during the first 24 hours and the remainder in the 24–48 hour period, with half-lives of 4–8 hours.

Overall, elimination of DINP and its metabolites after oral exposure is rapid and almost complete within the first 24 hours. Following single doses, about equal amounts are excreted by urinary and faecal routes at low doses, but more is excreted in faeces at high doses. Following repeated doses, excretion is higher in urine than in faeces. The urinary excretion in both rats and humans shows a biphasic pattern with an initial elimination phase occurring 8–24 hours post dosing and a second elimination phase commencing at 24 hours post dosing. Excretion after dermal exposure is higher in urine than in faeces but at a much slower rate. The presence of radioactivity in the faeces also implies excretion via the bile.

6.2 Effects on laboratory animals and other test systems

6.2.1 Acute toxicity

The acute toxicity of DINP has been evaluated in a number of species via the oral, dermal and inhalation routes of administration.

In acute oral studies (up to 40,000 mg/kg/bw) in rats, findings consisted of laboured respiration, dyspnea, apathy, alopecia, spastic gait, piloerection, tremors and organ discolouration. Moderate erythema and slight desquamation were reported following dermal application of up to 3,160 mg/kg DINP in rabbits. No mortality, bodyweight changes, gross lesions or microscopic alterations of the lungs were observed in rats following aerosol exposure of 4.4 mg/L of air during 4 hours. LD₅₀ and LC₅₀ values derived from these studies are shown in Table 6.1.

DINP has low acute oral (LD₅₀ > 10,000 mg/kg bw), dermal (LD₅₀ > 3,160 mg/kg bw) and inhalation toxicity (LC₅₀ > 4.4 mg/L).

Table 6.1—Summary of acute toxicity studies on DINP (adapted from ECB, 2003)

Study	Species	Results (LD50/LC50)	Test substances	References
Oral	Rat	> 10,000 mg/kg bw	CAS No. 68515-48-0	Hazleton (1968c*)
		> 50,000 mg/kg bw	CAS No. not stated	Hazleton (1980b*)
		> 40,000 mg/kg bw	CAS No. 28553-12-0	Midwest Research Institute (1981*)
		> 10,000 mg/kg bw	CAS No. 28553-12-0	BASF (1981a*)
		> 10,000 mg/kg bw	CAS No. 28553-12-0	Hüls (1985a*)
Inhalation (4 hours)	Rat	> 4.4 mg/L of air (analytical)	CAS No. not stated	Hazleton (1980a*)
Dermal	Rabbit	> 3,160 mg/kg bw	CAS No. 68515-48-0	Hazleton (1968a*)

Note: only validated studies were included.

6.2.2 Skin and eye irritation

Skin irritation

A study was conducted using undiluted DINP (CAS No. 68515-48-0) applied for 4 hours to the clipped intact skin of six male New Zealand white rabbits with a semi-occlusive dressing, followed by an observation period of 72 hours (Exxon Biomedical Sciences, 1996a*; ECB, 2003). One rabbit showed very slight erythema at 1 hour and another at 24 hours. All rabbits were free of erythema and oedema during the remainder of the study.

Two other studies on undiluted DINP (CAS No. 28553-12-0) were conducted in rabbits, including a study involving 24 hours exposure to abraded skin. Only slight erythema and oedema were observed (BASF, 1981b*; Hüls, 1985b*; ECB, 2003). All skin irritation effects were reversible at the end of the study period.

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

Eye irritation

Following single ocular application of undiluted DINP (CAS No. 68515-48-0) in six male and six female albino rabbits, irritation was confined to the conjunctivae which consisted of marked redness and slight discharge at 1 and 4 hours (score of 3), and slight redness only (moderate in one case) at 24 hours (score of 1). By 48 or 72 hours the irritation had completely subsided in all cases (Hazleton, 1968b*; ECB, 2003).

Single application of undiluted DINP (CAS No. 28553-12-0) to two male and four female white Vienna rabbits caused slight conjunctival redness (mean score 0.83) at 24 hours only and slight corneal opacity (mean score 0.5) at 72 hours only. The iris was unaffected. The reversibility of the corneal effects was not determined (BASF, 1981c*; ECB, 2003).

Another study (Hüls, 1985c*; ECB, 2003) on DINP (CAS No. 28553-12-0; undiluted) was conducted on small white Russian rabbits (three males and three females). There was no effect on the cornea and iris but, at 1 hour post exposure, slight to medium redness of the conjunctivae, accompanied by some discharge, was observed. The absolute score was 4.33 at this time, but returned to 0.33 at 24 hours and 0 at later times. The irritation index was calculated as 1.17/110.

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

6.2.3 Skin sensitisation

The skin sensitisation potential of DINP has been investigated using a number of standardised guinea pig test methods and other skin sensitivity tests. Data for respiratory sensitisation are not available.

Two Buehler tests on female guinea pigs using undiluted DINP (CAS No. 68515-48-0) were reported.

The earlier study was conducted of 40 animals (20 control and 20 treated) under occlusive bandaging, with a challenge application of undiluted DINP at 5% in peanut oil. Some evidence of sensitisation was seen and score 2 erythema was observed at day 37 in three of 20 animals (c.f. four of 10 control animals with score 1 on the same day) (Exxon Biomedical Sciences, 1992*; ECB, 2003). A second Buehler test was conducted in 20 control and 20 treated animals using undiluted DINP (CAS No. 68515-48-0) under occlusive bandaging, with a challenge application of undiluted DINP. No evidence of skin sensitisation was observed (Huntingdon Research Centre, 1998*; ECB, 2003).

In a mouse study (Larsen et al., 2002*; ECHA, 2010), the adjuvant effects of several phthalates including DINP were assessed by subcutaneously injecting concentrations of 2, 20, 200 or 2,000 µg/ml in the neck region of BALB/cJ mice together with ovalbumin. Additionally the mice were administered with either one or two booster injections of ovalbumin alone. For DINP, after the first booster injection, there was a significant adjuvant effect on IgE (Immunoglobulin E) and IgG (Immunoglobulin G) antibody levels at 200 mg/ml. After the second booster injection, there was dose-dependent increased production of IgG antibody level both at 200 and 2,000 mg/ml. No increase in IgE antibody levels was reported.

A study showed no significant elevations in total serum IgE, IL-4 or IL-13 (Interleukin-4 or 13) following dermal administration of undiluted DINP (CAS No. 68515-48-0) to B6C3F1 mice. Trimellitic anhydride, used as the positive control, showed statistically significant increases in all parameters (Butala et al., 2004).

In another mouse study (Lee MH et al., 2004 ND), the effects of DEHP and DINP on IL-4 production in CD4+ T cells (T helper cells) and IgE levels in serum *in vitro* and *in vivo* were studied. DINP significantly increased IL-4 production in activated CD4+ T cells and IgE levels. DINP also enhanced the activation of IL-4 production in EL4+ T cells via stimulation of NF-AT (nuclear factor of activated T cells) binding activity (Lee MH et al., 2004 ND). The results suggest that DINP elicited allergic responses via the enhancement of IL-4 production by CD4+ T cells.

The hypersensitisation potential of phthalates including DINP was tested in mice epicutaneously treated with fluorescein isothiocyanate (FITC). DINP did not show any hypersensitisation properties, compared with other phthalates tested (Imai et al., 2006 ND).

The effects of DINP on allergic diseases (e.g. atopic dermatitis) were investigated in mice (Koike et al., 2010 ND). DINP (doses 0, 0.15, 1.5, 15 or 150 mg/kg/d) was injected intraperitoneally. At 15 mg/kg/d, DINP caused aggravation of atopic dermatitis (AD)-like skin lesions which were consistent with eosinophilic inflammation, mast cell degranulation and thymic stromal lymphopoietin (TSLP) expression in the inflamed ear. These effects were mediated through the TSLP-related activation dendritic cells and by direct or indirect activation of the immune cells.

The new studies give some evidence of sensitising potential of DINP but these studies did not use standardised tests and would need to be validated for reliability. Overall, DINP shows no or only minimal skin sensitisation potential.

6.2.4 Repeat-dose toxicity

Several studies have been conducted with DINP in various animal species via the oral and dermal routes.

Oral route

Oral, repeat-dose studies on DINP were conducted in various animal species. A number of studies were conducted in rats to assess the effect of DINP on peroxisomal proliferation. A study on monkeys was conducted to elucidate the human relevance of liver effects observed in rats and mice. The findings and observations reported below do not cover neoplastic effects, which are reported separately in the carcinogenicity section (Section 6.2.6).

Conclusions from key studies are outlined below and summarised in Table 6.2.

Rats

A 13-week dietary study in Fischer 344 rats (15/sex/dose) administered DINP at 0, 0.1, 0.3, 0.6, 1 and 2% in the diet (approximately 77, 227, 460, 767, 1,554 mg/kg bw/d). It showed statistically significant increases in liver weights (dose related), liver enzymes and kidney weights with dose-related organ discolouration and urine chemistry changes consistent with organ toxicity from 0.3% and above. Statistically significant (dose-related) decreases in triglyceride (from 0.6%) and cholesterol (from 0.3%) levels were also reported (Bio/Dynamics 1982a*; ECB, 2003). The NOAEL was 0.1% (77 mg/kg bw/d) based on the increase in kidney and liver weights, and the decrease in cholesterol level at 0.3% (227 mg/kg bw/d).

Another 13-week dietary study in Fischer 344 rats (10/sex/dose) using doses of 0, 2,500, 5,000, 10,000, 20,000 ppm (approximately 0, 176, 354, 719, 1,545 (males) and 218, 438, 823, 1,687 mg/kg bw/d (females)) showed significantly increased absolute and/or relative liver and kidney weights from 2,500 ppm with changes in haematological and urine chemistry parameters from 5,000 ppm. Hepatocellular changes at 20,000 ppm and dose-related increases in granular casts and regenerative/basophilic tubules in kidney from 5,000 ppm were also noted. No NOAEL was identified. The LOAEL was 2,500 ppm (176–218 mg/kg bw/d) based on the increases in liver and kidney weights in males and females (Hazleton, 1991a*; ECB, 2003).

In a combined chronic/carcinogenicity study, Fischer 344 rats (110/sex/group) were fed diets containing DINP (CAS No. 68515-48-0) at 15, 152 and 307 mg/kg bw/d (males) and 18, 184 and 375 mg/kg bw/d (females) (0, 0.03, 0.3, 0.6%) for two years (Exxon Biomedical Sciences, 1986*; Lington et al., 1997). Preselected groups of 10 rats/sex/group were sacrificed after 6, 12 and 18 months on study. The remaining animals were sacrificed at 24 months (terminal sacrifice).

Both males and females from the mid-dose (152–184 mg/kg) and high-dose (307–375 mg/kg) groups exhibited statistically significant, dose-related increases in relative liver and kidney weights throughout most of the treatment period, including at study termination. At this time point, relative liver weight increases were approximately 31%. Absolute liver and kidney weights also demonstrated similar trends. At study termination, statistically significant increases in absolute and relative spleen weights and relative (but not absolute) adrenal weights were observed at the high dose (307–375 mg/kg) in both sexes. No treatment-related changes were observed in the absolute or relative weights for ovaries, testes, brain, heart or thyroid/parathyroid. Statistically significant decreases in red blood cell count, haemoglobin concentrations and haematocrit were seen in high-dose males (307 mg/kg) only at study termination. In addition, statistically significant increases in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase were seen in the mid- and high-dose males at some study intervals.

In the kidneys, despite relative organ weight increases of approximately 20% in high-dose animals at study termination, no clear treatment-related histological effects were reported. Some serum chemistry parameters were reportedly increased—e.g. albumin/globulin ratio and creatinine concentration—but were judged not to be of biological significance due to a lack of dose response.

At terminal sacrifice, increased incidences of non-neoplastic lesions in the liver were observed including regenerative nodules, hepatopathy associated with leukaemia, focal necrosis and spongiosis hepatitis in both sexes at mid and high doses (307–375 mg/kg). Hepatocellular enlargement was also observed in both sexes at high doses. No morphological evidence of peroxisome proliferation in the liver was found, even at the highest dose. A NOAEL was identified

for all biological endpoints of 15 and 18 mg/kg bw/d in males and females respectively (LOAELs of 152 and 184 mg/kg bw/d in males and females respectively).

In a 2-year dietary carcinogenicity study employing Fischer 344 rats (70–85/sex/group), DINP (CAS No. 68515-48-0) was administered at 0, 500, 1,500, 6,000 and 12,000 ppm (approx 0, 29–36, 88–108, 358–442, 733–885 mg/kg bw/d males–females respectively) for 104 weeks (Aristech Chemical Corporation, 1994*; Moore, 1998a*; ECB, 2003). A recovery high-dose group of 55 rats/sex was administered 12,000 ppm for 78 weeks followed by a 26-week recovery period. Additional analyses were conducted at weeks 1, 2, 13, 79 and 104 to evaluate chemically induced cell proliferation and peroxisome proliferation in the livers of the appropriate dose groups.

The liver and kidney were target organs for DINP. In both sexes, livers were enlarged with granular/pitted appearances at 6,000 ppm and 12,000 ppm. Statistically significant increases in mean absolute and/or relative liver weights were observed during the study and at termination. After one week of treatment, cell proliferation in the liver indicated by increases in number of mitotic cells and palmitoyl-CoA oxidase activity was observed in both sexes at the high dose. However, at subsequent time points, only diffuse hepatocellular enlargement was noted with no increase in the number of mitotic cells. At study termination, diffuse hepatocellular enlargement was observed in both sexes at high dose (12,000 ppm), but palmitoyl-CoA oxidase activity was again significantly elevated in both sexes at high dose (12,000 ppm) and in females at mid high dose (6,000 ppm), indicating peroxisome proliferation but not cellular proliferation was occurring at this time point. Palmitoyl-CoA oxidase activity was not evaluated in the recovery dose group, hence reversibility of this effect was not examined.

Liver enlargement appeared reversible, with absolute and relative liver weights in a high-dose recovery group comparable to control values. Increased serum AST and ALT were observed from 1,500 ppm in females (week 78) and from 6,000 ppm in both sexes from week 52 onwards. Increased liver cytoplasmic eosinophilia in both sexes at the highest dose and increased pigment in Kupffer cells/bile canaliculi from 6,000 ppm in both sexes were also observed. A subsequent review of liver lesions reportedly confirmed histopathological observations including a treatment-related increased incidence of spongiosis hepatitis in male rats only from 6,000 ppm at study termination.

