Priority Existing Chemical
Draft Assessment Report

Australian Government
Department of Health and Ageing
NICNAS

Diisononyl Phthalate

APRIL 2012

DRAFT

Any requests for variation must be made with respect to the draft report and accompanied by a completed application form (NICNAS Form 4a).

Form 4a is available from www.nicnas.gov.au/Forms/Existing_Chemicals/Form4a_PDF.pdf or from the address below.

Requests for variation should be sent to:

NICNAS, GPO Box 58, Sydney NSW 2001

Ph: 02 8577 8863

Fax: 02 8577 8888

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

GPO Box 58, Sydney NSW 2001 AUSTRALIA www.nicnas.gov.au

ISBN xxxxxxxxxxx

Table of Contents

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* (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
- 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 after 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 1000 - 9999 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.

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 polymers for 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) have been implemented in EU, USA and Canada on the use of DINP in toys and child care articles that can be placed in the mouth by children. 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 MCL, 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 liver and kidney weights, biochemical changes in enzymes of hepatic origin 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 (2500 mg/kg). No systemic effects were reported.

Overall, a 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 2600 mg/kg bw/d DINP 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 (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 ≥ 1000 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.

Decreased foetal testicular testosterone content and increased testicular and epididymal agenesis/atrophy in GD 21 male foetuses were also noted in rats exposed to DINP (≥ 600 mg/kg bw/d) from GD 7 to PND 17. Histopathological changes such as degeneration of meiotic spermatocytes and Sertoli cells and agenesis/atrophy of testes and epididymides were also reported at ≥ 1000 and ≥ 600 mg/kg bw/d, respectively. 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. 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 300 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 PND 21. Taking all together, the reduced pup weight is considered the most sensitive DINP-related adverse effects on offspring growth and development, and hence for the purposes of this review, the developmental NOAEL is established as 31 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, offspring growth and sexual differentiation 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 6 and 12 months of age. Based on these studies, for children aged 6-12 months, a reasonable worst-case exposure scenario considered a maximal mouthing time of 3 h/d and a typical exposure scenario considered a mean daily mouthing time of 0.8 h/d.

Given the low acute toxicity, low 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 2895 and 365, respectively. The MOE for reproductive and developmental effects for the typical scenario was 9868 and 1020, respectively and for the worst case scenario, 1243 and 128, respectively. 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 reproductive and developmental effects indicate low concern for children at the current reported levels of DINP in toys and child care article.

The effect of cumulative exposures to phthalates can arise from the effects of other components in a mixed phthalate used in toys and child care articles, and from the combined exposure scenarios or multiple sources. While 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, albeit marginally, 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 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*, 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; and
- additional information or events that change the assumptions for estimating the cumulative risk in this assessment.

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

Acronyms & Abbreviations

AGD	anogenital distance
BBP	butylbenzyl phthalate
bw	bodyweight
CAS	Chemical Abstracts Service
CERHR	Centre for the Evaluation of Risks to Human Reproduction (US)
CHAP	Chronic Hazard Advisory Panel (US)
CHO	Chinese hamster ovary
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
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
EU RAR	European Union Risk Assessment Report
FDA	Food and Drug Administration (US)
g	gram
GD	gestation day
GI	gastro-intestinal
GJIC	gap junctional intercellular communication

h	hour
hER	human oestrogen receptor
HMW	high molecular weight
HPVC	high production volume chemical
HVICL	High Volume Industrial Chemical List
IgE	immunoglobulin E
IgG	immunoglobulin G
IL	interleukin
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
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	postnatal 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)
USA	United States of America
US EPA	United States Environmental Protection Agency
wt	weight

1. Introduction

1.1 Declaration

Diisononyl phthalate (DINP) (CAS No. 68515-48-0 and 28553-12-0) was one of 9 phthalate chemicals declared as a Priority Existing Chemical (PEC) under the *Industrial Chemicals (Notification and Assessment) Act 1989* (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.

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 for information 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.

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 May 2011.

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

This assessment also incorporates hazard information from the 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 Industrial Chemicals (Notification and Assessment) Act 1989, NICNAS provided the applicants with a draft copy of the report for comment during the corrections/variations phase of the assessment. The applicants were as follows:

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

On the basis of the ester side chain length, 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 in PVC-containing school supplies (scented erasers, pencil case). DINP can be blended into a paste (plastisol), for coating (tapaulins, synthetic leather and wall covering) and rotomoulding (toys, play and exercise balls, hoppers) applications. In addition, DINP is also used in non-polymer 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) which 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 pacifier 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 phthalates 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 European Union in 2005 (Directive 2005/84/EC) and sets a content limit of 0.1% 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/):

Regulatory information on DINP was also available from the United States of America (USA).

In February 2007, the state of California (USA) 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 (USEPA) released a Phthalates Action Plan covering 8 phthalates including DINP. According to the plan, because of concerns over toxicity and evidence of human and environmental exposures to these phthalates, the USEPA intends to initiate action to address their manufacturing, processing, distribution and/or use. The action is part of a coordinated approach with the CPSC and the Food and Drug Administration (FDA). The USEPA stated that they intended to initiate rulemaking in 2010 to include these phthalates to the Concern List under TSCA section 5(b)(4) as chemicals that present, or may present, an unreasonable risk of injury to health or the environment. The USEPA 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 updates 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 (CIUCUS) in the US (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 the International Cosmetic Ingredient Dictionary (ICID), a list of chemicals in use in cosmetic products compiled by the Personal Care Products Council.

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% weight/weight) 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 6 months after the registration of Phthalates Regulations in December 2010.

Beyond the recent actions in EU, USA 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, NICNAS in 2008 released a series of hazard assessments on phthalates 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);
- Opinion of the European Food Safety Authority (EFSA) on the safety of DINP for use in food contact materials;
- 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 European Chemicals Agency (ECHA) (ECHA, 2010).

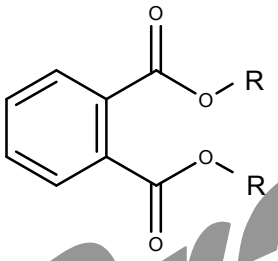
3. Identity, Properties and Analysis

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)
	1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters
	1,2-benzenedicarboxylic acid, diisononyl ester
Molecular Formula:	$C_{26}H_{42}O_4$ [average]
Molecular Weight:	420.6 [average]
Purity:	>99.5%

Structural Formula:



CAS Number: 68515-48-0

R = is an undefined branched alkyl chain comprising 8 to 10 carbon atoms, predominantly 9 carbon atoms

CAS Number: 28553-12-0

R = is an undefined branched alkyl chain comprising 9 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 which 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 coefficient 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/m^3

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 1000 and 9999 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.

4.2 Uses of DINP

4.2.1 Use 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 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% - 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/year (ECHA, 2010). The assessment undertaken by European Chemicals Bureau (ECB, 2003) and the DINP Information Centre (http://www.dinp-facts.com/), indicate that 95 per cent 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 1 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), which is 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 for Consumer Safety.