Kidney effects were also observed in both sexes, consisting of increased absolute and/or relative kidney weights in both sexes and some related effects, more marked in the males (increased serum urea nitrogen, increased urine volume and decreased urine potassium, calcium, creatinine and chloride, suggesting compromised tubular function) from week 79 up to the termination of the study. Histologically, kidney changes at study termination consisted of mineralisation of the renal papilla at 1,500 ppm and above in males and increased pigmented tubule cells at 6,000 and 12,000 ppm in both sexes. There was also an increase in the frequency and severity of chronic progressive nephropathy in males.

A NOAEL of 1,500 ppm (88–108 mg/kg bw/d males–female respectively) was established from this study based on liver and kidney toxicity consisting of increased liver and kidney weights, biochemical changes (increased serum ALT and AST) and histopathological findings at higher doses (LOAEL of 358–442 mg/kg bw/d). These observations did not appear directly related to peroxisome proliferative effects.

Mice

A 13-week dietary study was conducted in B6C3F1 mice fed diets containing 0, 1,500, 4,000, 10,000, 20,000 ppm (approx 0, 365, 972, 2,600, 5,770 mg/kg bw/d) (Hazleton 1992*, ECB, 2003). Additional groups of 15 mice/sex/group (satellite study) were treated with DINP and a positive control (WY 14463; 15 mice/sex/group) to evaluate the hepatocellular proliferation and peroxisome proliferation potential of DINP.

Increases in absolute and relative liver weights from 4,000 ppm were noted in both sexes. Enlarged livers were also observed from 4,000 ppm and above in males and from 10,000 ppm in females. Absolute and relative kidney weights were decreased in males only at 4,000 ppm with significant

decreases in urinary sodium, chlorides and creatinine at the highest dose in both sexes. At 20,000 ppm, moderate to severe hepatocellular enlargement, pigmented Kupffer cells and bile canaliculi and minimal to slight liver degeneration/necrosis were observed. Tubular necrosis in the kidney as well as immature/abnormal sperm, lymphoid depletion in spleen and thymus, hypoplasia in the uterus and absence of corpora lutea in the ovaries were also seen at this dose. At 10,000 ppm and higher, decreased (absolute) epididymis and testes weights were observed.

In the satellite study, test-related lesions were observed in the liver (hepatocyte enlargement, degeneration/necrosis and pigmented cells) at 10,000 ppm and at 1,000 ppm in the positive control. At 10,000 ppm, DINP-treated animals did not show any increase in cell proliferation even though an increase in palmitoyl-Co-A oxidase was observed.

The NOAEL from this study was 1,500 ppm (approx 365 mg/kg bw/d), based on increases in liver weights at 4,000 ppm (approx 972 mg/kg bw/d).

In a 2-year dietary carcinogenicity study, B6C3F1/Crl BR mice (70/sex/dose) were fed daily doses of 0, 500, 1,500, 4,000 and 8,000 ppm DINP (0, 90–112, 275–335, 742–910, 1,560–1,887 mg/kg bw/d, males–females respectively) for 104 weeks. A recovery high-dose group (55 mice/sex) was also treated with 8,000 ppm in the diet for 78 weeks followed by a 26-week recovery period (Aristech Chemical Corporation, 1995*; Moore, 1998b*; ECB, 2003).

At interim sacrifice (week 78), absolute and relative testis weights were decreased (respectively by 11.1, 20.2 11.8%) but with no associated histological changes at 4,000 and 8,000 ppm (including recovery group).

At week 78 and at study termination, statistically significant decreases in absolute kidney weights in males and increases in liver weights in females from 1,500 ppm and above were observed. In males, increased liver masses were reported from 1,500 ppm and statistically significant increases in absolute and/or relative liver weights were also noted at 4,000 ppm and above. Mean liver palmitoyl-CoA oxidase activities were statistically significantly increased in all high-dose animals (8,000 ppm) compared to controls, suggesting significant peroxisome proliferation.

The most substantial gross changes at termination were increased incidence of lung masses (primarily males), liver masses (most frequently seen at 4,000 ppm and above and high recovery group) in males, enlarged spleen in all groups, granular pitted/rough kidneys in females at 8,000 ppm (corresponding to increased incidence/severity of treatment-related nephropathy) and distended urinary bladder (most frequently seen in males at 4,000 ppm and above). Histological examination showed increased incidence of cytoplasmic eosinophilia, diffuse hepatocellular enlargement and pigment at the highest dose in both sexes.

A NOAEL of 500 ppm (90–112 mg/kg bw/d) was derived, based on decreased absolute kidney weights and increased incidence of liver masses in males, and increased absolute liver weights in females at 1,500 ppm (275–335 mg/kg bw/d).

Other species

In a 13-week feeding study, beagle dogs (groups of four dogs/sex) were fed diets containing 0, 0.125, 0.5 and 2% (approximately 0, 37, 160 and 2,000 mg/kg/d) DINP (Hazleton, 1971*; ECB, 2003). Dose levels were increased from 2% to 4% from weeks 9 to 13. At week 4, ALT was slightly to moderately increased at 0.125% in both sexes and the increase was dose-related in females only at week 13. These changes were believed to be associated with increases in absolute and relative liver weights at 0.5% and above in males and at 2% in females. At 2%, absolute and relative kidney weights were increased in a few animals in both sexes and hypertrophy of kidney tubular epithelial cells were noted. In females, kidney discolourations (pale to dark/brown, red/purple) were observed. Microscopic examination of the liver showed hepatocytic hypertrophy associated with decreased prominence of hepatic sinusoids. No NOAEL was established in this study due to the absence of statistical data and some inconsistencies in data reporting.

In marmoset monkeys, systemic toxic potential of DINP with particular focus on hepatic peroxisome proliferation was reported (Hall et al., 1999 ND). In this study, marmoset monkeys

gavaged with DINP using doses of 100, 500 and 2,500 mg/kg bw/d (four monkeys/sex/group) for 13 weeks showed no changes in biochemical parameters, hormonal concentrations (oestradiol and testosterone) and organ weights that were considered treatment-related. Bodyweight losses or low bodyweight gains were observed for both sexes at the highest dose. A slight increase in palmitoyl CoA oxidase and lauric acid 11- and 12-hydroxylase activity at the high-dose group only was reported. These effects were not considered biologically significant due to the wide range of individual variations, absence of statistical significance and absence of concomitant increases in liver weights and histopathological changes. There was no indication that DINP acted as a peroxisome proliferator following dosing at levels of up to 2,500 mg/kg/d. A NOAEL of 500 mg/kg bw/d and LOAEL of 2,500 mg/kg bw/d based on decreases in bodyweight and bodyweight gain were assigned in this study.

In a subsequent study designed to assess the effects of DINP on peroxisomal proliferation, cynomolgus monkeys were given 500 mg/kg bw/d DINP by gavage for 14 days (Pugh et al., 2000 ND). No effects on food consumption, bodyweight and organ weights (liver, kidney, testes/epididymis, thyroid/parathyroid weights) were noted. Histopathological examination of the tissues from these animals revealed no treatment-related effects in the liver, kidney or testes. However, statistically significant increases in neutrophil count and decreases in lymphocyte counts were observed. There were no changes in any of the hepatic markers (replicative DNA synthesis and peroxisomal beta oxidation) for peroxisomal proliferation observed. A LOAEL of 500 mg/kg/d was reported in this study.

Dermal route

A six-week dermal study was undertaken in New Zealand white rabbits with groups of four animals each receiving doses of 0.5 or 2.5 mL/kg bw DINP or 2.5 mL/kg bw mineral oil as control (Hazleton, 1969*; ECB, 2003). Applications were made for 24 hours on abraded and intact skin, five days a week for a total of 30 exposures. DINP effects were confined to gross alterations of the skin. At the lowest dose, mild dermal irritation occurred which was slightly more severe than mineral oil vehicle alone. At the high dose, slight or moderate erythema and slight desquamation were observed. There were no systemic effects. A NOAEL of 0.5 mL/kg (approx 500 mg/kg) was established for local effects.

Summary of repeat-dose toxicity

Overall, repeat dosing of DINP via oral route resulted in adverse effects to the liver and kidneys of rodents. These effects were less pronounced in other experimental animals including dogs and primates. Rodent studies showed peroxisome proliferator effects of DINP, while studies in primates did not show that DINP was a peroxisome proliferator. Repeated exposure to DINP via the dermal route did not produce systemic effects.

Table 6.2—Summary of key repeated dose toxicity studies (adapted from ECB, 2003)

Species, study duration and test substances	Doses (mg/kg bw/d) and administration mode	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) and effects observed	References
<i>Oral</i>				
Rat Fischer 344 13-week study CAS No. 68515-48-0	0, 0.1, 0.3, 0.6, 1, 2% (0, 77, 227, 460, 767, 1,554 (both sexes)) in diet	77	227 in both sexes; ↑ kidney, liver weights; ↓ cholesterol	Bio/Dynamics (1982a*)
Rat Fischer 344 13-week study CAS No. 28553-12-0	0, 2,500, 5,000, 10,000, 20,000 ppm (0, 176–218, 354–438, 719–823, 1,545–1,687 (m-f)) in diet	NE	176–218; ↑ kidney, liver weights	Hazleton (1991a*)
Rat Fischer 344 2-year study CAS No. 68515-48-0	0, 0.03, 0.3, 0.6% (0, 15–18, 152–184, 307–375 (m-f)) in diet	15–18	152–184; ↑ kidney, liver weights; ↑ incidence of non-neoplastic changes in kidney & liver	Exxon Biomedical Sciences (1986*); Lington et al. (1997)
Rat Fischer 344 2-year study CAS No. 68515-48-0	0, 500, 1,500, 6,000, 12,000 ppm (0, 29–36, 88–108, 358–442, 733–885 (m-f)) in diet	88–108	358–442; ↑ kidney, liver weights in both sexes; ↑ AST & ALT with histopathological findings	Aristech Chemical Corporation (1994*); Moore (1998a*)
Mouse B6C3F1 13-week study CAS No. 28553-12-0	0, 1,500, 4,000, 10,000, 20,000 ppm (0, 365, 972, 2,600, 5,770 (both sexes)) in diet	365	972 in both sexes; Enlarged liver; ↑ absolute and relative liver weights; 2,600; ↓ absolute epididymis & testes weight	Hazleton (1992*)
Mouse B6C3F1 2-year study CAS No. 68515-48-0	0, 500, 1,500, 4,000, 8,000 ppm (0, 90–112, 275–335, 742–910, 1,560–1,887 (m-f)) in diet	90–112	275–335; ↑ absolute liver weights in females; ↑ liver masses and ↓ absolute kidney weights in males; 742; ↓ absolute & relative testes weight	Aristech Chemical Corporation (1995*); Moore (1998b*)

Species, study duration and test substances	Doses (mg/kg bw/d) and administration mode	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) and effects observed	References
Dog beagle 13-week study CAS No. 68515-48-0	0, 0.125, 0.5, 2% (0, 37, 160, 2,000) in diet	NE	37; ↑ ALT in both sexes	Hazleton (1971*)
Monkey marmoset (16–25-month old); 13-week study CAS No. not specified	0, 100, 500, 2,500 gavage	500	2,500; ↓ bodyweight; ↓ bodyweight gain	Huntington Life Sciences (1998*); Hall et al. (1999 ND)
Monkey cynomolgus males 2-week study CAS No. not specified	0, 500 gavage	NE	500; ↑ neutrophil count; ↓ lymphocyte count	Pugh et al. (2000)

Dermal

Rabbit New Zealand White 6-week study CAS No. 68515-48-0	0, 0.5, 2.5 mL/kg bw	500 (0.5 mL/kg bw)	2,500 (2.5 mL/kg bw); slight or moderate erythema, and slight desquamation	Hazleton (1969*)
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NE = not established; ↓ = decreased; ↑ = increased; m-f = male–female.

6.2.5 Genotoxicity

Several *in vitro* and *in vivo* assays have been conducted to assess the genotoxic effects of DINP (CAS No. 68515-48-0 and 28553-12-0). Conclusions from key studies are outlined below and summarised in Table 6.3.

In vitro

In a bacterial mutation assay (the Ames test), no positive responses were observed with any of the bacterial strains tested (*Salmonella typhimurium* TA98, 100, 1535, 1537, 1538), either in the presence or absence of metabolic activation (Exxon Biomedical Sciences, 1996b*; ECB, 2003).

A mouse lymphoma forward mutation assay (Hazleton, 1986*; ECB, 2003), found that DINP (CAS No. 68515-48-0) did not induce increases in mutant frequency at any dose in either the presence or the absence of metabolic activation.

DINP (CAS No. 68515-48-0) was also tested for clastogenic activity in cultured Chinese hamster ovary (CHO) cells in the presence or absence of metabolic activation (Exxon Biomedical Sciences, 1996c*; ECB, 2003). There was a statistically significant increase in the percentage of aberrant cells in the absence of metabolic activation. However, the percentage of aberrant cells was within the normal range of the vehicle control, not dose related and did not exceed 5%, which is the defined threshold to be considered as a positive result. Therefore, DINP was considered negative for clastogenicity in this study.

DINP (CAS No. 28553-12-0) was found to be inactive in a primary rat hepatocyte unscheduled DNA synthesis assay (Litton Bionetics, 1981*; ECB, 2003).

In vivo

In an *in vivo* cytogenetic assay, DINP (CAS No. 28553-12-0) was administered orally to three groups of Fischer 344 rats over five days (Microbiological Associates, 1981a*; ECB, 2003). Samples of femoral bone marrow were analysed for chromosomal aberrations after the treatment period. There was no evidence that DINP was active in this assay.

**Table 6.3—Summary of gene mutation and cytogenetic assays on DINP
(adapted from ECB, 2003)**

Genetic toxicity tests and test substances	Test system	Doses	Results	References
<i>In vitro</i>				
Bacterial test (gene mutation) CAS No. 68515-48-0	Salmonella typhimurium TA 98, 100, 1535, 1537, 1538	0.5 to 5,000 µg/plate ± S9	Negative	Exxon Biomedical Sciences (1996b*)
Mouse lymphoma assay CAS No. 68515-48-0	L5178 TK ±	1,500 to 8,000 nL/mL without metabolic activation and 500 to 6,000 nL/mL with metabolic activation	Negative	Hazleton (1986*)
Cytogenetic assay CAS No. 68515-48-0	CHO cells	5, 10, 20, 40, 80, 160 µg/mL ± S9	Negative	Exxon Biomedical Sciences (1996c*)
Mammalian test (Unscheduled DNA synthesis assay) CAS No. 28553-12-0	Rat hepatocytes	0.625 to 10 µg/mL	Negative	Litton Bionetics (1981*)
<i>In vivo</i>				
Cytogenetic assay	Rat Fischer 344 bone marrow cells	0.5, 1.7 and 5 mg/kg bw/d during 5 days via oral route	Negative	Microbiological Associates (1981a*)

Note: only validated studies included.