Consumption of DINP in the US was estimated to be 178,000 metric 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 include stationary and 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, under body coatings, and sealants (carpet backing) (CERHR 2003). The use of DINP in toys represents <1% 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 compilation of ingredients used in cosmetics (CIUCUS) in the US (Personal Care Products Council, 2011). The CIUCUS provides a compilation of ingredients that have documented use in cosmetics by the Food and Drug Administration (FDA). It is also not listed in the International Cosmetic Ingredient Dictionary (ICID), a list of chemicals in use in cosmetic products compiled by the Personal Care Products Council.

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 1 to 13 carbons, phthalates exhibit a number of orders of magnitude increase in the octanol-water partition coefficient (K_{ow}) and a ten-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 from 9 mPa.s for DEP to 52 mPa.s for DINP and up to 190 mPa.s for ditridecyl phthalate (Eastman, 2002).

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

Little information is available in open literature on the subject of substitutability of phthalates. A number of phthalates and their functions are listed in the International Cosmetic Ingredients Dictionary and Handbook (Gottschalck & McEwen, 2006 ND), 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 ND) 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 a high molecular weight phthalate and thus it is not likely to substitute for DEP – a low molecular weight phthalate commonly used in cosmetics. Furthermore, there is no evidence to suggest that the high molecular weight 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 high molecular weight 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 International Cosmetic Ingredient Dictionary and Handbook (The Personal Care Products Council, 2010). Cosmetic uses were not identified in the EU RAR 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 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 childrens' 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 studies based on data for PVC plasticised with DEHP (NICNAS, 2010).

International biomonitoring data provide estimation of overall exposure of the general population or specific subpopulations 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 for these exposures 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 Sections 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 (total of 35 samples) that are likely to be mouthed by children in the USA, and to determine the amount of phthalate migration from these products using in vitro and in vivo tests. The products include soothers, teethers, nipples, pacifiers, books, handbag, and a variety of toys. In vitro tests were conducted either by shaking a PVC sample in a saliva stimulant or subjecting cut samples of PVC to impaction applied by a piston. For in vivo tests, human volunteers gently chewed/mouthed a polyethylene disk from a toy duck for four 15 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%-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% non-PVC) purchased in 17 countries including 5 purchased in Australia. The country of origin was also stated, with 41/71 toys purchased worldwide being made in China, including 4/5 purchased in Australia. For the remaining toy purchased in Australia, the origin was not determined. The country of origin data seen in this 2000 study for the Australian purchased toys was anecdotally confirmed to be relevant for the majority of toys currently being imported to Australia (Australian Toy Association, 2009).

DINP was the phthalate most frequently found in the toy samples (64%) and tended to be present at the highest concentration (up to 51% w/w). DEHP was the next most frequently found in the tested toys (up to 48%) with concentrations ranging from 0.008% to 35.5% w/w. However, few of the sampled toys contained DEHP as the dominant phthalate plasticiser (8%, with a variety of countries of origin), with the majority of the remainder having <1% DEHP in conjunction with higher levels of DINP. Other phthalates found included diethyl phthalate (DEP), di-n-butyl phthalate (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 3 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 industrialized countries. This review indicated that DINP may be present in certain children toys at weight concentrations exceeding 40%.

The phthalate levels of toys available in the Indian market were investigated with most of the toys analysed were for children ages 3 years and below. A total of 15 soft and 9 hard toys were tested wherein all of the samples have been reported to contain phthalates. The predominant phthalates in the soft toys were DINP and DEHP. DINP was found in 40% (6/15) of the soft toys and 44% (4/9) of the hard toys. The highest DINP concentration was 16.2% in a soft toy marketed for children 3-18 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 4 (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%-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 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 A. The results demonstrate that migration rate of phthalate plasticisers from plastic toys into saliva through biting and chewing is the combined effect of molecular diffusion and mechanical action with the latter the likely dominating factor. The phthalate migration rate from articles appears largely determined by the magnitude of the mechanical force applied to an article and the properties of the PVC grade comprising the article, and less so by the physicochemical characteristics or concentration of the particular phthalate.

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 body weight of children, estimated mouthing duration, and phthalate migration rate from toys. The main estimate is for a 6 month old infant, based on the studies which demonstrate that 6 month

old infants are within an age range showing maximum mouthing behaviour, and have the lowest body weight in this age range (Appendix 2). The following assumptions were also used:

- A child of 6 months weighs 7.5 kg. The mean body weight is based on the 50th percentile weight of 6-month old children (combined sexes) (USEPA, 2006);
- The surface area of a child's open mouth and the typical surface of an article available for mouthing at any one time is approximately 10 cm^2 (LGC, 1998);
- The maximum time the child spends mouthing toys is 3 h/d and a typical mouthing time is around 0.8 h/d (Appendix 2); and
- Phthalate bioavailability via the oral route is 100% (Section 7.1).

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

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

Where:

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

M = Migration rate of DINP from toys, µg/cm^2/h

S_{mouth} = Surface area of a child's open mouth, cm^2

t = Mouthing time, h

n = Frequency/day

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

BW = Child bodyweight, kg

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

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

	M (µg/cm^2/h)	**BW (kg)**	**S_{mouth} (cm^2)**	**t · n* (h/d)**	**$D_{int.oral}$ (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	3	231.7

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

The estimate of daily exposure using the worst-case scenario is comparable with the estimate in the EU RAR (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 cm^2) with ^{14}C-DEHP (total of 40.4% DEHP w/w) were applied to shaved backs of 8 male rats in two separate experiments. The mean dermal absorption of DEHP in rats was determined to be 0.24 $\mu g/cm^2/h$ (Section 6.1).

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

In this scenario, exposure is proportional to the amount of time spent handling the toys, the internal dose is dependent on the time handling the toys and the rate of dermal absorption. Dermal exposure to 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 6 months weighs 7.5 kg (USEPA, 2006);
- The maximum time the child spends handling toys is 3 h/d and a typical contact time is around 0.8 h/d (Appendix 2); and
- The contact surface area is 100 cm^2 based on exposure to lips and hands (Exponent, Inc., 2007).

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

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

Where:

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

R_{derm} = Dermal absorption rate of DINP in skin, $\mu g/cm^2/h$

S_{derm} = Surface area of a child's lips and hands, cm^2

t = Time of contact, h

n = Frequency/day

BW = Child bodyweight, kg

The exposure factors and calculations of 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 calculated daily internal doses from dermal exposure to children mouthing toys and child care articles

	R_{derm} (μg/cm²/h)	BW (kg)	S_{derm} (cm²)	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	3	9.6

* 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	231.7
Dermal	2.6	9.6
Combined	30.4	241.3

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 demonstrates that DINP is rapidly excreted and does not appear to accumulate in tissues (Section 6.1), and therefore single day measurements approximate the daily dosing. The analytical approaches and uncertainties associated with biomonitoring data limit their use in exposure and human health risk assessments (Albertini et al., 2006). It is not possible to determine the relative contribution of different exposure routes directly from population biomonitoring data. 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 to 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 indicate 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 which 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.

Wittasek & Angerer (2008) examined non-oxidised and oxidative metabolites of a range of phthalates in 102 subjects aged between 6 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.

In a similar study, Frederiksen et al. (2011) analysed phthalate metabolites from urine of 129 Danish children and adolescents, with ages ranging from 6-21 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). 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 calculated 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 6 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 November 2011.