Overall, DINP tested negative in an *in vitro* bacterial mutation assay, *in vitro* mammalian gene mutation assays and a cytogenetic assay in CHO cells. DINP was also not clastogenic in an *in vivo* bone marrow assay in Fischer 344 rats. DINP is not considered to be genotoxic.

6.2.6 Carcinogenicity

The carcinogenicity of DINP has been investigated *in vitro* and *in vivo*. Conclusions from key studies are outlined below and summarised in Table 6.4.

Cell transformation assays

DINP has been subjected to several *in vitro* cell transformation assays using Balb/c-3T3 mouse cells (clone 1-13) under different conditions (ECB, 2003; Barber et al., 2000). Four of the eight tests were negative and three were doubtful (slight increases in transforming activity without statistical significance).

A single study with concentrations of DINP ranging from 0.03 to 1 µL/mL found statistically significant and dose-dependent type III transforming activity in 3T3 cells in the absence of metabolic activation (Microbiological Associates, 1981b*; ECB, 2003).

Two-year carcinogenicity studies

In vivo carcinogenicity studies in animals include three 2-year dietary studies in rats and a 2-year dietary study in mice.

In a combined chronic/carcinogenicity study, Fischer 344 rats (110/sex/group) were fed diets containing DINP (CAS No. 68515-48-0) at 15, 152 and 307 mg/kg bw/d (males) and 18, 184 and 375 mg/kg bw/d (females) for two years (Exxon Biomedical Sciences, 1986*; Lington et al., 1997).

MCL was the common cause of unscheduled deaths. Tubular cell pigmentation in the kidney was increased in severity in animals with advanced MCL. Statistically significant increases in MCL were observed in both sexes at mid (152–184 mg/kg) and high (307–375 mg/kg) doses. Renal neoplasms (transitional cell carcinomas and tubular cell carcinomas) were present in three mid-dose and two high-dose male rats respectively.

A retrospective evaluation of kidney tissue from this study was conducted using immunohistochemical techniques (Caldwell et al., 1999a). Results showed a dose-dependent alpha 2µ-globulin accumulation in specific regions of male kidneys where increases in cellular proliferation were noted. These findings were attributed to a gender- and species-specific alpha 2µ-globulin tumourigenic mechanism in male rat kidneys that is not regarded as relevant to humans (Caldwell et al., 1999a; ECB, 2003).

A NOAEL of 15–18 mg/kg bw/d was established, based on increased incidence of MCL at doses of 152–184 mg/kg bw/d and above.

Another study using Sprague Dawley CD rats (70/sex/dose) was performed with a non-commercial, branched DINP (CAS No. 71549-78-5) in the diet at dose levels of 0, 500, 5,000, 10,000 ppm for a period of 2 years.

An increased incidence of hepatocellular carcinomas was found in both sexes of the mid- and high-dose groups leading to a NOAEL of 500 ppm (27–33 mg/kg bw/d males–females respectively). Also, increased incidence of testicular cell hyperplasia, slightly increased incidences of pancreatic islet cell tumours and parathyroid gland hyperplasia were observed in high dose males. Endometrial hyperplasia was observed in high-dose females (Bio/Dynamics, 1986*; ECB, 2003).

In a 2-year carcinogenicity study (CAS No. 68515-48-0), Fischer 344 rats were administered daily with dietary concentrations of 0, 500, 1,500, 6,000 and 12,000 ppm (approximately 29–36, 88–108, 358–442, 733–885 mg/kg bw/d, males–females, respectively) of DINP for 104 weeks (Aristech Chemical Corporation, 1994*; Moore, 1998a*; ECB, 2003).

Animals in the recovery group received 12,000 ppm for 78 weeks followed by a 26-week recovery period. Ancillary analyses were also conducted to evaluate chemically-induced cell proliferation and peroxisome proliferation in the livers of the appropriate dose groups.

At 12,000 ppm, increased incidence of MCL (46% in both sexes vs 34% and 26% in control males and females, respectively) and hepatocellular neoplasms in both sexes were observed. Increased incidence of renal carcinomas (transitional cell carcinomas and tubule cell carcinomas) was also reported only in males. Histologic and biochemical analyses indicated the presence of hepatocellular proliferation during Week 1. Thereafter, palmitoyl CoA oxidase activity was significantly increased in both sexes at the highest dose. These results showed evidence of peroxisome proliferation associated with cell proliferation only at Week 1.

At 6,000 ppm, the incidence of MCL was 49% and 45% in males and females respectively.

After the 26-week recovery period, MCL in both sexes and kidney neoplasms in males were not reversible. Reversibility of liver neoplasms could not be determined since these were only found in animals treated with DINP over the last 26 weeks of the study.

A NOAEL for carcinogenicity was established at 88 mg/kg bw/d, based on increased incidence of MCL observed at higher doses (LOAEL of 6,000 ppm or 358–442 mg/kg bw/d).

In a 2-year dietary carcinogenicity study, B6C3F1/Crl BR mice (70/sex/dose) were fed daily doses of 0, 500, 1,500, 4,000 and 8,000 ppm DINP (0, 90–112, 275–335, 742–910, 1,560–1,887 mg/kg bw, males–females respectively) for 104 weeks.

A recovery high-dose group (55/sex) was also treated with 8,000 ppm in the diet for 78 weeks followed by a 26-week recovery period (Aristech Chemical Corporation, 1995*; Moore, 1998b*; ECB, 2003).

Neoplastic changes consisting mainly of hepatocellular neoplasia (adenoma and carcinoma combined) were reported in both sexes at 8,000 ppm. The total incidence of hepatocellular neoplasia was significantly increased in males from 4,000 ppm (47%) and in females from 1,500 ppm (17%). Hepatocellular carcinoma was increased in both sexes at 4,000 and 8,000 ppm, and in females in the recovery group. In males, there were no significant increases in the total incidence of liver adenoma in any dose group including the recovery group. In females, a significant increase in the total incidence of liver adenoma was observed at 8,000 ppm and in the recovery group.

The above results led to the establishment of a NOAEL of 500 ppm (112 mg/kg bw/d), with a LOAEL of 1,500 ppm (335 mg/kg bw/d) for females and a NOAEL of 1,500 ppm (275 mg/kg bw/d) and a LOAEL of 4,000 ppm (742 mg/kg bw/d) for males based on observed increases in total hepatocellular neoplasms.

Ancillary studies showed high levels of peroxisome proliferation in high-dose animals as indicated by significant increases in palmitoyl-CoA oxidase activity. This suggested that the liver carcinogenicity was linked to peroxisome proliferative effects.

Table 6.4—Summary of key in vivo carcinogenicity studies (adapted from ECB, 2003)

Species, study duration and test substances	Doses (mg/kg bw/d) and administration mode	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) and effects observed	References
Rat Fischer 344 2-year study CAS No. 68515-48-0	0, 0.03, 0.3, 0.6% (0, 15–18, 152–184, 307–375 (m-f)) in diet	15–18	152–184; ↑ MCL, renal neoplasms (transitional cell carcinomas and tubular cell carcinomas)	Exxon Biochemical Sciences (1986*); Lington et al. (1997)
Rat Sprague Dawley 2-year study CAS No. 71549-78-5	0, 500, 5,000, 10,000 ppm (0, 27–33, 271–331, 553–672 mg/kg bw/d (m-f)) in diet	27–33	271–333; hepatocellular carcinomas (No MCL)	Bio/Dynamics (1986*)
Rat Fischer 344 2-year study CAS No. 68515-48-0	0, 500, 1,500, 6,000, 12,000 ppm (0, 29–36, 88–108, 358–442, 733–885 (m-f)) in diet	88–108	358–442; ↑ MCL 733–885; hepatocellular neoplasia, renal tubule cell carcinomas	Aristech Chemical Corporation (1994*); Moore 1998a*
Mouse B6C3F1 2-year study CAS No. 68515-48-0	0, 500, 1,500, 4,000, 8,000 ppm (0, 90–112, 275–335, 742–910, 1,560–1,887 (m-f)) in diet	112 (f); 275 (m)	335 (f) and 742 (m); ↑ total hepatocellular neoplasms (adenomas and carcinomas combined)	Aristech Chemical Corporation (1995*); Moore (1998b*)

↑ = increased; m-f = male–female.

Other data

Benford et al. (1986*; ECB, 2003) investigated the peroxisome proliferative potential of DINP and its metabolites, monoisodecyl phthalate (MIDP) and MINP, in primary monolayer cultures of rat and marmoset monkey hepatocytes. Mono-(2-ethylhexyl) phthalate (MEHP) was used as a positive control. Parameters measured included peroxisomal palmytoyl-CoA (PCoA) oxidation, laurate 11-12 hydroxylation (LAH) and the protein content of the homogenate. In cultured rat hepatocytes, both MIDP and MINP induced dose-related increases in PCoA oxidation, with fewer increases observed in DINP. There were no significant increases in LAH activity reported for the metabolites. In cultured marmoset hepatocytes, minimal changes in PCoA oxidation activity were observed; however, MIDP and MINP metabolites caused significant increases in LAH activity.

The gap junctional intercellular communication (GJIC) effects of metabolites (MINP-M and MINP-S) of two forms of DINP (CAS No. 68515-49-0 and CAS No. 71549-78-5) were examined in hepatocytes of rats, mice, hamsters and humans (Baker et al., 1996*; ECB, 2003). The GJIC assay has been reported to have good cancer predictive potential for phthalates (Kalimi et al., 1995*; ECB, 2003). Compounds that block GJIC and increase replicative DNA synthesis appear to function at the tumour promotion phase of the chemical carcinogenesis process. Alteration of these hepatic markers has been implicated in peroxisome proliferator-induced hepatocarcinogenesis in rodents (Pugh et al., 2000 ND). In rat hepatocytes, metabolites of both forms of DINP inhibited GJIC, while only MINP-S inhibited GJIC in mouse hepatocytes. In hamster or human hepatocytes and in human liver cell line, none of the monoesters inhibited GJIC at non-toxic doses.

The above results indicated a significant species difference in the peroxisome proliferative and GJIC effects of DINP and its metabolites.

Overall, the available data do not indicate a carcinogenic potential in humans for DINP. MCL was not found in other mammalian species and has no comparable type in humans. Kidney tumours were attributed to alpha 2 μ globulin tumourigenic mechanism specific in male rats. Liver carcinogenicity was related to peroxisome proliferative effects, which is regarded as not relevant to human health.

6.2.7 Reproductive toxicity

Reproductive toxicity associated with DINP has been examined in one- and two-generation studies in rats, in specific studies on testicular function, in pre-natal and post-natal developmental toxicity studies and in studies that focus on possible modes of action. They are presented below in chronological order for each type of study.

One-/two-generation reproductive toxicity studies

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

A good laboratory practice (GLP) compliant one-generation reproductive toxicity study administered dietary levels of 0, 0.5%, 1.0% and 1.5% (equivalent to 0, 301–923, 622–1,731, 966–2,246 mg/kg bw/d) DINP (CAS No. 68515-48-0) to SD rats (30/sex/group) from 10 weeks before mating and throughout the mating period (~ 3 weeks). The males were sacrificed after mating while dosing continued in the females through gestation and lactation until weaning of the offspring on PND 21. Statistically significant decreases in bodyweight were observed in the mid- and high-dose male and female parental (F0) animals. There were statistically significant dose-related increases in the absolute and/or relative liver and kidney weights of both sexes at all dose levels tested. Increased weights of left and right testes and right epididymis and decreased weights of left and right ovaries were also statistically significant in the high-dose animals compared to controls. Histopathological changes were not examined and thus significance of organ weight changes could not be assessed.

DINP had no significant effects on mating, fertility, fecundity, gestational length or index. Effects on offspring such as live birth index (No. of live pups at birth / No. of pups born) and survival of offspring during lactation were significantly reduced at 1.5%. Dose-related decreases in mean offspring bodyweight were also observed during lactation in both sexes at all dose levels on PND 0 and PND 14–21. At $\geq 1.0\%$, the pup weight reduction was sustained in both sexes throughout PND 0–21. Pup weights were below the historical range at the highest dose (1.5%). These findings were considered a result of decreased maternal bodyweight and/or from direct effects of DINP on pup milk consumption via exposure through lactation. Given the lack of conclusive causal evidence, the reduced pup weight was assessed as a DINP-related effect. In this study, the NOAEL for fertility-related toxicity was 1.5% (966–2,246 mg/kg bw/d), the highest dose tested. The NOAEL for developmental toxicity could not be established due to decreased pup weight at the lowest dose tested (301–923 mg/kg bw/d) (Waterman et al., 2000).

A GLP compliant two-generation reproductive rat study was also conducted by Waterman et al. (2000) with DINP (CAS No. 68515-48-0) at dietary levels of 0, 0.2%, 0.4% and 0.8% (equivalent to 0, 114–395, 235–758, 467–1,541 mg/kg bw/d). F0 and F1 parents (30/sex/group) were treated for 10 weeks before mating, through mating, gestation and lactation until weaning. Necropsy of males was after the delivery of the last litter and of females after weaning the litters on PND 21. F0 bodyweights were unaffected except for a reduced dam weight at 0.8% during PND 14–21. F1 bodyweights at 0.8% were significantly below control values throughout the pre-mating and mating period for males, and GD 14 and PND 4–21 (lactation period) for females. Reductions in

bodyweight of F1 males at 0.4% only achieved statistical significance sporadically. Statistically significant increases in absolute kidney weights were observed at $\geq 0.2\%$ in F0 females, $\geq 0.4\%$ in F0 males and 0.8% in F1 males. Statistically significant increases in absolute liver weights were observed at $\geq 0.4\%$ in F0 females, 0.8% in F0 males and 0.8% in F1 females. Histopathological examination revealed minimally to moderately increased cytoplasmic eosinophilia in the livers (at all dose levels in both sexes and both generations) and minimally to moderately increased renal pelvis dilatation (at 0.4–0.8% in F1 males only). No significant weight changes or histological changes were seen in any of the reproductive organs from either generation.

There were no significant differences between F0 and F1 animals and the controls with regard to the reproductive indices (such as mating, fertility, fecundity, gestational length and index) and offspring indices measured (such as live birth index and offspring survival during lactation) in this two-generation study. Similar to the results of one-generation study, dose-related decreases in mean offspring bodyweight were observed at all doses on PND 21 (m-f, F1) and PND 7 (f, F2), and at $\geq 0.4\%$ on PND 7–21 in both sexes and both generations (F1, F2). The NOAEL for fertility-related toxicity was 0.8% (467–1,541 mg/kg bw/d) based on no significant effects reported at the highest dose tested. The NOAEL for developmental effects was not established and the LOAEL was 0.2% (114–395 mg/kg bw/d) based on reduced pup weights on PND 21 (m-f, F1) and PND 7 (f, F2).

Studies on testes and testicular function

Pregnant Wistar rats (8/group) were exposed by gavage to DINP (CAS No. 28553-12-0) at 0 or 750 mg/kg bw/d during GD 7–21. DINP was shown to statistically significantly reduce testicular testosterone content and production (*ex vivo*) of GD 21 male foetuses. Reductions in plasma testosterone levels and elevation of foetal plasma luteinizing hormone (LH) concomitantly with the suppression of testosterone synthesis, whether alone or in combination with DEHP (statistically significant), were also observed. The authors hypothesised that DEHP and DINP reduced testosterone by mechanism of action via a functional feedback loop from the gonads to the hypothalamus and pituitary (Borch et al., 2004).

Testicular toxicity via an antiandrogenic mechanism was investigated by Hershberger assay in castrated immature SD rats (6/group) for DINP and six other phthalates. DINP was administered by gavage at 20, 100 or 500 mg/kg bw/d in combination with testosterone at 0.4 mg/kg bw/d, subcutaneous, for 10 consecutive days. Testosterone alone as an androgen agonist was administered as a positive control. No effects were observed on animal bodyweight, liver, kidney or adrenal weights. Weights of accessory sex organs such as seminal vesicles and levator ani/bulbocavernosus (LABC) were significantly decreased by DINP at ≥ 20 and 500 mg/kg bw/d respectively (c.f. DEHP at ≥ 100 and 500 mg/kg bw/d respectively). The inconsistency in the relative potency between DINP and DEHP in this study compared with other studies precluded making conclusion about this publication (Lee and Koo, 2007 ND).

In pregnant SD rats (7–8/group), exposure to DINP at 0, 250 or 750 mg/kg bw/d by gavage during GD 13–17 did not cause statistically significant reduction in testicular testosterone content of GD 19 male foetuses. However, a statistically significant increase in foetal testicular transcript levels of P450 side-chain cleavage (P450scc), insulin-like factor 3 (InsI3) and GATA binding protein 4 (GATA4) was observed at 750 mg/kg bw/d, possibly due to a rebound effect on steroidogenesis. The authors also suggested the shorter exposure time used in this study (GD 13–17 or five days vs GD 7–21 or 15 days) could be the reason for different outcomes from the study by Borch et al. above (Adamsson et al., 2009 ND).

Pregnant SD rats (3–6/group) were dosed orally for a short exposure duration (five days) from GD 14–18 with 0, 500, 750, 1,000 or 1,500 mg/kg bw/d DINP (CAS No. 68515-48-0 or 28553-12-0). There were dose-related decreases in foetal testicular testosterone production at ≥ 500 mg/kg bw/d and transcript levels of StAR and Cyp11a (genes involved in androgen synthesis) at $\geq 1,000$ mg/kg bw/d with no differences between DINP chemical formulations. By comparing dose-response curves of DINP and DEHP for these effects, the authors concluded that

DINP and DEHP shared a similar pattern of foetal endocrine alterations although quantitatively DINP was less potent than DEHP, e.g. 2.3-fold less in reducing foetal testicular testosterone production (Hannas et al., 2011 ND).

In another study by Hannas et al. (2012 ND) using a targeted gene array approach for defining relative potency of phthalates, DINP and several other phthalates were dosed to pregnant SD rats as in preceding study. DINP was shown to down-regulate the expression of *Ins13* and other foetal testicular genes involved in androgen synthesis and cholesterol transport (at ≥ 500 mg/kg bw/d), although DINP was less potent than the other four phthalates tested (DPeP>DHP>DIBP \geq DHeP>DINP).

Pre-natal developmental toxicity studies

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

SD rats (25/group) were dosed by oral gavage with DINP (CAS No. unspecified) at 0, 10, 500 and 1,000 mg/kg on GD 6–15. The dams were examined twice daily and sacrificed on GD 20. No statistically significant DINP-related effects were noted with respect to maternal or foetal toxicity and thus the relevant NOAELs were determined as 1,000 mg/kg bw/d, the highest dose tested (Hazleton, 1981*; ECB, 2003).

In another prenatal toxicity study, each of three DINP variants (CAS No. 68515-48-0 and two others with CAS No. 28553-12-0) was administered at gavage doses of 0, 40, 200 and 1,000 mg/kg bw/d to Wistar rats (8–10/group) on GD 6–15. For CAS No. 68515-48-0 (DINP1), a statistically significant increased occurrence of foetal skeletal variations, consisting mainly of rudimentary cervical and accessory 14th ribs at 1,000 mg/kg bw/d, led to a NOAEL for developmental toxicity of 200 mg/kg bw/d. The NOAEL for maternal toxicity was 200 mg/kg bw/d and the LOAEL was 1,000 mg/kg bw/d based on a slightly decreased food consumption and an increased relative kidney weights. For CAS No. 28553-12-0 (DINP2), a NOAEL for developmental toxicity was established at 200 mg/kg bw/d based on an increased incidence of skeletal variations (rudimentary cervical and accessory 14th ribs) at 1,000 mg/kg bw/d. The NOAEL for maternal toxicity was assessed as 200 mg/kg bw/d based on the occurrence of vaginal haemorrhage, albeit in one dam, at 1,000 mg/kg bw/d. The increased skeletal variations with DINP1 and DINP2 were statistically significant on a per-litter basis and distinctly above historical control values and were thus considered slight developmental effects. Results on DINP3 were also reported, but this material has not been manufactured since 1995, so it is not included here for consideration (Hellwig et al., 1997; ECB, 2003).

Waterman et al. (1999) conducted a developmental toxicity study using SD rats (23–25/group) administered DINP (CAS No. 68515-48-0) at gavage doses of 0, 100, 500 or 1,000 mg/kg bw/d on GD 6–15. There were no maternal effects except for statistically significant decreases in bodyweight gain and food consumption at 1,000 mg/kg bw/d during the treatment period, leading to a reported NOAEL for maternal toxicity of 500 mg/kg bw/d. The high-dose dams had regained bodyweight and food consumption after exposure ceased, possibly indicating a recovery effect. Foetal observation showed a significantly increased incidence of skeletal (rudimentary lumbar ribs) and visceral (dilated renal pelvis) variations at 1,000 mg/kg bw/d on a per-litter basis. These variations are relatively common in rodents; however, the induced frequencies (78% vs 25% control for rudimentary lumbar ribs, and 26% vs 0% control for dilated renal pelvis) were outside historical control ranges and thus interpreted as indicative of slight developmental effects. The NOAEL for developmental toxicity was assessed as 500 mg/kg bw/d.

In an unpublished study by Clewell et al. (2011a ND), pregnant SD rats (8/group) were dosed from GD 12–19 via oral gavage with 0, 50, 250 or 750 mg/kg bw/d DINP (CAS No. 68515-48-0). Maternal bodyweight or weight gain was not altered by DINP, but absolute and relative maternal liver weights were increased at ≥ 250 mg/kg bw/d. Three markers of male reproductive tract development were examined in the male pups, namely: AGD; testosterone concentration in the

foetal testes; and histopathology of the foetal testes. There was no change in absolute AGD or scaled AGD (i.e. AGI = AGD divided by the cube root of the bodyweight). Foetal testicular testosterone was reduced (statistically significantly) at 2 hours post dosing (GD 19) and increased (not statistically significantly) at 24 hours post dosing (GD 20) in the mid- and high-dose animals (50% to 60% reduction respectively). There were no other testosterone measurements between the 2-hour and 24-hour time points to determine the extent of variation and/or fluctuation of testicular testosterone levels in foetuses. DINP also increased multinucleated gonocytes (MNG) at ≥ 250 mg/kg bw/d and increased number of gonocytes and size of Leydig cell aggregates at 750 mg/kg bw/d in the foetal testes (GD 20). Effects on seminiferous tubule diameter were not seen. The maternal and developmental NOAELs in this study were 50 mg/kg bw/d based on increased maternal liver weights and reduced foetal testicular testosterone to an adverse level of 50–60% compared to the control at 250 mg/kg bw/d.

Post-natal developmental toxicity studies

The post-natal developmental toxicity studies examine the in-utero and early post-natal developmental effects of DINP administered daily to female animals through gestation, lactation and weaning.

In a study using a range of phthalates, DINP (CAS No. 68515-48-0) was administered by gavage in SD dams (6–8/dose) at 0 or 750 mg/kg bw/d from GD 14 to PND 3. There was no overt maternal toxicity or reduced litter size, although DINP reduced pregnancy weight gain to GD 21. There were no treatment-related effects on foetal bodyweight or AGD on PND 2. As infants, males in the DEHP, BBP and DINP groups were reported as displaying female-like areolas (87%, 70%, 22% respectively and reported as statistically significant). All three phthalates that induced areolas also induced reproductive malformations (DEHP, 82%; BBP, 84%; and DINP, 7.7%). Two of 52 animals (from 2/14 litters) displayed permanent nipples (number of nipples = 1 and 6 for each of the two males). The males affected by DINP treatment displayed diverse malformations. Four of 52 adult males (from three litters) exhibited malformation such as: small and atrophic testis; flaccid, fluid-filled testis; bilateral testicular atrophy and unilateral epididymal agenesis with hypospermatogenesis; and scrotal fluid-filled testis devoid of spermatids. There were no treatment-related effects reported in androgen-sensitive tissue weights: testes, LABC, seminal vesicles, ventral prostrate, glans penis and epididymis. The authors concluded that DINP did display antiandrogenic activity, but it was about 20-fold less potent than DEHP (Gray et al., 2000).

In a follow-up study by the same group, DINP was administered by gavage in SD dams from GD 14 to PND 3 using higher dosage levels of 1,000 and 1,500 mg/kg bw/d to confirm its antiandrogenic action in utero. At PND 2, males exposed to 1,500 mg DINP displayed reduced AGD while female AGD was unaffected. DINP also increased the percentage of males with areolas on PND 13 in a dose-related fashion (14%, 55% and 75% in the control, 1,000 mg and 1,500 mg DINP groups, respectively). Maternal toxicity was not reported (Ostby et al., 2001*; CPSC, 2010 ND).

The potential impact of dietary exposure to DINP (CAS No. 28553-12-0) at doses of 400, 4,000 and 20,000 ppm (or 31–66, 307–657 and 1,165–2,657 mg/kg bw/d) from GD 15 to PND 10 was evaluated in pregnant SD rats (5/group). Decreases in maternal bodyweight gain and food consumption were observed at 20,000 ppm. Litter size was slightly decreased but not statistically significantly, even at the highest dose. Reduction of foetal bodyweight gain was noted at 20,000 ppm in both sexes during PND 2–10 (with recovery after cessation of exposure PND 10–21) and in male pups only during PND 21–42. AGD measured on PND 2 was not significantly changed at all doses in either sex. At pre-pubertal necropsy on PND 27, reduced weights of male and female pups and absolute and/or relative brain, testes, ovaries and uterus weights were statistically significantly at 20,000 ppm. The bodyweight of male pups PND 27 at 4,000 ppm was also significantly reduced. No obvious effects on onset of puberty such as preputial separation or vaginal opening were observed. On PND 77, testes and prostate weights were not affected. However, histopathology showed non-significant degeneration of stage XIV meiotic spermatocytes and vacuolar degeneration of Sertoli cells or decreased corpora lutea at 20,000 ppm. The NOAEL

for maternal toxicity was 307–657 mg/kg bw/d, based on decreased weight gain and food consumption at the high dose. The developmental NOAEL for male rats was 31–66 mg/kg bw/d (based on the reduced pup weight at mid dose) and for female rats was 307–657 mg/kg bw/d (based on reduced pup and reproductive organ weights at the high dose) (Masutomi et al., 2003).

In another study by Masutomi et al. (2004) pregnant rats were exposed to DINP at doses of 400, 4,000, or 20,000 ppm via the diet between GD 15 and PND 10. At both PNW 3 and 11, DINP had no effect on pituitary cells positive for luteinizing hormone, follicle stimulating hormone or prolactin in male and female animals. Additionally, there was no effect on pituitary weight in either sex at this time point.

Pregnant Wistar rats were given a diet containing DINP (CAS No. 28553-12-0) at 0, 40, 400, 4,000 and 20,000 ppm (equivalent to 0, 2, 20, 200 and 1,000 mg/kg bw/d according to ECHA (2010 ND)) from GD 15 to PND 21 to assess its potential endocrine disrupting effects. There were no effects on litter size or sex ratio. At all dose levels, significantly reduced foetal bodyweights were seen in both sexes and reduced AGD and AGI in exposed males. On PND 7, DINP resulted in significant increases in hypothalamic gene expression of p130 and granulin mRNA levels in males and females respectively. Decreased copulatory behaviour was noted only in the 40 ppm group males and not in a dose-dependent manner. Females at all doses showed a dose-dependent decreased lordosis quotient (LQ—the number of lordosis reflexes or postures adopted by the female rat per 10 mounts during mating \times 100%). Serum levels of LH and FSH in both sexes, testosterone in males and oestradiol in females were not affected by treatment. It was suggested that inappropriate expression of granulin and/or p130 genes in the brain of neonatal rats following perinatal exposure to DINP may exert permanent effects on the hypothalamus, thereby decreasing sexual behaviour after maturation. Maternal toxicity was not reported in this study (Lee et al., 2006 ND).

Boberg et al. (2011 ND; Hass et al., 2003* and CPSC, 2010 ND) studied DINP effects on reproduction and sexually dimorphic behaviour by administering pregnant Wistar rats (16/group) with gavage doses of 0, 300, 600, 750 or 900 mg/kg bw/d during GD 7—PND 17. In male offspring, DINP (CAS No. 28553-12-0) caused a dose-dependent increase in nipple retention on PND 13 (\geq 600 mg/kg bw/d; statistically significant at 750 mg/kg bw/d) and a statistically significant decrease in AGD and AGI on PND 1 (\geq 900 mg/kg bw/d). Four animals had permanent malformations such as epididymal and testicular dysgenesis, as well as permanent nipples. Reduced sperm motility (\geq 600 mg/kg bw/d) and increased sperm count (\geq 900 mg/kg bw/d) were also seen. In addition, a tendency towards reduced testicular testosterone content and production (ex vivo) was noted in the DINP-exposed male fetuses on GD 21, but this was not statistically significant except for reduced testosterone content at 600 mg/kg. Also, although not being statistically significant, it was reported that mean testicular testosterone content in the highest-dose group was only 63% of control levels on PND 90. Altered testicular histology was observed in GD 21 fetuses including increased multinucleated gonocytes (MNG) and enlarged seminiferous tubule at \geq 600 and \geq 750 mg/kg bw/d respectively. Bodyweight on PND 13 was significantly reduced (male pups at 900 mg/kg and female pups at 750 mg/kg). Pup retrieval by mothers was significantly delayed at 600 mg/kg, suggesting either maternal toxicity or inadequate nutrition at these doses. DINP affected spatial learning on the first day of memory testing, given female offspring performed better than controls and similarly to control males. The effect was dose-related and became statistically significant at \geq 900 mg/kg bw/d. Although the implications of the observed effects are inconclusive, hormone alteration within a critical period of brain development might cause masculinisation of behaviour in DINP-exposed female rats. Maternal bodyweight, weight gain during pregnancy, gestational length, litter size and sex ratio were not affected by treatment. Therefore, the NOAEL for maternal toxicity was 900 mg/kg bw/d. The NOAEL for developmental toxicity for male rats was set at 300 mg/kg bw/d based on testicular pathology, reduced testicular testosterone content and sperm motility at \geq 600 mg/kg bw/d, and for female rats at 600 mg/kg bw/d based on reduced pup weight at 750 mg/kg bw/d.