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 2500 mg/kg bw/d for 6 d. Within 72 h, about 85% of the administered dose was excreted in the faeces and 12% in the urine, most within the first 24 h. 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 (4 males and 20 females per dose) or 5 daily doses of 50, 150 or 500 mg/kg bw (15 males per dose). Excretion after 72 h was 49%-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 h 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 h (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 and 48 h 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 h was 33% and was considered in agreement between these two human studies with more than 90% of the excretion occurred within the first 24 h and the remainder in the 24-48 h 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 3 groups of male F344 rats. Two groups were received a single application of radiolabelled ^{14}C-DINP (6 animals at 1.2 mL/kg bw and 3 animals at 0.6 mL/kg bw), whereas the other group were pre-treated (conditioned) with non-labelled DINP for 3

d prior to the application of labelled DINP (6 animals at 1.2 mL/kg bw). All applications were occlusive and remained on the skin for 1, 3 or 7 d prior to sacrifice. The dermal absorption rate was slow as indicated by the total recovery (approximately 0.3%-0.6% per day) of the applied dose in urine, faeces, GIT 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%-4% of the applied dose over the 7-d period. Most of the radioactivity was recovered from the application sites (93%-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/cm^2). 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 d, 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 ND). 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 4 times less than through rat skin (5.6 vs. 22.4 µg/cm^2/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/cm^2/h) (Barber et al., 1992 ND).

Deisinger et al. (1997 ND) investigated the in vivo percutaneous absorption of DEHP leaching from plastics. Sheets of PVC film (15 cm^2) 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 h, the application site was rewrapped, and the excreta were collected daily for 7 d while Study II terminated at 24 h, the application site was washed and the excreta collected. A similar absorption rate of 0.24 µg/cm^2/h was calculated from both studies based on the sum of radioactivity at the exposure site and that absorbed systemically and 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%-4% over 7 d) 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/cm^2/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 6 daily doses of 2500 mg/kg bw ^{14}C-DINP, only trace amounts of the radioactivity were found in tissues after 72 h 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 5 daily doses of 50, 150 or 500 mg/kg bw ^{14}C-DINP, radioactivity peaked at 1-4 h and appeared higher after repeated than single dosing. The levels were greatest in the liver, followed by blood and kidney, and declined to 6%-24% of peak values by 24 h. 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 appears 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. 5 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 (MCINP, mean 127 µg/mL) as the major metabolite, followed by hydroxy-MINP (MHINP, 12 µg/mL) and oxo-MINP (MOINP, 5 µg/mL). Although most metabolites contained the same alkyl side chain length of 9 as the parent compound, metabolites with shorter or longer side chain 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 either as a conjugate (glucuronidated) or 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 1 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 20-29 years (Wittassek et al., 2007 ND).

In 399 urine samples collected over 7-8 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 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 high molecular weight 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 (2500 mg/kg bw/d for 6 d) was rapidly excreted with 85% in faeces and 12% in the urine (Hazleton, 1972*; ECB, 2003). In another study, excretion of radioactivity at 72 h 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 h 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 gastrointestinal 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 h with the half-lives for elimination of MCINP, MOINP and MHINP being 7.6, 8.3 and 8.6 h, respectively (Silva et al., 2006a ND).

In a human volunteer, elimination of DINP metabolites also followed a biphasic pattern after single oral doses of d_4-DINP. For the 1st phase (8-24 h post dosing), half-lives were estimated as 3 h for the monoester MINP and 5 h for the oxidative metabolites. In the 2nd phase (beginning 24 h post dosing), estimated half-lives were 5 h for MINP, 12 h for MHINP and MOINP, and 18 h for MCONP (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 h and the remainder in the 24-48 h period with half-lives of 4-8 h.

Overall, elimination of DINP and its metabolites after oral exposure is rapid and almost complete within the first 24 h. 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 h and a second elimination phase commencing at 24 h 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 40000 mg/kg/bw) in rats, findings consisted of laboured respiration, dyspnea, apathy, alopaecia, spastic gait, piloerection, tremors and organ discolouration. Moderate erythema and slight desquamation were reported following dermal application of up to 3160 mg/kg DINP in rabbits. No mortality, body weight 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 >10000 mg/kg bw), dermal (LD50 >3160 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	>10000 mg/kg bw	CAS 68515-48-0	Hazleton (1968c*)
		>50000 mg/kg bw	CAS Not stated	Hazleton (1980b*)
		>40000 mg/kg bw	CAS 28553-12-0	Midwest Research Institute (1981*)
		>10000 mg/kg bw	CAS 28553-12-0	BASF (1981b*)

		>10000 mg/kg bw	CAS 28553-12-0	Hüls (1985a*)
Inhalation (4-h)	Rat	>4.4 mg/L of air (analytical)	CAS Not stated	Hazleton (1980a*)
Dermal	Rabbit	>3160 mg/kg bw	CAS 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 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 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, 1981a*; 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 68515-48-0) in 6 male and 6 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 h the irritation had completely subsided in all cases (Hazleton, 1968b*; ECB, 2003).

Single application of undiluted DINP (CAS 28553-12-0) to 2 male and 4 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, 1981b*; ECB, 2003).

Another study (Hüls, 1985c*; ECB, 2003) on DINP (CAS 28553-12-0; undiluted) was conducted on small white Russian rabbits (3 males and 3 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 68515-48-0) were reported.

The earlier study was conducted in 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, with score 2 erythema was observed at day 37 in 3/20 animals (cf. 4/10 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 68515-48-0) under occlusive bandaging, with a challenge application of undiluted DINP.

No evidence of skin sensitisation was observed (Huntingdon Research Centre, 1994*; 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 2000 µg/ml in the neck region of BALB/cJ mice together with ovalbumin. Additionally the mice were administered with either one or two booster injection 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 2000 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 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 (approx 77, 227, 460, 767, 1554 mg/kg bw/d), showed statistically significant increases in liver weights (dose related), liver enzymes, 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 1982b*; 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, 2500, 5000, 10000, 20000 ppm (approximately 0, 176, 354, 719, 1545 (males) and 218, 438, 823, 1687 mg/kg bw/d (females)) showed significantly increased absolute and/or relative liver and kidney weights from 2500 ppm with changes in haematological and urine chemistry parameters from 5000 ppm. Hepatocellular changes at 20000 ppm and dose related increases in granular casts and regenerative/basophilic tubules in kidney from 5000 ppm were also noted. No NOAEL was identified. The LOAEL was 2500 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 2 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 (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 hepatis 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, 1500, 6000 and 12000 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 12000 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 6000 ppm and 12000 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 (12000 ppm), but palmitoyl-CoA oxidase activity was

again significantly elevated in both sexes at high dose (12000 ppm) and in females at mid high dose (6000 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 1500 ppm in females (week 78) and from 6000 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 6000 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 hepatis in male rats only from 6000 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 1500 ppm and above in males and increased pigmented tubule cells at 6000 and 12000 ppm in both sexes. There was also an increase in the frequency and severity of chronic progressive nephropathy in males.