DINP effects on male sexual development were measured in the male offspring of rats (20/group) administered 0, 50, 250 or 750 mg/kg bw/d (CAS No. 68515-48-0) in the diet from GD 12–19

(Clewell et al. 2011b—unpublished study). The transitional phthalate DBP at 500 mg/kg bw/d was used as a positive control. Both food consumption and maternal weight were decreased in the high-dose group and the authors commented that food palatability could be responsible for the reduced maternal weight. Increased liver weight was not seen with DINP in this study. DINP at 750 mg/kg bw/d decreased AGD and AGI on PND 14. On PND 49, neither DBP nor DINP was associated with decreased AGD/AGI. Testicular testosterone was also not significantly different between the control, DBP or DINP treatment groups on PND 2 and 49. Testicular pathology associated with DINP on PND 2 included increased MNG at ≥ 250 mg/kg bw/d and increased incidence and severity of large Leydig cell aggregates at 750 mg/kg bw/d. Pup weights were reduced at 750 mg/kg bw/d on PND 2, at ≥ 250 mg/kg bw/d on PND 14 and comparable to controls on PND 49. A NOAEL for maternal toxicity was established at 250 mg/kg bw/d (based on the reduced food consumption and bodyweight at the high dose) and for developmental toxicity at 50 mg/kg bw/d (based on the increased MNG on PND 2 and reduced pup weight on PND 14 at the mid dose).

Mode of action studies

DINP showed extremely weak oestrogenic or antiandrogenic activity in both recombinant and two-hybrid yeast assays (Harris et al., 1997; Zacharewski et al., 1998; Nishihara et al., 2000; Kolle et al., 2010 ND). DINP did not demonstrate receptor-mediated oestrogenic or antiandrogenic activity in recombinant receptor/reporter gene assays using either human breast cancer (MCF-7), human cervical carcinoma (HeLa) or Chinese hamster ovary (CHO-K1) cells transfected with respective expression vectors (Harris et al., 1997; Zacharewski et al., 1998; Takeuchi et al., 2005; Kruger et al., 2008 ND; Ghisari & Bondefeld-Jorgensen, 2009 ND). In contrast, proliferation of ZR-75 (another human breast cancer cell line with higher oestrogen specificity) was induced by DINP at concentrations from 10^{-7} to 10^{-5} M to a significantly greater extent than the control 17β -oestradiol (endogenous oestrogen) (Harris et al., 1997).

DINP (10^{-6} to 10^{-3} M) was shown to compete ineffectively with 17β -oestradiol for binding to the rat uterine oestrogen receptor (ER). DINP (10^{-8} to 10^{-4} M) also did not alter basal progesterone or oestradiol production by porcine ovarian granulosa cells after 72 hours of culture in the absence of human recombinant follicle-stimulating hormone (hFSH). However, DINP tended to amplify progesterone production (not statistically significant) and suppress oestradiol production (statistically significant) in the presence of hFSH. Although the molecular mechanism involved in these alterations of steroid hormone production was unclear, the results indicated that ovarian steroidogenesis might be one of the possible processes affected in the endocrine disrupting actions of DINP and other phthalates tested (Mynarcikova et al., 2007 ND).

In vivo, DINP exhibited no significant ER-mediated increases in uterine wet weight or vaginal epithelial cell cornification (which occurs during oestrus) in ovariectomised SD rats treated with gavage doses of 20, 200 or 2,000 mg/kg bw/d for 4 days (Zacharewski et al., 1998).

In conclusion, the data on the oestrogenic or antiandrogenic potency of DINP are limited and equivocal, and hence the exact mechanism of DINP effects on the male reproductive system such as increased nipple retention, testicular and epididymal agenesis/atrophy, reduced testosterone and sperm quality cannot be determined, although DINP does appear to interfere with endocrine function.

The DINP effects on reproductive endpoints in rodents are summarised in Table 6.5.

Table 6.5—Summary of the fertility and developmental effects of DINP

Study design	Species & route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & endpoint	Reference
Reproductive toxicity studies (one-generation)					
16 weeks (10 weeks prior to mating till weaning, males sacrificed after mating) 30/sex/group	Rat SD Diet	DINP CAS No. 68515-48-0 0, 0.5, 1, 1.5% (0, 301–923, 622–1,731, 966–2,246)	Maternal: NE	Maternal: 301–923: ↑ liver & kidney weights; 622–1,731: ↓ bodyweight (m-f)	Waterman et al., 2000; CERHR, 2003; ECB, 2003
			Fertility-related parameters: 966–2,246	Fertility-related parameters: NE	
			Developmental: NE	Developmental: 301–923: ↓ pup weight PND 0 & PND 14-21 (m-f); 622–1,731: ↓ pup weight PND 0-21 (m-f)	
(Two-generation)					
32 weeks (10 weeks prior to mating till weaning—similar for F0 & F1, males sacrificed after delivery of the last litter) 30/sex/group	Rat SD Diet	DINP CAS No. 68515-48-0 0, 0.2, 0.4, 0.8% (0, 114–395, 235–758, 467–1,541)	Maternal: 114 (m); NE (f)	Maternal: 114–395: ↑ kidney weight (f, F0); 235–758: ↑ liver (f, F0) & kidney (m, F0) weights; 467–1,541: ↓ bodyweight during lactation PND 14–21 (f, F0), during (pre-) mating (m, F1) & lactation PND 4–21 (f, F1)	Waterman et al., 2000; CERHR, 2003; ECB, 2003
			Fertility-related parameters: 467–1,541	Fertility-related parameters: NE	
			Developmental: NE	Developmental: 114–395: ↓ pup weight PND 21 (m-f, F1) & PND 7 (f, F2); 235–758: ↓ pup weight PND 7–21 (m-f, F1, F2)	
Studies on testes and testicular function					
GD 7–21 8/group	Rat; Wistar; Gavage	DINP CAS No. 28553-12-0 0, 750	Fertility-related parameters: NE	Fertility-related parameters: 750: ↓ foetal testicular testosterone content & production (GD 21)	Borch et al., 2004

Study design	Species & route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & endpoint	Reference
10 days (Hershberger assay) 6/group	Rat (castrated immature); SD; Gavage	DINP (CAS No. unspecified) 0, 20, 100, 500 (in combination with testosterone 0.4 mg/kg bw/d, sc)	Fertility-related parameters: NE	Fertility-related parameters: 20: ↓ seminal vesicle weight 500: ↓ LABC	Lee and Koo, 2007 ND
GD 13–17 7–8/group	Rat; SD; Gavage	DINP (CAS No. unspecified) 0, 250, 750	Fertility-related parameters: 250	Fertility-related parameters: 750: ↑ foetal testicular gene expression GD 19 (P450ssc, Ins13 & GATA4)	Adamsson et al., 2009 ND
GD 14–18 3–6/group	Rat; SD; Gavage	DINP CAS No. 68515-48-0 & 28553-12-0 0, 500, 750, 1,000, 1,500	Fertility-related parameters: NE	Fertility-related parameters: 500: ↓ foetal testicular testosterone production (GD 14–18); 1,000: ↓ foetal testicular gene expression (StAR & Cyp11a) (GD 14-18)	Hannas et al., 2011 ND
GD 14–18 3–6/group	Rat; SD; Gavage	DINP CAS No. 68515-48-0 & 28553-12-0 0, 500, 750, 1,000, 1,500	Fertility-related parameters: NE	Fertility-related parameters: 500: ↓ Ins13 & other foetal testicular gene expression for androgen synthesis and cholesterol transport (GD 14–18)	Hannas et al., 2012 ND
<i>Prenatal developmental toxicity studies</i>					
GD 6-15 (dams sacrificed GD 20) 25/group	Rat; SD; Gavage	DINP (CAS No. unspecified) 0, 10, 500, 1,000	Maternal: 1,000	Maternal: NE	Hazleton, 1981*; ECB, 2003
			Developmental: 1,000	Developmental: NE	
GD 6–15 8–10/group	Rat; Wistar; Gavage	DINP1 (CAS No. 68515-48-0), DINP2 (CAS No. 28553-12-0) 0, 40, 200, 1,000	Maternal: 200	Maternal: 1,000: ↓ food consumption & ↑ relative kidney weights with DINP1; 1,000: vaginal haemorrhage in one dam with DINP2	Hellwig et al., 1997; CERHR, 2003; CPSC, 2010 ND
			Developmental: 200	Developmental: 1,000: ↑ skeletal variations (rudimentary cervical & accessory 14th ribs) with DINP1 & DINP2	

Study design	Species & route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & endpoint	Reference
GD 6–15 23–25/group	Rat; SD; Gavage	DINP CAS No. 68515-48-0 0, 100, 500, 1,000	Maternal: 500	Maternal: 1,000: ↓ weight gain & food consumption	Waterman et al., 1999; CERHR, 2003
			Developmental: 500	Developmental: 1,000: ↑ skeletal (rudimentary lumbar ribs) & visceral (dilated renal pelvis) variations	
GD 12–19 8/group	Rat; SD; Gavage	DINP CAS No. 68515-48-0 0, 50, 250, 750	Maternal: 50	Maternal: 250: ↑ liver weight	Clewell et al., 2011a ND
			Developmental: 50	Developmental: 250: ↑ testicular pathology (↑ MNG, GD 20), ↓ foetal testicular testosterone GD 19 (but ↑ on GD 20, not statistically significant); 750: ↑ testicular pathology (↑ No. of gonocytes & size of Leydig cell aggregates, GD 20)	

Post-natal developmental toxicity studies

GD 14 – PND 3 6–8/group	Rat; SD; Gavage	DINP CAS No. 68515-48-0 0, 750	Maternal: NE	Maternal: 750: ↓ weight gain to GD 21	Gray et al., 2000
			Developmental: NE	Developmental: 750: ↑ nipple retention, ↑ testicular & epididymal pathology (agenesis/atrophy)	
GD 14 – PND 3 No. of dams unspecified	Rat; SD; Gavage	DINP CAS No. 68515-48-0 0, 1,000, 1,500	Maternal: NE	Maternal: Not reported	Ostby et al., 2001*; CPSC, 2010 ND
			Developmental: NE	Developmental: 1,000: ↑ nipple retention; 1,500: ↓ AGD	
GD 15 – PND 10 5/group	Rat; SD; Diet	DINP CAS No. 28553-12-0 0, 400, 4,000, 20,000 ppm (0, 31–66, 307–657, 1,165–2,657 as calculated for gestational period GD 15–20 & lactational period PND 2–10, respectively)	Maternal: 307–657	Maternal: 1,165–2,657: ↓ weight gain & food consumption	Masutomi et al., 2003
			Developmental: 31–66 (m); 307–657 (f)	Developmental: 307–657: ↓ pup weight (m) PND 27; 1,165–2,657: ↓ brain & pup weights (m-f), ↓ testis, absolute ovarian & uterus weights PND 27, ↑ testicular pathology (degeneration of meiotic spermatocytes & Sertoli cells), ↓ corpora lutea in the ovary PND 77	

Study design	Species & route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & endpoint	Reference
GD 15 – PND 21 No. of dams unspecified	Rat; Wistar; Diet	DINP CAS No. 28553-12-0 0, 40, 400, 4,000, 20,000 ppm (0, 2, 20, 200, 1,000)	Maternal: NE	Maternal: Not reported	Lee et al., 2006; ECHA, 2010 ND
			Developmental: NE	Developmental: 2: ↓ pup weight (m-f), ↓ AGD & AGI, ↑ hypothalamic p130 (m) & granulin (f) gene expression PND 7, ↓ copulatory behaviour (m) & lordosis quotient (f) after maturity	
GD 7 – PND 17 16/group	Rat; Wistar; Gavage	DINP CAS No. 28553-12-0 0, 300, 600, 750, 900	Maternal: 900	Maternal: NE	Hass et al., 2003*, Boberg et al., 2011 ND, CPSC, 2010 ND
			Developmental: 300 (m); 600 (f)	Developmental: 600: ↑ nipple retention, ↑ testicular pathology (↑ MNG), ↓ testicular testosterone content & production (not statistically significant), ↓ sperm motility; 750: ↓ pup weight PND 13 (f), ↑ testicular pathology (enlarged seminiferous tubule); 900: ↓ pup weight PND 13 (m), ↓ AGD & AGI, ↓ testicular testosterone PND 90 (37%, not statistically significant), ↑ sperm counts, masculinised learning behaviour (f)	
GD 12 – PND 14 20/group	Rat; SD; Diet	DINP CAS No. 68515-48-0 0, 50, 250, 750	Maternal: 250	Maternal: 750: ↓ bodyweight & food consumption	Clewell et al., 2011b ND
			Developmental: 50	Developmental: 250: ↓ pup weight PND 14 (m), ↑ testicular pathology (↑ MNG, PND 2); 750: ↓ pup weight PND 2 (m), ↓ AGD & AGI, ↑ testicular pathology (enlarged Leydig cell aggregates)	

F0 = parental generation; F1= first filial/offspring generation; F2 = second filial/offspring generation; m-f = male–female; No. = number; sc = subcutaneous; ↓ = decreased; ↑ = increased; AGD = anogenital distance; AGI = anogenital index (AGD divided by cubic root of bodyweight); GATA4 = GATA binding protein 4; GD = gestational day; InsI3 = insulin-like factor 3; LAABC = levator ani/bulbocavernosus; LH = luteinising hormone; m-f = male–female; MNG = multinucleated gonocytes; NE = not established; P450ssc = P450 side-chain cleavage; PND = post-natal day; SD = Sprague-Dawley.