A NOAEL of 1500 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, 1500, 4000, 10000, 20000 ppm (approx 0, 365, 972, 2600, 5770 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 4000 ppm were noted in both sexes. Enlarged livers were also observed from 4000 ppm and above in males and from 10000 ppm in females. Absolute and relative kidney weights were decreased in males only at 4000 ppm with significant decreases in urinary sodium, chlorides and creatinine at the highest dose in both sexes. At 20000 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 10000 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 10000 ppm and at 1000 ppm in the positive control. At 10000 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 1500 ppm (approx 365 mg/kg bw/d), based on increases in liver weights at 4000 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, 1500, 4000 and 8000 ppm DINP (0, 90-112, 275-335, 742-910, 1560-1887 mg/kg bw/d, males-females, respectively) for 104 weeks. A recovery high dose group (55 mice/sex) was also treated with 8000 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 4000 and 8000 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 1500 ppm and above were observed. In males, increased in liver masses was reported from 1500 ppm and statistically significant increases in absolute and/or relative liver weights were also noted at 4000 ppm and above. Mean liver palmitoyl-CoA oxidase activities were statistically significantly increased in all high dose animals (8000 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 4000 ppm and above and high recovery group) in males, enlarged spleen in all groups, granular pitted/rough kidneys in females at 8000 ppm (corresponding to increased incidence/severity of treatment-related nephropathy) and distended urinary bladder (most frequently seen in males at 4000 ppm and above). Histological examination showed increased incidence of cytoplasmic eosinophilia, diffuse hepatocellular enlargement and pigment were also observed 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 1500 ppm (275-335 mg/kg bw/d).

Other species

In a 13-week feeding study, beagle dogs (groups of 4 dogs/sex) were fed diets containing 0, 0.125, 0.5 and 2% (approximately 0, 37, 160 and 2000 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 2500 mg/kg bw/d (4 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. Body weight losses or low body weight 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 2500 mg/kg/d. A NOAEL of 500 mg/kg bw/d and LOAEL of 2500 mg/kg bw/d based on decreases in body weight and body weight 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, body weight 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 was 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 4 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
Oral				
Rat Fischer 344; 13-week study; CAS 68515-48-0	0, 0.1, 0.3, 0.6, 1, 2% (0, 77, 227, 460, 767, 1554 (both sexes)) in diet	77	227 in both sexes; ↑ kidney, liver weights, ↓ cholesterol	Bio/Dynamics (1982b*)
Rat Fischer 344; 13-week study; CAS 28553-12-0	0, 2500, 5000, 10000, 20000 ppm (0, 176-218, 354-438, 719-823, 1545-1687 (m-f)) in diet	NE	176-218; ↑ kidney, liver weights	Hazleton (1991a*)
Rat Fischer 344; 2-year study; CAS 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 68515-48-0	0, 500, 1500, 6000, 12000 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 28553-12-0	0, 1500, 4000, 10000, 20000 ppm (0, 365, 972, 2600, 5770 (both sexes)) in diet	365	972 in both sexes; Enlarged liver; ↑ absolute and relative liver weights; 2600; ↓ absolute epididymis & testes weight	Hazleton (1992*)
Mouse B6C3F1; 2-year study; CAS 68515-48-0	0, 500, 1500, 4000, 8000 ppm (0, 90-112, 275-335, 742-910, 1560-1887 (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*)
Dog beagle; 13-week study; CAS 68515-48-0	0, 0.125, 0.5, 2% (0, 37, 160, 2000) in diet	NE	37; ↑ ALT in both sexes	Hazleton (1971*)
Monkey marmoset (16-25-month old); 13-week study; CAS Not specified.	0, 100, 500, 2500 gavage	500	2500; ↓ body weight; ↓ body weight gain	Huntington Life Sciences (1998*); Hall et al. (1999 ND)
Monkey cynomolgus males; 2-week study; CAS Not specified.	0, 500 gavage	NE	500 ↑ neutrophil count ↓ lymphocyte count	Pugh et al. (2000)
Dermal				
Rabbit New Zealand White; 6-week study; CAS 68515-48-0	0, 0.5, 2.5 mL/kg bw	500 (0.5 mL/kg bw)	2500 (2.5 mL/kg bw); slight or moderate erythema, and slight desquamation	Hazleton (1969*)

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

6.2.5 Genotoxicity

Several in vitro and in vivo assays have been conducted to assess the genotoxic effects of DINP (CAS 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 68515-48-0) did not induce increases in mutant frequency at any dose, either in the presence or absence of metabolic activation.

DINP (CAS 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 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 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
In vitro				
Bacterial test (gene mutation) CAS 68515-48-0	Salmonella typhimurium TA 98, 100, 1535, 1537, 1538	From 0.5 to 5000 µg/plate ± S9	Negative	Exxon Biomedical Sciences (1996b*)
Mouse lymphoma assay CAS 68515-48-0	L5178 TK ±	From 1500 to 8000 nL/mL without metabolic activation and from 500 to 6000 nL/mL with metabolic activation	Negative	Hazleton (1986*)
Cytogenetic assay CAS 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 28553-12-0	Rat hepatocytes	From 0.625 to 10 μg/mL	Negative	Litton Bionetics (1981*)
In vivo				
Cytogenetic assay	Fischer 344 rat bone marrow cells	5-1.7 and 0.5 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 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 8 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 2 years (Exxon Biomedical Sciences, 1986*; Lington et al., 1997).

Mononuclear cell leukaemia (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 ND). 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 ND; ECB, 2003).

A NOAEL of 15-18 mg/kg bw/d was established, based on increased incidence of mononuclear cell leukaemia (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, 5000, 10000 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, 1500, 6000 and 12000 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 12000 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 12000 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 6000 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 6000 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, 1500, 4000 and 8000 ppm DINP (0, 90-112, 275-335, 742-910, 1560-1887 mg/kg bw, males-females respectively) for 104 weeks. A recovery high dose group (55/sex) was also treated with 8000 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 8000 ppm. The total incidence of hepatocellular neoplasia was significantly increased in males from 4000 ppm (47%) and in females from 1500 ppm (17%). Hepatocellular carcinoma was increased in both sexes at 4000 and 8000 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 8000 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 1500 ppm (335 mg/kg bw/d) for females and a NOAEL of 1500 ppm (275 mg/kg bw/d) and a LOAEL of 4000 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 durationand 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	0, 0.03, 0.3, 0.6% (0, 15-18,	15-18	152-184;	Exxon Biochemical

344; 2-year study; CAS 68515-48-0	152-184, 307-375 (m-f)) in diet		↑ MCL, Renal neoplasms (transitional cell carcinomas and tubular cell carcinomas) n	Sciences (1986*); Lington et al. (1997)
Rat Sprague Dawley; 2-year study; CAS 71549-78-5	0, 500, 5000, 10000 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 68515-48-0	0, 500, 1500, 6000, 12000 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 68515-48-0	0, 500, 1500, 4000, 8000 ppm (0, 90-112, 275-335, 742-910, 1560-1887 (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 include 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 less 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 DINP (CAS No. 68515-49-0 and CAS No. 71549-78-5) were examined in hepatocytes of rats, mice, hamster 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 2μ 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 prenatal and postnatal developmental toxicity studies, and in studies which focus on possible modes of action. They are presented below in chronological order for each type of study.

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 GLP compliant one-generation reproductive toxicity study administered dietary levels of 0, 0.5%, 1.0% and 1.5% (equivalent to 0, 301-923, 622-1731, 966-2246 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 postnatal day (PND) 21. Statistically significant decreases in body weight 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 body weight were also observed during lactation in both sexes at all dose levels on PND 0 and PND 14-21. At ≥ 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 body weight 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 DINP-related effects. In this study, the NOAEL for fertility-related toxicity was 1.5% (966-2246 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, 467-1541 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 body weights were unaffected except a reduced dam weight at 0.8% during PND 14-21. F1 body weights at 0.8% were significantly below control values throughout the premating and mating period for males, and GD 14 and PND 4-21 (lactation period) for females. Reductions in

body weight of F1 males at 0.4% only achieved statistical significance sporadically. Statistically significant increases in absolute kidney weights were observed at ≥ 0.2% in F0 females, ≥ 0.4% in F0 males and 0.8% in F1 males. Statistically significant increases in absolute liver weights were observed at ≥ 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 body weight were observed at all doses on PND 21 (m-f, F1) and PND 7 (f, F2), and at ≥ 0.4% on PND 7-21 in both sexes and both generations (F1, F2). The NOAEL for fertility-related toxicity was 0.8% (467-1541 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. Given DINP and DEHP elevated foetal plasma luteinizing hormone (LH) concomitantly with the suppression of testosterone synthesis whether alone or in combination (statistically significantly), the authors hypothesised that both reduced testosterone by a similar 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. 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 (cf. 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 (Insl3) 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 5 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 (5 days) from GD 14-18 with 0, 500, 750, 1000 or 1500 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 ≥1000 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., 2011a ND).

In another study by Hannas et al. (2011b 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 Insl3 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 4 phthalates tested (DPeP>DHP>DIBP≥DHeP> DINP).

Prenatal developmental toxicity studies

These studies are designed to examine the effects of prenatal 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 1000 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 1000 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 1000 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 1000 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 1000 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 1000 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 thus considered slight developmental effects. Results on DINP3 were also reported, but this material has not been manufactured since 1995, 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 1000 mg/kg bw/d on GD 6-15. There were no maternal effects except for statistically significant decreases in body weight gain and food consumption at 1000 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 body weight 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 pelves) variations at 1000 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 pelves) 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.

Postnatal developmental toxicity studies

The postnatal developmental toxicity studies examine the in utero and early postnatal 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. Male offspring in the DINP treatment group displayed female-like areolas/nipples (22%) and reproductive malformations (7.7%), including bilateral atrophic testes, flaccid or fluid-filled

testes, unilateral epididymal agenesis with hypospermatogenesis and scrotal fluid-filled testis devoid of spermatids. Preputial separation was not delayed by DINP treatment. Anogenital distance (AGD), offspring body and non-reproductive organ weights were also unaffected. 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 1000 and 1500 mg/kg bw/d to confirm its antiandrogenic action in utero. At PND 2, males exposed to 1500 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, 1000 mg and 1500 mg DINP groups, respectively). Maternal toxicity was not reported (Ostby et al., 2001*; CPSC, 2010 ND).

Groups of 12 Wistar rats were gavaged with 0, 300, 600, 750 or 900 mg/kg bw/d DINP (CAS No. unspecified) from GD 7 through PND 17. AGD was significantly decreased at birth in male pups at ≥ 600 mg/kg. After adjusting for birth weight, reductions in AGD were statistically significant only at 900 mg/kg. A significant dose-related increase of nipple retention on PND 13 was also observed in male pups at ≥ 600 mg/kg. Pup retrieval by mothers was significantly delayed at 600 mg/kg, suggesting either maternal toxicity or inadequate nutrition at these doses. The maternal and developmental NOAELs in this study were 300 mg/kg bw/d. The authors concluded that DINP induced antiandrogenic effects similar to those seen for DEHP, but at higher doses. (Hass et al., 2003*; CPSC, 2010 ND).

The potential impact of dietary exposure to DINP (CAS No. 28553-12-0) at doses of 400, 4000 and 20 000 ppm (or 31-66, 307-657 and 1165-2657 mg/kg bw/d) from GD 15 to PND 10 was evaluated in pregnant SD rats (5/group). Decreases in maternal body weight 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 body weight 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 prepubertal necropsy on PND 27, reduced weights of male and female pups, absolute and/or relative brain, testes, ovaries and uterus were statistically significantly at 20 000 ppm. The body weight of male pups PND 27 at 4000 ppm was also significantly reduced. No obvious effects on onset of puberty such as preputial separation or vaginal opening were observed. Histopathology showed non-significant degeneration of stage XIV meiotic spermatocytes and vacuolar degeneration of Sertoli cells or decreased corpora lutea at 20 000 ppm on PND 77. 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).

Pregnant Wistar rats were given a diet containing DINP (CAS No. 28553-12-0) at 0, 40, 400, 4000 and 20 000 ppm (equivalent to 0, 2, 20, 200 and 1000 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 body weights were seen in both sexes and reduced AGD and anogenital index (AGI = AGD divided by the cube root of the body weight) 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. After maturation, male rats at 40 ppm displayed decreased copulatory behaviours while 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 x 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).

Recently, Boberg et al. (2011 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 statistically significant and dose-dependent increase in nipple retention on PND 13 (≥ 600 mg/kg bw/d) and a statistically significant decrease in AGD and AGI at birth (≥ 900 mg/kg bw/d). Reduced sperm motility (≥ 600 mg/kg bw/d) and increased sperm count (≥ 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 foetuses GD 21, but this was not statistically significant except for a reduced testosterone content at 600 mg/kg. Altered histology was observed in GD 21 male foetuses including testicular and epididymal agenesis/atrophy at ≥ 600 mg/kg bw/d. Body weight on PND 13 was significantly reduced (male pups at 900 mg/kg and female pups at 750 mg/kg). DINP also affected spatial learning and increased masculinisation of behaviour (≥ 900 mg/kg bw/d) in female offspring. Maternal body weight, weight gain during pregnancy, gestational length, litter size or sex ratio was not affected by treatment. Therefore, the NOAEL for maternal toxicity was the highest dose tested, i.e. 900 mg/kg bw/d. The NOAEL for developmental toxicity for male rats was set at 300 mg/kg bw/d based on increased nipple retention, testicular and epididymal agenesis/atrophy GD 21, reduced testicular testosterone content and sperm motility at ≥ 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.

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 nor 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 h 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 (Miynarcikova 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 2000 mg/kg bw/d for 4 d (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 rats 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, 622-1731, 966-2246)	Maternal: NE Fertility-related parameters: 966-2246 Developmental: NE	Maternal: 301-923: ↑ liver & kidney weights 622-1731: ↓ body weight (m-f) Fertility-related parameters: NE Developmental: 301-923: ↓ pup weight PND 0 & PND 14-21 (m-f) 622-1731: ↓ pup weight PND 0-21 (m-f)	Waterman et al., 2000; CERHR, 2003; ECB, 2003
(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, 467-1541)	Maternal: 114 (m) NE (f) Fertility-related parameters: 467-1541 Developmental: NE	Maternal: 114-395: ↑ kidney weight (f, F0) 235-758: ↑ liver (f, F0) & kidney (m, F0) weights 467-1541: ↓ body weight during lactation PND 14-21 (f, F0), during (pre)mating (m, F1) & lactation PND 4-21 (f, F1) Fertility-related parameters: NE Developmental: 114-395: ↓ pup weight PND 21 (m-f,	Waterman et al., 2000; CERHR, 2003; ECB, 2003