6.3 Effects observed in humans

Only limited information is available on the health effects of DINP (CAS No. 68515-48-0) in humans. No information is available for DINP (CAS No. 28553-12-0) in humans.

6.3.1 Skin irritation

In humans, DINP (CAS No. 68515-48-0) was applied undiluted for 24 hours to the skin of volunteers, followed by an observation period of 24 hours (Hill Top Research, 1995*; ECB, 2003). Positive and negative controls were included. Mild to moderate erythema was observed with the positive control but not with the test substance.

DINP did not cause skin irritation in humans.

6.3.2 Sensitisation

A human study (28 subjects in the pilot study and 76 subjects in the definitive study) using DINP (CAS No. 68515-48-0) involved the administration of the substance neat, with induction applications being made three times per week for three successive weeks. A challenge application was made after a 10- to 17-day rest period. There was no evidence of sensitisation (Hill Top Research, 1995*; ECB, 2003).

There have been reports of dermal reactions among children handling the internal contents of a toy ball containing DINP as an ingredient. However, it is possible that other ingredients of the material or attempts to remove the sticky material from the skin using detergents and cleaners may have caused the reactions. Unfortunately, no patch test was performed to clarify the hypothesis (Brodell and Torrence, 1992*; ECB, 2003).

Overall, DINP (CAS No. 68515-48-0) is unlikely to cause skin sensitisation.

6.3.3 Human studies

Fertility-related effects

Breast milk samples were analysed for six different phthalate monoesters in a Danish–Finnish cohort study in which serum measurements for gonadotropins (e.g. FSH and LH), inhibin B, sex-hormone-binding globulin and testosterone were also taken from newborn three-month old boys (62 cryptorchid and 68 healthy boys). No associations between any phthalate monoesters and cryptorchidism (testis maldescent) were found, but MINP (a metabolite of DINP) showed positive and statistically significant dose-dependent correlations with LH levels (Main et al., 2006).

Non-reproductive effects

In 845 Danish children of four to nine years of age, creatinine-uncorrected urinary metabolites of DINP (MCIOP, MHINP, MOINP and MINP, measured eight other phthalate metabolites) were negatively associated with serum levels of insulin-like growth factor I and thyroid hormones (free and total T3, but not free and total T4). The association reached statistical significance primarily in boys and this was only for MCIOP—a shared metabolite between DINP and DEHP. There were also overall negative associations (not statistically significant) between DINP metabolites with absolute values of height, weight, body mass index (BMI) and body surface area (BSA) in both sexes of this cohort (Boas et al., 2010 ND).

In conclusion, until the mechanism underlying a possible association between DINP or its metabolites with these non-reproductive effects are better understood, the implications of these findings are unclear.

7. Human health hazard characterisation

This section provides a brief overview of the main features of the toxicity data, identifies the critical endpoints and the 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 DINP, the hazard profile is based principally on animal data. In addition, for those toxicological endpoints where the data are incomplete or unavailable, information from structurally similar phthalates was used to examine the potential toxicity. This information was obtained from other NICNAS assessment reports for relevant phthalates. The NICNAS Phthalates Hazard Compendium (NICNAS, 2008b) contains a comparative analysis of toxicity endpoints across 24 ortho-phthalates, including DINP. DINP has predominantly 7- to 9-carbon backbone and is considered to be an HMW phthalate (Phthalate Esters Panel HPV Testing Group, 2001 & 2006; OECD, 2004).

The findings below are representative for DINP in general, as the two chemical formulations of DINP (CAS No. 68515-48-0 and 28553-12-0) show no statistically distinguishable differences in their toxicological profile.

7.1 Toxicokinetics

Orally administered DINP is rapidly absorbed based on the urinary excretion data in animal and human studies. Following high single doses or repeated dosing, the oral absorption of DINP may become saturated and incomplete. No information on the total excretion via all routes and/or the extent of faecal excretion (whether as the result of bile elimination or saturated urinary excretion) is available. For the purposes of this review, the oral bioavailability of DINP is considered to be 100% for both adults and children.

The available data suggest that dermal absorption of DINP through human skin may be significantly less than that of rat skin. Quantitative dermal absorption data for DINP are limited, thus the mean dermal absorption rate of 0.24 µg/cm²/h for DEHP migrated from the PVC film is considered appropriate to apply to DINP without the need for use of a correction factor to extrapolate from rats to humans.

Following oral and/or dermal administration in animals, DINP is widely distributed to tissues with no evidence of accumulation. The highest concentrations are observed in liver, blood and kidney, which rapidly decrease to trace amounts after 24 hours.

DINP is rapidly metabolised first to the monoester MINP, which is further metabolised by oxidation to form oxidative metabolites (mainly carboxy-MINP, hydroxy-MINP and oxo-MINP) or by hydrolysis to phthalic acid. In humans, carboxy-MINP is excreted mostly as free form, oxo-MINP mostly glucuronidated, and hydroxy-MINP equally in either forms. This metabolic profile of DINP is considered similar to those of DEHP and other HMW phthalates, with the monoester being only a minor urinary metabolite.

The urinary excretion of DINP after oral exposure shows a biphasic pattern in both rats and humans, with the majority excreted during the first 24 hours (1st phase) and the remainder in the 24–48 hour period (2nd phase). Excretion after dermal exposure is higher in urine than in faeces but at a much slower rate. The presence of radioactivity in the faeces also implies excretion via the bile.

7.2 Acute toxicity, irritation and sensitisation

In experimental animals, DINP exhibits low acute oral, dermal and inhalation toxicity. It caused minimal skin and eye irritation, and these were reversible. DINP showed no or minimal skin sensitisation potential. Therefore, DINP is expected to have low acute toxicity in humans.

7.3 Repeated dose toxicity

The toxicity of DINP has been evaluated in a number of non-primate animal species in both short-term (few weeks) and long-term studies (up to 2 years) by oral and dermal routes of exposure. Short-term studies in monkeys have also been conducted. Rodent studies reveal that repeated doses of DINP have effects mainly on the liver, kidney and testes. In the case of liver and kidney, increased absolute and/or relative organ weights and biochemical and histological changes were observed repeatedly with oral DINP administration in rats and mice. Decreased absolute testes weights were also reported at high doses, but only in mice.

Oral administration of DINP in monkeys for up to 13 weeks with doses up to 2,500 mg/kg bw/d produced no treatment-related changes in organ weights, biochemical parameters or histological findings. Although studies in non-human primates are clearly considered of greater relevance to humans, available rodent studies of DINP are more appropriate because of the longer-term administration and significantly greater numbers of animals tested.

7.3.1 Liver and kidney effects

Based on OECD guidance for quality of data (OECD, 2005), two critical studies of repeat-dose toxicity were identified. Both are 2-year dietary studies in Fischer 344 rats reported by Lington et al. (1997) and Moore et al. (1998a) in which liver and kidney effects dominated the toxicity profile for DINP.

The Lington et al. study (1997) administered three doses of DINP (CAS No. 68515-48-0) (15–18, 152–184 and 307–375 mg/kg bw/d) to rats. Both males and females from the mid- and high-dose groups exhibited dose-related increases in absolute and relative liver and kidney weights. At mid and high doses, increased incidences of non-neoplastic lesions were observed in the liver including focal necrosis in both sexes and spongiosis hepatis only in males. Based on these hepatic and renal effects, NOAELs of 15–18 mg/kg bw/d were derived.

In the study by Moore et al. (1998a), four doses of DINP (CAS No. 68515-48-0) (0, 500, 1,500, 6,000 and 12,000 ppm approx 0, 29–36, 88–108, 358–442 and 733–885 mg/kg bw/d) were used to treat rats. A recovery high-dose group was administered 12,000 ppm for 78 weeks followed by a 26-week recovery period. At ≥ 358 –442 mg/kg bw/d, dose-related increases in absolute and relative liver weights, serum ALT and AST and histopathological alterations were observed. Increased absolute/relative kidney weights in both sexes were also seen, with related biochemical changes more marked in males. There was also an increase in the frequency and severity of chronic progressive nephropathy in males. Based on these hepatic and renal effects, NOAELs of 88–108 mg/kg bw/d were derived.

Peroxisome proliferation occurs in the rodent liver in response to DINP but the extent to which it contributes to the toxicity profile of DINP is unclear. No morphological evidence of peroxisome proliferation in the liver even at the highest dose was reported by Lington et al. (1997) but Moore et al. (1998a) reported increases in numbers of mitotic cells and palmitoyl-CoA oxidase activity in the livers of high-dose male and female rats after one week of treatment. However, at subsequent time points, only diffuse hepatocellular enlargement was noted at this high dose, although palmitoyl-CoA oxidase activity was still elevated in all high dose treated males and females and mid high dose treated females at study termination.

Levels of peroxisome proliferation-activated receptors (PPAR) vary among different organs and are species dependent. However, all subtypes, PPAR α , PPAR β and PPAR γ , are found in multiple organs in both rodents and humans. Limited studies with DINP in cynomolgus and marmoset monkeys did not report convincing evidence of peroxisome proliferation. Slight changes in palmitoyl CoA oxidase activity and lauric acid 11- and 12-hydroxylase activity were reported in marmosets, but these were not regarded as biologically significant. Studies with hypolipidaemic agents in humans have provided no evidence of peroxisome proliferation or increased hepatocyte division (Bentley et al., 1993*; Ashby et al., 1994*; Cattley et al., 1998*; ECB, 2003). The comparative unresponsiveness of the primate liver to peroxisome proliferators has been explained

on the basis of decreased tissue levels of PPAR α , genotypic variations rendering the primate liver receptor less active compared to rodents, and species differences in phthalate hydrolysis and production of active phthalate metabolites (Tugwood et al., 1996; Palmer et al., 1998; Woodyatt et al., 1999).

Although peroxisome proliferation is not considered relevant to human health, the hepatomegaly seen in rat studies following administration of DINP did not appear solely related to peroxisome proliferation.

As well as liver weight increases, spongiosis hepatitis was reported late in life (only > 18 months) in both long-term rat studies and confirmed by a subsequent histopathology peer review (EPL, 1999*; ECB, 2003). Spongiosis hepatitis is a spontaneous, chronic liver lesion of ageing, particularly in male rats. It has no comparable lesion type in humans (Karbe and Kerlin, 2002) and was not reported in other DINP studies including similar long-term studies in the mouse. There was no evidence of a lesion resembling spongiosis hepatitis in a review of 163 human livers conducted by members of the Bannasch laboratory (Su Q et al., 1997*; ECPI, 2009). Historically, the lesion is associated with studies of hepatocarcinogens in rats and fish and has been described as a pre-neoplastic and/or neoplastic lesion (Stroebel et al., 1995; Bannasch 2003). More recently, it has been described as a cystic degenerative lesion (Karbe and Kerlin, 2002; Kerlin and Karbe, 2004).

Despite questions regarding the lack of a comparable lesion in humans or non-human primates, absence from other DINP studies including other rodent species and prevalence following DINP exposure only in older male rats, the incidence of spongiosis hepatitis in these studies has been used to model risk levels for human health risk assessment of DINP, including that for children, in other international reports (CPSC 1998, 2010; CSTEE, 2001). In the current assessment, spongiosis hepatitis was not considered relevant as a critical endpoint for human health risk assessment for the above reasons.

Increased absolute and/or relative kidney weights were also reported in both long-term rat studies. In the kidneys, despite relative organ weight increases, no clear treatment-related histological effects were reported; however, chronic progressive nephropathy was observed in most rats (Lington et al., 1997). A retrospective histochemical evaluation of kidney lesions in male rats in this study noted consistency with a specific male rat alpha 2 μ globulin nephropathy not regarded as relevant to humans (Caldwell et al., 1999a). Chronic progressive nephropathy was also reported by Moore et al. (1998a) with mineralisation of the renal papilla in mid-, high- and high-recovery dose males and of increased pigmented tubule cells at mid-, high- and high-recovery dose animals of both sexes. Non-neoplastic lesions in female rats in both studies have been attributed to an exacerbation of chronic progressive nephropathy common in aged rodents (Caldwell et al., 1999a) but the exact mechanism by which DINP may facilitate this is uncertain.

In deciding a NOAEL for risk characterisation from a number of studies, a NOAEL is selected to be the highest value below the lowest LOAEL from studies with a similar design. The advantage of a NOAEL over other methods, such as a benchmark dose for delineating the lower end of a dose-response relationship, is the lack of requirement for defining the nature or steepness of the dose-response curve (WHO, 1999). For the repeat-dose toxicity of DINP, a NOAEL can be selected based on a combination of the two complementary, well-performed rat chronic studies reporting similar adverse hepatic and renal effects. The Lington et al. (1997) study presents the lowest LOAEL for hepatic effects (152–184 mg/kg bw/d). The NOAEL in this study was 15–18 mg/kg bw/d. However, the Moore et al. (1998a) study reports two higher doses that were similarly without effect (29–36 mg/kg bw/d and 88–108 mg/kg bw/d). Consequently, a NOAEL of 88 mg/kg bw/d is selected for repeat-dose effects, noting that the Moore et al. study included two dose levels between the NOAEL and LOAEL of the Lington et al. study.

7.4 Genotoxicity and carcinogenicity

DINP exhibits little or no evidence of genotoxicity in available studies.

In rat carcinogenicity studies, increased incidences of MCL, kidney and liver neoplasia were observed. MCL was observed in DINP toxicological studies with Fischer 344 rats but not with Sprague Dawley rats. MCL is a common neoplasm in Fischer 344 rats with no comparable tumour type in humans and its increased incidence after chronic exposure to some substances is considered to be a strain-specific effect (Caldwell DJ, 1999b*). Therefore, MCL observed in Fischer 344 rats is not regarded as relevant to humans.

Incidences of kidney neoplasia were also observed in rodent carcinogenicity studies. However, these tumours were regarded as of limited relevance to humans. Retrospective histochemical studies of kidneys in male rats exposed to DINP noted accumulation of alpha 2μ-globulin in areas of cellular proliferation accompanying renal tubular nephropathy. Consequently, kidney tumours in male rats appear consistent with a specific gender- and species-specific alpha 2μ-globulin accumulation mechanism that is not regarded as relevant to humans (Caldwell et al., 1999a).

Liver neoplasia was also reported in rat studies accompanied by evidence of peroxisome proliferation in some but not all studies. Several studies performed specifically to assess the peroxisomal proliferation potential of DINP revealed biochemical evidence of peroxisomal proliferation in rodents (Hüls, 1992*). In contrast, there was no evidence of carcinogenic effects, and little biochemical evidence of peroxisome proliferation in cynomolgus or marmoset monkeys following oral administration of DINP for 2 and 13 weeks respectively.