				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	Borch et al., 2004
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 (P450ssc, Insl3 & 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, 1000, 1500	Fertility-related parameters: NE	Fertility-related parameters: 500: ↓ foetal testicular testosterone production 1000: ↓ foetal testicular gene expression (StAR & Cyp11a)	Hannas et al., 2011a ND
GD 14-18 3-6/group	Rat SD Gavage	DINP CAS No. 68515-48-0 & 28553-12-0 0, 500, 750, 1000, 1500	Fertility-related parameters: NE	Fertility-related parameters: 500: ↓ Insl3 & other foetal testicular gene expression for androgen synthesis and cholesterol transport	Hannas et al., 2011b ND

Prenatal developmental toxicity studies					
GD 6-15 (dams sacrificed GD 20) 25/group	Rat SD Gavage	DINP (CAS No. unspecified) 0, 10, 500, 1000	Maternal: 1000 Developmental: 1000	Maternal: NE Developmental: NE	Hazleton, 1981*; ECB, 2003
GD 6-15 8-10/group	Rat Wistar Gavage	DINP1 (CAS No. 68515-48-0), DINP2 & DINP3 (CAS No. 28553-12-0) 0, 40, 200, 1000	Maternal: 200 Developmental: 200	Maternal: 1000: ↓ food consumption & ↑ relative kidney weights with DINP1 1000: vaginal haemorrhage in one dam with DINP2 Developmental: 1000: ↑ skeletal variations (rudimentary cervical & accessory 14th ribs) with DINP1 & DINP2	Hellwig et al., 1997; CERHR, 2003; CPSC, 2010
GD 6-15 23-25/group	Rat SD Gavage	DINP CAS No. 68515-48-0 0, 100, 500, 1000	Maternal: 500 Developmental: 500	Maternal: 1000: ↓ weight gain & food consumption Developmental: 1000: ↑ skeletal (rudimentary lumbar ribs) & visceral (dilated renal pelves) variations	Waterman et al., 1999; CERHR, 2003
Postnatal developmental toxicity studies					
GD 14 – PND 3 6-8/group	Rats SD Gavage	DINP CAS No. 68515-48-0 0, 750	Maternal: NE Developmental: NE	Maternal: 750: ↓ weight gain to GD 21 Developmental: 750: ↑ malformations (↑ nipple retention, testicular & epididymal agenesis/atrophy)	Gray et al., 2000
GD 14 – PND 3	Rats SD	DINP CAS No. 68515-48-0	Maternal: NE	Maternal: Not reported	Ostby et al., 2001*; CPSC,

No. of dams unspecified	Gavage	0, 1000, 1500	Developmental: NE	Developmental: 1000: ↑ nipple retention 1500: ↓ AGD	2010 ND
GD 7 – PND 17 12/group	Rat Wistar Gavage	DINP (CAS No. unspecified) 0, 300, 600, 750, 900	Maternal: 300 Developmental: 300	Maternal: 600: delayed pup retrieval by mothers Developmental: 600: ↑ nipple retention 900: ↓ AGD	Hass et al., 2003*; CPSC, 2010 ND
GD 15 – PND 10 5/group	Rat SD Diet	DINP CAS No. 28553-12-0 0, 400, 4000, 20 000 ppm (0, 31-66, 307-657, 1165-2657 as calculated for gestational period GD 15-20 & lactational period PND 2-10, respectively)	Maternal: 307-657 Developmental: 31-66 (m) 307-657 (f)	Maternal: 1165-2657: ↓ weight gain & food consumption Developmental: 307-657: ↓ pup weight (m) PND 27 1165-2657: ↓ brain & pup weights (m-f), ↓ testis, absolute ovarian & uterus weights PND 27. Degeneration of meiotic spermatocytes & Sertoli cells, ↓ corpora lutea in the ovary PND 77	Masutomi et al., 2003
GD 15 – PND 21 no. of dams unspecified	Rat Wistar Diet	DINP CAS No. 28553-12-0 0, 40, 400, 4000, 20 000 ppm (0, 2, 20, 200, 1000)	Maternal: NE Developmental: NE	Maternal: Not reported Developmental: 2: ↓ pup weight (m-f), ↓ AGD & AGI, ↑ hypothalamic p130 (m) & granulin (f) gene	Lee et al., 2006; ECHA, 2010 ND

				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 Developmental: 300 (m) 600 (f)	Maternal: NE Developmental: 600: ↑ malformations (↑ nipple retention, testicular & epididymal agenesis/atrophy GD 21), ↓ testicular testosterone content & production (not statistically significant), ↓ sperm motility 750: ↓ pup weight PND13 (f) 900: ↓ pup weight PND13 (m), AGD & AGI, ↑ sperm counts, masculinised learning behaviour (f)	Boberg et al., 2011 ND

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 body weight); GATA4 = GATA binding protein 4; GD = gestational day; Insl3 = insulin-like factor 3; LABC = levator ani/bulbocavernosus; LH = luteinising hormone; NE = not established; P450ssc = P450 side chain cleavage; PND = postnatal day; SD = Sprague-Dawley.

6.3 Effects observed in humans

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

6.3.1 Skin irritation

In humans, DINP (CAS 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 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 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 3-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, statistically significant dose-dependent correlations with LH levels (Main et al., 2006).

Non-reproductive effects

In 845 Danish children 4-9 years of age, creatinine-uncorrected urinary metabolites of DINP (MCIOP, MHINP, MOINP and MINP, measured among other 8 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 no observed adverse effect levels (NOAELs), and discusses the relevance of the effects observed in animal studies to humans.

Given that there is limited information available from human studies on the potential health effects associated with exposure to 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 a high molecular weight 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/cm^2/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 extrapolating 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 h.

DEP 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 high molecular weight 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 h (1st phase) and the remainder in the 24-48 h 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 2500 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.

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 3 doses of DINP (CAS No. 68515-48-0) (15-18, 152-184 and 307-375mg/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, 1500, 6000 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 liver 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, PPARα, PPARβ and PPARγ, 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 PPARα, genotypic variations rendering the primate liver receptor less active compared to rodents, and species differences in phthalate hydrolysis and production of active phthalate metabolites (Tugwood et al., 1996; Palmer et al., 1998; 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 hepatis 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 hepatis is a spontaneous, chronic liver lesion of ageing, particularly 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. 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, absence from other DINP studies including other rodent species and prevalence following DINP exposure only in older male rats, the incidence of spongiosis hepatis 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 2001, 2010; CSTEE 2001). In the current assessment spongiosis hepatis 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 2µ globulin nephropathy not regarded as relevant to humans (Caldwell et al., 1999a ND). 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 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 ND) 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 2 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 ND).

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*) 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 68515-48-0 and CAS 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*). The GJIC assay has been claimed to have good cancer predictive potential for phthalates (Kalimi et al., 1995*). 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 agenesis/atrophy and decreased AGD/AGI in male offspring and increased masculinisation of behaviour in female offspring) 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 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 2600 mg/kg bw/d DINP were reported in repeated dose studies in mice but not in rats (Hazleton, 1992*; Lington et al., 1997; 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.

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., 2011a). 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., 2011a,b). 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 Insl3 (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 four 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 and agenesis/atrophy of testes and epididymides were also reported at ≥ 1000 and ≥ 600 mg/kg bw/d, respectively (Masutomi et al., 2003; Boberg et al., 2011).

In the study by Boberg et al (2011), decreased foetal testicular content and increased testicular and epididymal agenesis/atrophy in GD 21 male foetuses were also noted in rats exposed to DINP (≥ 600 mg/kg bw/d) from GD 7 to PND 17. At ≥ 900 mg/kg bw/d increased masculinisation of behaviour in female offspring was also reported. In this study, the NOAEL for fertility-related toxicity (or sexual developmental toxicity) was established as 300 mg/kg bw/d

Lee et al. (2006) also reported decreased AGD and AGI, and increased expression of genes involved 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 in rodent studies, such as reduced testosterone content and production and altered reproductive organ weights (with or without histopathologies), were among the fertility-related effects seen for DEHP (NICNAS, 2010). For all fertility-related effects, the potency of DINP is much lower than DEHP. Similar to the assessment for DEHP, these effects are regarded as relevant to a human risk assessment. An overall NOAEL for fertility-related or sexual developmental effects was determined to be 300 mg/kg bw/d based on the collective study results of Boberg et al. (2011) and Hannas et al. (2001a).

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. ≤ C3 and ≥ 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 the reproductive and behavioural effects, DINP at ≥ 900 mg/kg bw/d affected spatial learning in rats (Boberg et al., 2011).

When evaluating weight of evidence based on the three studies (Waterman et al., 2000; Masutomi et al., 2003; and Boberg et al., 2011) collectively, 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 PND 21. Taking all together, the reduced pup weight is considered the most sensitive DINP-related adverse effects on offspring growth and development, and hence for the purposes of this review, the developmental NOAEL is derived as 31 mg/kg bw/d based on reduced pup weights at approximately 100 mg/kg bw/d and above.

After prenatal exposure in rats at high doses (e.g. 1000 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 were 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, the reproductive and developmental effects of DINP observed in rat studies such as reduced testicular testosterone, decreased pup weight, decreased AGD/AGI, increased nipple retention and testicular and epididymal agenesis/atrophy are regarded as relevant to a human risk assessment.

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). 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 high molecular weight phthalates such as DINP is not certain. Compared to certain transitional phthalates, there is a paucity of information to examine the mode of action of DINP with respect to reproductive effects.

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 similar pattern of endocrine alterations in male rat foetuses 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., 2011a).

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 but also the amount of the shorter side chains (e.g. C4 - C6) in the mixture.

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 reproductive/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 therefore considered relevant to humans for this risk assessment

Antiandrogenic 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 male reproductive system and sexual differentiation during development in a number of rodent studies (e.g. increased nipple retention, testicular and epididymal agenesis/atrophy, decreased AGD/AGI in male offspring and increased masculinisation of behaviour in female offspring) which are components of the antiandrogenic pattern observed with DEHP (a known endocrine disruptor in humans). 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) as a possible rebound mechanism on testicular steroidogenesis following exposure to DINP at high doses (e.g. $\geq$ 750 mg/kg bw/d).

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 a much lower dose of DINP (approximately 100 mg/kg bw/d) in both sexes, both one and two generations of rats and in the absence of overt maternal toxicity. The pup weight reduction was also sustained and not considered solely related to low birth weight. 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. 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
Repeated dose (increased organ weights)	88	358: ↑ liver and kidney weights	Rat Fischer 344 adults	Moore et al., 1998a
Fertility-related parameters (reduced testosterone)	300	500: ↓ testicular testosterone content &/or production	Rat SD or Wistar foetuses	Boberg et al., 2011; Hannas et al., 2011a
Development (reduced pup weight)	31	~100: ↓ pup weight PND 21 (F1) & PND 7 (F2)	Rat SD newborn	Waterman et al., 2000; Masutomi et al., 2003

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

8. Human Health Risk Characterisation

8.1 Methodology

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

1. Identification of critical health effect(s)
2. Identification of the most appropriate/reliable NOAEL (if available) for the critical effect(s).
3. Where appropriate, comparison of the estimated or measured human dose or exposure (EHD) to provide an MOE:
4. MOE = NOAEL/EHD
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/inter species 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 - Health Hazard Assessment and Section 7 - Health Hazard Characterisation). These effects are repeated dose toxicity (increased liver and kidney weights with histopathological findings), 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), 300 mg/kg bw/d (fertility-related toxicity) and 31 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 3 h/d identified for children aged 6-12 months. The typical scenario considers the mean daily mouthing time of 0.8 h/d calculated as an average across several studies examining mouthing behaviours in the same age group. These scenarios are based on international literature examining mouthing behaviour in children in different age groups from 0 to 36 months of age. Overall, these studies demonstrate that mouthing times are highest for children aged 6-12 months and they decrease with increasing age. In the absence of Australian information, these mouthing behaviours are assumed applicable to Australian children.

Additional assumptions considered are as follows:

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

Table 8.1 - Estimated total internal exposure for children

Route of Exposure	Typical D_{int} (μg/kg bw/d)	Worst-case D_{int} (μg/kg bw/d)
Oral	27.8	231.7
Dermal	2.6	9.6
Combined	30.4	241.3

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 margins of exposure (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 mg/kg bw/d	MOE for typical exposure scenario	MOE for worst-case exposure scenario
Repeated dose (increased organ liver & kidney weights)	88	2895	365
Fertility-related parameters (reduced testosterone)	300	9868	1243
Development (reduced pup weight)	31	1020	128

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 although being reduced from 1020 to 128 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, albeit marginally, above 100 (Appendix 1, Table 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, USA 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, USA (California) and Canada are 3, 3 and 4 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 an 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 contains a list of prohibited or restricted cosmetic chemicals in Australia. DINP is not currently listed.

Current EU, USA 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 ND). 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 chemical, can be added and a combined MOE determined. It should be noted that the hazard index for individual chemical calculated in this way is the inverse of the MOE (i.e. HI = 1/MOE). Equations for calculating the combined MOE are provided in the Appendix 4 – *Mixture risk assessment methodology – evaluating the health risk due to exposure to mixtures of chemicals* in the Sixth Framework Programme of the Health and Environment Integrated Methodology and Toolbox for Scenario Development (HEIMTSA) (Sarigiannis et al., 2010). This includes a number of different equations for determining cumulative risk and the choice of the most appropriate equation depends on the available input data. For the current calculations, the equation used is:

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

The calculations for combined exposure were undertaken for three scenarios:

- combined exposure to DINP in toys and child care articles and DEP in cosmetics (Table A.1.1);
- combined exposure to a mixed plasticiser containing 42% DINP and 1% DEHP used in toys and child care articles (Table A1.2); and
- 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 =31 mg/kg bw/d) is more potent than that of DEP (NOAEL = 197 mg/kg bw/d). Relevant exposure estimates for 6 month old infant are 241.3 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 128 (DINP) and 1022 (DEP). The respective hazard indices are 1/128 (DINP) and 1/1022 (DEP) and cumulative hazard index is the sum (1/128 + 1/1022) equaling 1/114.