Benford et al. (1986*, ECB 2003) studied the peroxisome proliferative potential of DINP and its metabolites *in vitro*. In cultured rat hepatocytes, these compounds induced increased peroxisomal palmitoyl-CoA oxidation. In contrast, in cultured marmoset monkey hepatocytes, only minimal changes in peroxisomal palmitoyl-CoA oxidation activity were observed with DINP and its metabolites, whereas there was a considerable increase in laurate 11-hydroxylation and 12-hydroxylation. Results suggested a significant species difference in the peroxisomal proliferative effects of DINP and its metabolites.

Metabolites of two types of DINP (CAS No. 68515-48-0 and CAS No. 71549-78-5) were also studied for their effects on gap junctional intercellular communication (GJIC) effects *in vitro* in hepatocytes of various species including humans, rats and mice (Baker et al., 1996*, ECB 2003). The GJIC assay has been claimed to have good cancer predictive potential for phthalates (Kalimi et al., 1995*, ECB 2003). Metabolites of both forms of DINP inhibited GJIC in rat hepatocytes. In contrast, none of the metabolites of either form of DINP inhibited GJIC in human hepatocytes at non-cytotoxic doses.

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

7.5 Reproductive toxicity

Following one-/two-generational and pre-/post-natal exposure of rats to DINP, effects on reduced pup weight, testosterone content and production and altered sexual differentiation and development (e.g. increased nipple retention, testicular and epididymal dysgenesis or agenesis/atrophy and decreased AGD/AGI in male offspring) and testicular pathology (increased MNG, number of gonocytes and size of Leydig cell aggregates; degeneration of meiotic spermatocytes and Sertoli cells and enlarged seminiferous tubule) were observed.

7.5.1 Effects related to fertility and sexual development

Changes in testicular testosterone levels and sexual differentiation malformations such as increased nipple retention, testicular and epididymal agenesis/atrophy, testicular pathology and decreased AGD/AGI were commonly reported effects following post-natal exposure to DINP in rodent studies.

DINP has no effects on mating, fertility, fecundity, gestational length or index in rat studies. Therefore, the NOAEL for fertility was determined to be 966 and 467 mg/kg bw/d respectively in one- and two-generation reproductive toxicity studies (Waterman et al., 2000).

Reduced testis weights (without histopathological changes) from 742 mg/kg bw/d and epididymis weights from 2,600 mg/kg bw/d DINP were reported in repeated-dose studies in mice but not in rats (Hazleton, 1992*; Moore et al., 1998b*; ECB, 2003). There was a report of increased weights of testes and epididymis in rats at 966 mg/kg bw/d, but, without histopathology examination, the significance of these organ weight changes could not be assessed (Waterman et al., 2000; CERHR, 2003; ECB 2003).

In rats, DINP was also shown to reduce testicular testosterone content and/or production (ex vivo) by male foetuses (GD 21) after gavage exposure during GD 7–21 (at 750 mg/kg bw/d) and GD 14–18 (at ≥ 500 mg/kg bw/d) in a similar pattern as observed with DEHP (Borch et al., 2004; Hannas et al., 2011). Foetal expression of genes involved in androgen synthesis such as StAR and Cyp11a were also reduced at ≥ 500 mg/kg bw/d (Hannas et al., 2011; 2012). However, in another study in rats, there were no decreases in testosterone production in male foetuses (GD 19) at 750 mg/kg bw/d after GD 13–17 exposure, although increases in gene expression levels of P450scc, GATA4, and particularly Ins13 (a foetal Leydig cell product critical for testis descent) were seen as a possible rebound mechanism on testicular steroidogenesis (Adamsson et al., 2009).

In at least three rat studies, DINP caused nipple retention at doses of ≥ 600 mg/kg bw/d and decreased AGD and/or AGI at ≥ 900 mg/kg bw/d in male offspring (Gray et al., 2000; Ostby et al., 2001; Hass et al., 2004; Boberg et al., 2011). Histopathological changes such as degeneration of meiotic spermatocytes and Sertoli cells at $\geq 1,000$ mg/kg bw/d, increased dysgenesis or agenesis/atrophy of testes and epididymis, increased size of Leydig cell aggregates and enlarged seminiferous tubule at ≥ 750 mg/kg bw/d were also reported (Masutomi et al., 2003; Gray et al., 2000; Boberg et al., 2011 and Clewell et al., 2011a,b). Increase in number of gonocytes were also reported at ≥ 250 mg/kg bw/d (Clewell et al., 2011 a,b; Boberg et al., 2011).

In the study by Boberg et al. (2011), decreased foetal testicular content in GD 21 male foetuses was also noted in rats exposed to DINP (≥ 600 mg/kg bw/d) from GD 7 to PND 17. In this study, the NOAEL for fertility-related toxicity (or sexual developmental toxicity) was established as 300 mg/kg bw/d.

In an unpublished study by Clewell et al. (2011a), foetal testicular testosterone was statistically significantly reduced (50% reduction) at ≥ 250 mg/kg bw/d on GD19. The NOAEL for fertility-related toxicity in this study was established as 50 mg/kg bw/d.

Lee et al. (2006) also reported decreased AGD and AGI, decreased copulatory behaviour in males (not dose dependent), and increased expression of genes involved in sexually dimorphic behaviour (e.g. hypothalamic p130 and granulin mRNA) at 40 ppm. No calculation of corresponding doses in mg/kg bw/d was provided in the study. According to ECHA (2010), a dose of 40 ppm is likely to be equivalent to 2 mg/kg bw/d. This LOAEL is considered inconsistent with the dose ranges reported from other rat studies and thus it is not included in the NOAEL derivation for risk assessment of DINP.

In humans, breast milk levels of MINP (a metabolite of DINP) were reported to be positively and dose-dependently correlated with levels of LH. Physiologically, there is a negative feedback between pituitary LH secretion and serum testosterone levels; however, reductions in testosterone did not reach statistical significance in this study (Main et al., 2006). This finding in human studies is very limited by questions concerning the reliability of breast milk samples as indicators of DINP

exposure and by other confounding factors such as the measured presence of other phthalate metabolites.

The observations for DINP on fertility-related parameters at high doses in rodents, such as reduced testosterone content and production and altered reproductive organ weights (with or without histopathologies), were of effects seen in numerous studies on transitional phthalates, including DEHP at much lower doses (NICNAS, 2010). The more severe effects reported for transitional phthalates at higher doses are not expected to be seen following treatment with DINP at achievable doses. An overall NOAEL for fertility-related or sexual developmental effects was determined to be 50 mg/kg bw/d based on the collective results of all available studies, including Boberg et al. (2011), Clewell et al. (2011a) and Hannas et al. (2011). Consistently, in a review of the category approach for reproductive effects of phthalates, Fabjan et al. (2006) also indicated that phthalates with shorter and longer side-chains (i.e. $\leq C3$ and $\geq C7$ respectively) might also produce some less severe reproductive effects or effects at higher doses.

7.5.2 Other developmental effects

In the study on reproductive and behavioural effects, DINP affected spatial learning on the first day of memory testing given female offspring performed better than controls and similarly to control males. The effect was dose-related and became statistically significant at ≥ 900 mg/kg bw/d. Although the implications of the observed effects are inconclusive, hormone alteration within a critical period of brain development might cause masculinisation of behavior in DINP-exposed female rats.

Changes in pup weight were observed in both sexes, in both one and two generations of rats exposed to DINP and at a much lower dose of approximately 100 mg/kg bw/d (Waterman et al., 2000; Masutomi et al., 2003). In addition, there was no overt maternal toxicity at this dose level where reduced pup weights were observed. The pup weight reduction was also sustained after birth and continued to PND 21. In the study of Clewell et al. (2011b), both food consumption and maternal weight were decreased at 750 mg/kg bw/d due to food palatability. Pup weights were also reduced at 750 mg/kg bw/d on PND 2 and at ≥ 250 mg/kg bw/d on PND 14. Taking all together, the reduced pup weight is considered the most sensitive DINP-related adverse effects on offspring growth and development. For the purposes of this review, the developmental NOAEL is derived as 50 mg/kg bw/d based on studies by Clewell et al. (2011b) from which the highest NOAEL for reduced pup weight was chosen and Waterman et al. (2000), which provided the lowest LOAEL.

After prenatal exposure in rats at high doses (e.g. 1,000 mg/kg bw/d), an increased frequency of skeletal and/or visceral variations (such as accessory 14th ribs, rudimentary cervical or lumbar ribs and/or dilated renal pelves) were reported but these effects generally occurred at or above maternally toxic doses (Hellwig et al., 1997; Waterman et al., 1999). The increase in supernumerary ribs (either cervical or lumbar) is one of the common anomalies seen in developmental toxicity studies in rodents (Chernoff & Rogers, 2004; Daston & Seed, 2007; NICNAS, 2008b). In view of the lack of conclusive evidence to assign the skeletal defects to maternal toxicity, together with the induced frequencies being outside historical control ranges, these skeletal variations in rats were interpreted as indicative of slight developmental effects.

No human data were available for developmental effects of DINP.

7.5.3 Relevance to humans

Overall, although the available human data are limited and do not provide sufficient evidence for a causal relationship between exposure to DINP and possible adverse health effects, elements of a plausible mode of action for the effects of DINP on male reproductive system (reduced testicular testosterone), offspring growth (decreased pup weight) and sexual differentiation (decreased AGD/AGI and increased nipple retention) are considered parallel in rats and humans if the exposure to DINP is high and within a critical window of development. Therefore, these effects observed in animal studies are regarded as relevant to humans for risk characterisation.

7.5.4 Mode of action

Historically, health impacts associated with phthalates have been linked most strongly to reproductive effects. The majority of data on the mode of action of phthalates in inducing reproductive effects involve studies of mid molecular weight (so-called ‘transitional’) phthalates such as DEHP (reviewed by Foster, 2005*; Ge et al., 2007; Hu et al., 2009*, NICNAS 2010). These studies support a mode of action for transitional phthalates in rodents involving effects on steroidogenesis and expression of genes critical for development of the reproductive system. The extent to which this mode of action for transitional phthalates is reflective of the mode of action for HMW phthalates such as DINP is not certain. Although data on the overall reproductive effects of DINP in rodents are adequate, there is insufficient information to examine the mode of action of DINP on male reproductive tract development and sexual function in comparison with transitional phthalates.

In *in vitro* studies, DINP was shown not to display affinity for oestrogen or androgen receptors but did induce the proliferation of ZR-75 (a human breast cancer cell line with higher estrogen specificity) to a significantly greater extent than the control 17β -oestradiol (endogenous oestrogen) (Harris et al., 1997). DINP also tended to amplify progesterone production (not statistically significant) and suppress oestradiol production (statistically significant) by porcine ovarian granulosa in the presence of hFSH, although the mode of action involved in ovarian steroidogenesis was unclear (Mlynarcikova et al., 2007).

In *in vivo* studies, dose-response curves for antiandrogenic activities such as reduced testicular testosterone levels and related gene expression of DINP and DEHP suggested that both shared a similar pattern of endocrine alterations in male rat fetuses, although quantitatively DINP was less potent than DEHP (e.g. 20-fold less in inducing nipple retention and testicular and epididymal agenesis/atrophy and 2.3-fold less in reducing foetal testicular testosterone production (Gray et al., 2000; Hannas et al., 2011)).

Overall, there are uncertainties with respect to the exact mechanism of DINP effects on fertility-related parameters and development in rodents; however, the mechanism appears to involve alterations of endocrine function. In addition, the chemical composition of DINP with side-chains made up of 5–10% methylethylhexyl (Section 3.1) indicates that a minor component of DINP meets the definition of a ‘transitional phthalate’ as the side-chain length in this case is six. Transitional phthalates are postulated to have antiandrogenic activity (Phthalate Esters Panel HPV Testing Group, 2006; NICNAS, 2008b). Fajan et al. (2006), when reviewing a category approach for reproductive effects of phthalates, also suggested that when assessing complex phthalate mixtures it is not enough to determine the predominant side-chain length—the amount of the shorter side-chains (e.g. C4–C6) in the mixture must also be determined.

7.6 Non-reproductive effects

Recent human studies suggested some statistical correlations between creatinine-uncorrected urinary metabolites of DINP (MCIOP, MHINP, MOINP and MINP) and possible decreased thyroid activity, increased adiposity and insulin resistance that may be related with low testosterone in adult males. However, these findings are preliminary and provide insufficient basis for risk assessment.

7.7 Summary

The critical toxicity endpoints for DINP in animal studies are repeated-dose toxicity (increased liver and kidney weights with histopathological findings in the liver) and fertility-related/developmental toxicity (reduced pup weight, testosterone and altered sexual differentiation).

Although some studies reported the association between liver toxicity and peroxisome proliferation, there is no morphological evidence to explain the mechanism of liver enlargement seen following repeated DINP dietary exposure. On this basis, this organ effect did not appear directly related to peroxisome proliferation and is therefore considered relevant to humans for this risk assessment.

The effects of DINP on fertility-related parameters such as reduced testosterone content and production and altered reproductive organ weights (with or without histopathologies) have been demonstrated in rats. Although quantitatively being less potent, DINP has exhibited adverse effects on the male reproductive system and sexual differentiation during development in a number of rodent studies (e.g. increased nipple retention, testicular pathology and decreased AGD/AGI in male offspring), which are components of the antiandrogenic pattern observed with DEHP (a known reproductive toxicant). Foetal expression of genes involved in androgen synthesis such as StAR and Cyp11a were also reduced. There was also a report of increased gene expression levels of InsL3 (a foetal Leydig cell product critical for testis descent) that may infer the impaired testicular steroidogenesis following exposure to DINP at high doses (e.g. ≥ 750 mg/kg bw/d). Reduced InsL3 was also reported in numerous studies with DEHP.

Considering the chemical composition of DINP, which is represented as mixed phthalates with side-chains made up of 5–10% methylethylhexyl, limited evidence of the toxicological properties of transitional phthalates may be expected at high doses of DINP tested.

The reduced pup weight was observed at approximately 100 mg/kg bw/d in both sexes, both in one- and two-generation reproductive studies in rats, in the absence of overt maternal toxicity. The pup weight reduction was also sustained and not considered solely related to low birth weight. In a post-natal toxicity study, reduced pup weight was also reduced at ≥ 250 mg/kg bw/d. Therefore, this adverse effect of DINP is assessed as the most sensitive endpoint on offspring development.

Overall, the available human data do not provide sufficient evidence for a causal relationship between exposure to DINP and adverse health effects in humans. There is also insufficient information to examine the mode of action of DINP on male reproductive tract development and sexual function in comparison with transitional phthalates. However, elements of the plausible mode of action for DINP effects on the male reproductive system, offspring growth and sexual differentiation are considered likely to be parallel in rats and humans if the exposure to DINP is high and within a critical window of development. Therefore, the effects observed in animal studies are regarded as relevant to a human risk assessment.

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

Table 7.1—Endpoints selected for risk characterisation of DINP

Toxicity	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & endpoints	Species & age at treatment	Reference
Systemic effects (increased liver & kidney weights)	88	358: \uparrow liver and kidney weights	Rat; Fischer 344; Adults	Lington et al., 1997; Moore et al., 1998a
Fertility-related effects (reduced testosterone)	50	250: \downarrow testicular testosterone content &/or production	Rat; SD or Wistar; Foetuses	Boberg et al., 2011; Clewell et al., 2011a; Hannas et al., 2011
Developmental effects (reduced pup weight)	50	~ 100 : \downarrow pup weight PND 21 (F1) & PND 7 (F2)	Rat; SD; Newborn	Waterman et al., 2000; Clewell et al., 2011b

F1= first filial/offspring generation; F2 = second filial/offspring generation; m-f = male–female; \downarrow = decreased; \uparrow = increased; PND = post-natal day; SD = Sprague-Dawley

8. Human health risk characterisation

8.1 Methodology

An MOE methodology is used frequently in international assessments to characterise risks to human health associated with exposure to chemicals (ECB, 2003). The risk characterisation is conducted by comparing quantitative information on exposure to 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) to provide an MOE;
4. $MOE = NOAEL/EHD$; and
5. Characterisation of risk, by evaluating whether the MOE indicates a concern for the human population under consideration.

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

In this assessment, the MOE methodology was used for characterising the public health risks to children from DINP exposure through use of toys and child-care articles.

8.2 Critical health effects

The analyses of the toxicological effects of DINP, including the identification of key studies and health effects relevant to humans, reveal three critical health effects for risk characterisation (see Section 6—Human Health Hazard Assessment and Section 7—Human Health Hazard Characterisation). These effects are repeated-dose toxicity (increased liver and kidney weights with histopathological findings), and effects on fertility-related parameters and development (reduced testicular testosterone and pup weight) observed in rodents. The NOAELs for risk characterisation are 88 mg/kg bw/d (repeated dose toxicity), 50 mg/kg bw/d (fertility-related toxicity) and 50 mg/kg bw/d (developmental toxicity) (Table 7.1).

8.3 Risk estimates

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

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

The combined internal dose for children, arising from contact with toys and child-care articles, is discussed in Section 5.2.5 and summarised in Table 8.1. Two exposure scenarios are considered for children using toys and child-care articles: a ‘typical’ and a reasonable ‘worst-case’ scenario. The reasonable worst-case scenario takes into account the maximal mouthing time of 2.2 hours/day identified for children aged six months to 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 to 36 months of age.

Overall, these studies demonstrate that mouthing times are highest for children aged six months to 12 months and they decrease with increasing age. In the absence of Australian information, these mouthing behaviours are assumed applicable to Australian children.

Additional assumptions considered are as follows:

- Maximal and typical migration rate for DINP 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 $\mu\text{g}/\text{cm}^2/\text{h}$. The mean migration rate, which is applied to the typical exposure scenario, is 26 $\mu\text{g}/\text{cm}^2/\text{h}$ (Chen, 1998).
- Bioavailability of DINP via the oral route is assumed to be 100%.
- Dermal absorption of DINP from PVC matrix is 0.24 $\mu\text{g}/\text{cm}^2/\text{h}$.

Table 8.1—Estimated total internal exposure for children

Route of exposure	Typical D_{int} ($\mu\text{g}/\text{kg bw}/\text{d}$)	Worst-case D_{int} ($\mu\text{g}/\text{kg bw}/\text{d}$)
Oral	27.8	169.9
Dermal	2.6	7.0
Combined	30.4	176.9

Estimation of margin of exposure

Risk estimates take into account the likelihood for adverse effects on liver and kidneys and reproduction/development at future life stages related to long-term exposure through repeated handling and mouthing of toys. Table 8.2 provides the MOE estimated from the internal DINP dose in children and the dose at which no adverse effects were observed on the liver, kidney, fertility-related parameters and growth of the offspring in experimental animals, i.e. the NOAEL.

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

Toxicity endpoints	NOAEL $\text{mg}/\text{kg bw}/\text{d}$	MOE for typical exposure scenario	MOE for worst- case exposure scenario
Systemic effects (increased liver & kidney weights)	88	2,895	497
Fertility-related effect (reduced testosterone)	50	1,645	283
Developmental effect (reduced pup weight)	50	1,645	283

The risk estimates for DINP-induced effects on the liver, kidney, fertility-related parameters and growth of the offspring in both scenarios of toy use by children give MOEs above 100 (Table 8.2) and hence indicate low risk of adverse effects on these organs, reproductive system and growth.

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 DINP arise mainly from inadequate data and include:

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

Areas of concern

The risk estimates above do not indicate particular areas of concern from exposure of children to DINP via handling/mouthing of toys and child-care articles. The MOE for pup weight effects and foetal testicular testosterone, although being reduced from 1645 in the typical case scenario to 283 in the reasonable worst-case scenario, is still an adequate safety margin.

Risks from cumulative exposure of children to DINP in toys and child-care articles with or without DEHP at maximum 1% together with co-exposure to DEP in cosmetics at maximum 0.5% in body lotions are considered low, as cumulative MOEs for the three critical health effects identified are all above 100 (Appendix 1, Tables A1.1, 1.2 and 1.33), which indicate an adequate safety margin. However, as the concentration of DEP in body lotion increases from 0.5% to 0.75% and 1%, the cumulative MOE from combined exposure to DINP and DEP decreases.

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 DINP.

9.1 Current public health risk standards

9.1.1 Toys and child-care articles

In Australia, DINP was identified as being in use or with the potential for use in children's toys and child-care articles including play and exercise balls, pacifiers, teething rings and squeeze toys. Data from the 2006 voluntary call for information on phthalates in articles indicate that DINP is present in imported toys at a concentration range of 0.005–35%.

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

In contrast, current EU, US and Canadian legislation restricts the use of DINP to less than 0.1% w/w of the plastic used in toys and child-care articles that can be mouthed by children. The age cutoffs in EU, US (California) and Canada are three, three and four years old, respectively.

9.1.2 Cosmetics

There was no information on the use of DINP in cosmetics from information provided by Australian industry. There is no available information to indicate the use of DINP in cosmetic products or any evidence to suggest that DINP is used in cosmetics in Australia or overseas.

There are currently no restrictions on the use of DINP in cosmetics in Australia. The NICNAS Cosmetics Guidelines 2007, published in 2007 and modified in 2008, contain a list of prohibited or restricted cosmetic chemicals in Australia. DINP is not currently listed.

Current EU, US and Canadian legislation has no restrictions on the use of DINP in cosmetic products.

Appendix 1—Risk estimate from cumulative exposures

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

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). The assumption is made that the phthalates operate by a similar mode of action for each of the three endpoints considered (systemic toxicity, fertility-related and developmental effects) 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 an 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:

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

The calculations for combined exposure were undertaken for three scenarios:

- combined exposure to DINP in toys and child-care articles and DEP in cosmetics (Table A.1.1)
- exposure to a mixed plasticiser containing 42% DINP and 1% DEHP used in toys and child-care articles (Table A1.2)
- combined exposure to a mixed DINP/DEHP plasticiser in toys and child-care articles and DEP in cosmetics (Table A1.3).

An example calculation can be given for combined developmental toxicity (pup weight) of DINP in toys and child-care articles and DEP in cosmetics. For this endpoint, the toxicity of DINP (NOAEL = 50 mg/kg bw/d) is more potent than that of DEP (NOAEL = 197 mg/kg bw/d). Relevant exposure estimates for a six-month-old infant are 176.9 µg/kg bw/d for DINP (at maximum 43%) from toys and child-care articles and 192.8 µg/kg bw/d for DEP (at maximum 0.5%) from baby lotions. The relevant MOEs are therefore 283 (DINP) and 1,022 (DEP). The respective hazard indices are 1/283 (DINP) and 1/1,022 (DEP) and the cumulative hazard index is the sum ($1/283 + 1/1,022$) equalling 1/221.

The cumulative MOE is calculated from the equation above— $1/(1/MOE \text{ (DINP)} + 1/MOE \text{ (DEP)})$ —as 221. The other values are calculated in a similar manner, with adjustment, where necessary, for relative concentrations and combinations (Tables A1.2 and A1.3).

Risks from cumulative exposure of children to DINP in toys and child-care articles with or without DEHP at maximum 1% together with co-exposure to DEP in cosmetics at maximum 0.5% in body

lotions are considered low, as cumulative MOEs for the three critical health effects identified all indicate an adequate safety margin (Tables A1.1, A1.2 and A1.3). These MOEs are specifically calculated for six-month-old infants because the mouthing time studies (Appendix 2) indicate that newborn babies are unlikely to use teethingers or child-care articles, while MOE estimates for older babies (e.g. 12-month-old infants) are expected to be higher based on their higher bodyweights.

As the concentration of DEP in body lotion increases from 0.5% to 0.75% and 1%, the cumulative MOE for pup weight reduces from 221 to 199 and 182 respectively (detailed calculations not shown). Similarly, the MOE for reduced testes weight and/or testosterone reduces from 109 to 87 and 72 respectively (detailed calculations not shown). Therefore, at 0.75% DEP and above, the cumulative MOE for risk of fertility-related effects in six-month-old infants is below 100 and is of concern. In Australia, the maximum allowable concentration of DEP for use in body lotions is 0.5%.

Table A1.1—Calculated cumulative MOEs for combined exposure to DINP in toys and child-care articles and DEP in cosmetics

Toxicity	DINP ^a		DEP ^b		Cumulative MOE ^c
	NOAEL	MOE	NOAEL	MOE	
Systemic effects (enlarged liver &/or kidney)	88	497	150	778	303
Fertility-related effect (reduced testes weight &/or testosterone)	50	283	40	207	120
Developmental effect (reduced pup weight)	50	283	197	1,022	221

^a From Table 8.2 of the DINP risk characterisation.

^b From the DEP PEC assessment report (NICNAS, 2011) based on the daily internal DEP doses for six-month-old infants estimated from dermal exposure to body lotions containing 0.5% DEP.

^c Calculated from the formula $1/(1/\text{MOE of DINP} + 1/\text{MOE of DEP})$.

Table A1.2—Calculated cumulative MOEs for exposure to a mixed plasticiser containing 42% DINP and 1%DEHP used in toys and child-care articles

Toxicity	DINP ^a		DEHP ^b		Cumulative MOE ^c
	NOAEL	MOE	NOAEL	MOE	
Systemic effects (enlarged liver &/or kidney)	88	497	28.9	163	475
Fertility-related effects (reduced testes weight &/or testosterone)	50	283	4.8	27	232
Developmental effect (reduced pup weight)	50	283	46 ^d	260	282

^a From Table 8.2 of the DINP risk characterisation.

^b From Table 8.3 of the DEHP PEC assessment report (NICNAS, 2010).

^c Calculated from the formula $1/[(42/\text{MOE of DINP} + 1/\text{MOE of DEHP})/43]$.

^d A NOAEL for reduced pup weight derived from Wolfe & Layton's (2003) study reviewed in the DEHP PEC assessment report (NICNAS, 2010).

Table A1.3—Calculated cumulative MOEs for combined exposure to a mixed DINP/DEHP plasticiser in toys and child-care articles and DEP in cosmetics

Toxicity	42% DINP/ 1% DEHP ^a	DEP ^b		Cumulative MOE ^c
	MOE	NOAEL	MOE	
Systemic effects (enlarged liver &/or kidney)	475	150	778	295
Fertility-related effects (reduced testes weight &/or testosterone)	232	40	207	109
Developmental effect (reduced pup weight)	282	197	1,022	221

^a From Table A1.2 above.

^b From the DEP PEC assessment report (NICNAS, 2011) based on the daily internal DEP doses for six-month-old infants estimated from dermal exposure to body lotions containing 0.5% DEP.

^c Calculated from the formula $1/(1/\text{MOE of mixed DINP/DEHP} + 1/\text{MOE of DEP})$.

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 young children aged between three and 36 months, for five categories of objects: pacifiers, teethingers, fingers, toys and non-toys. Ten 15-minute observations of mouthing behaviour were conducted by parents 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 teethingers and other mouthing objects (excluding pacifiers) was 2 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 to 36 months of age.

Juberg et al. (2001) reported an observational study of the mouthing behaviour of children in the US with pacifiers, teethingers, 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; teethingers, 6 minutes; and other objects, 2 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, 2 minutes; teethingers, 0 minutes; and other objects, 2 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 2 hours per day. The report noted considerable variations in mouthing behaviour between children, and in day-to-day mouthing behaviour in individual children.

Kiss (2001) conducted an observational study of children's mouthing activity in the 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 4 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 1 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 3 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/cm²/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 10 cm². Initially all 20 volunteers were asked to suck and bite on the standard PVC disk 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/cm²/h (range 1.8–49.8 µg/cm²/h) for the standard PVC disk, 14.64 µg/cm²/h (range 5.4–53.4 µg/cm²/h) for the teething ring and 9.78 µg/cm²/h (range 5.4–34.2 µg/cm²/h) for the disk 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 teething rings (36% DINP) were used separately. Each specimen had a surface area of 10–15 cm². The volunteers were asked to suck only or chew the samples separately for 1–3 hours. Saliva samples were collected and analysed. For DINP, the mean release rate (sucking for 1 hour) was 8.33 µg/cm²/h (range 2.97–14.52 µg/cm²/h). Higher values were recorded from chewing. The mean release rate for DINP (chewing for 1 hour) was 13.3 µg/cm²/h (range 7.68–21.52 µg/cm²/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 cm². 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 cm². 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/cm²/h (SD 2.8 µg/cm²/h). The highest migration rate was for the PVC plate sample at 32.6 µg/cm²/h (SD 2.6 µg/cm²/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 plasticizers from in vivo testing

Study	PVC product	Phthalate	Wt. %	Test condition	Migration rate (SD) ($\mu\text{g}/\text{cm}^2/\text{h}$)	
					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 different test articles may depend more on the properties of the PVC grade comprising the article.
- The amount of phthalate released into saliva through biting and chewing exceeded its expected solubility in water in all *in vivo* studies, indicating that migration is not merely a simple diffusion process.
- Migration rates are proportional to the amplitude of mechanical action 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 $\mu\text{g}/\text{cm}^2/\text{h}$ 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 $\mu\text{g}/\text{cm}^2/\text{h}$ (Chen, 1998), which is similar to the highest mean migration rate of 32.6 $\mu\text{g}/\text{cm}^2/\text{h}$ (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.

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