The cumulative MOE is calculated from the equation above, 1/(1/MOE (DINP) + 1/MOE (DEP)), as 114. The other values are calculated in a similar manner, with adjustment, where necessary, for relative concentrations and combinations (Table 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 are all indicate an adequate safety margin (Table A1.1, 1.2 and 1.3). These MOEs are specifically calculated for 6-month infants because the mouthing time studies (Appendix 2) indicate that newborn babies are unlikely to use teethers or child care articles while MOE estimates for older babies (e.g. 12-month infants) are expected to be higher based on their higher body weights.

As the concentration of DEP in body lotion increases from 0.5% to 0.75% and 1%, the cumulative MOE reduces from 114 to 108 and 103 respectively (detailed calculations not shown). Therefore, at 0.75% DEP and above, the cumulative MOE for risk of developmental (pup weight) effects in 6-month infants is marginally above 100 and is of concern.

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

	DINP[a]		DEP[b]		
Toxicity	**NOAEL**	**MOE**	**NOAEL**	**MOE**	**Cumulative MOE[c]**
Systemic (Enlarged liver &/or kidney)	88	365	150	778	248
Fertility-related (Reduced testes weight &/or testosterone)	300	1243	40	207	178
Developmental (Reduced pup weight)	31	128	197	1022	114

[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 6-month infants estimated from dermal exposure to body lotions containing 0.5% DEP
[c] Calculated from the formula 1/(1/MOE of DINP + 1/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

	DINP[a]		DEHP[b]		
Toxicity	**NOAEL**	**MOE**	**NOAEL**	**MOE**	**Cumulative MOE[c]**
Systemic (Enlarged liver &/or kidney)	88	365	28.9	120	348
Fertility-related (Reduced testes weight &/or testosterone)	300	1243	4.8	20	512
Developmental (Reduced pup weight)	31	128	46[d]	191	129

[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/MOE of DINP + 1/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

	42% DINP/ 1% DEHP[a]	DEP[b]		
Toxicity	**MOE**	**NOAEL**	**MOE**	**Cumulative MOE[c]**
Systemic (Enlarged liver &/or kidney)	348	150	778	241
Fertility-related (Reduced testes weight &/or testosterone)	512	40	207	148
Developmental (Reduced pup weight)	129	197	1022	115

[a] From Table A1.2 above
[b] From the DEP PEC assessment report (NICNAS, 2011) based on the daily internal DEP doses for 6-month infants estimated from dermal exposure to body lotions containing 0.5% DEP
[c] Calculated from the formula 1/(1/MOE of mixed DINP/DEHP + 1/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 3-36 months, for five categories of objects: pacifiers, teethers, fingers, toys and non-toys. Ten 15-minute observations of mouthing behaviour were conducted by parents over 2 days with a total of 42 children aged between 3-6, 6-12, 12-18 and 18-36 months. Of the 4 age-groups observed, children 6-12 months of age showed the greatest daily mouthing times for objects excluding pacifiers, averaging 44 minutes/d (range 2.4 - 171.5 minutes/d). The average mouthing time across the 4 groups was 26.7 minutes/d. Differences in mouthing times between individuals were large.

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

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

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

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

DTI (2002) presented the findings of an investigation into the mouthing behaviour of 236 children aged 1-60 months in the UK. The study found that nearly all items a child came into contact with were mouthed. Mean estimated daily mouthing time on toys and other objects (excluding pacifiers) peaked at age 6-9 months (at approximately 1 hour) and decreased as children grow older. The maximum daily mouthing time for toys and other objects (excluding pacifiers) for children ages 6-9 months was 297 minutes.

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

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

Study	Number of children	Age (months)	Object mouthed	Daily mouthing times (mins)		
				Mean	Max	SD
Groot et al. (1998)	5	3-6	Toys meant for mouthing, toys not meant for mouthing & non-toys & fingers (excludes pacifiers).	36.9	67.0	19.1
	14	6-12		44.0	171.5	44.7
	12	12-18		16.4	53.2	18.2
	11	18-36		9.3	30.9	9.8
Health Canada (1998)	Not reported	3-12	Teethers and other mouthing products (excluding pacifiers)	120	180	-
Juberg et al. (2001)	107	0-18	Plastic toys	17		-
			Teethers	6		-
			Other objects (excludes pacifiers & fingers)	9	NR	-
	110	19-36	Plastic toys	2		-
			Teethers	0		-
			Other objects (excludes pacifiers & fingers)	2		-
	168	3-18	All objects, excluding pacifiers	36		48
Kiss (2001)	169 (total)	3-12	All objects, excluding pacifiers	70		-
		12-24	All objects, excluding pacifiers	48	NR	-
		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 believed 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 ages 3-36 months. Also, several studies noted that mouthing times decrease with increasing age (Groot et al. 1998; Kiss, 2001).

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

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

Extractability of phthalate plasticizers

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

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

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

DINP is the most prevalent phthalate in children's toys and the migration of this chemical from plastics has been studied most extensively. The studies demonstrate that migration of phthalates from plastic products is determined more by the magnitude of mechanical action applied to the plastic rather than the chemical diffusive properties determined by the physicochemical characteristics of the substrate or concentration of phthalate.

Chen (1998) conducted an in vivo study in the US with adult volunteers and an in vitro study using impaction methods and saliva simulants. In the in vivo study, two plastic disks (each with a surface area of approximately 10.3 cm^2) were cut from each of 5 identical PVC toy ducks each containing 43% DINP by weight. Ten US Consumer Product Safety Commission (CPSC) staff volunteers were asked to gently chew the disks for four 15-minute intervals. Saliva samples were collected after each chewing interval and analysed for DINP. Migration rates varied substantially from individual to individual. The average DINP migration rate across all time periods from volunteers was 26.03 $\mu g/cm^2/h$ (range 6.14 – 57.93 $\mu g/cm^2/h$). In vivo migration rates also averaged 39.5 times higher than rates obtained from the in vitro impaction study. In vitro impaction studies of phthalate release rates (range 0.1 to 4.4 $\mu g/cm^2/h$) from samples of children's toys or child care products showed poor correlation between release rates and the amount of phthalate present in samples.

Meuling and Rijk (1998) conducted an in vivo study in the Netherlands with 20 adult volunteers and an in vitro study with a simulant of saliva using shaking, head over heels mixing and ultrasound methods. In the in vivo study, three specimens were used: a standard PVC disk (38.5% DINP), part of a PVC teething ring (43% DINP), and a disk punched from the same teething ring (43% DINP). Each specimen had a surface area of $10cm^2$. Initially all 20 volunteers were asked to

suck and bite on the standard PVC disc for four 15-minute intervals. Saliva samples were collected after each biting interval and analysed for DINP. Subsequently, the volunteers were divided into two groups of 10. One group repeated the test using part of the teething ring while the other group used the disk punched from the teething ring. In the in vivo study, the mean release rates were: 8.28 μg/cm^2/h (range 1.8 - 49.8 μg/cm^2/h) for the standard PVC disc, 14.64 μg/cm^2/h (range 5.4 - 53.4 μg/cm^2/h) for the teething ring and 9.78 μg/cm^2/h (range 5.4 - 34.2 μg/cm^2/h) for the disc punched from the teething ring. The researchers noted that the amount of DINP released into saliva exceeded its expected solubility and that mechanical force was required in the in vitro studies in order to attain migration rates comparable to that obtained from the in vivo studies.

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

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

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

The results of the five in vivo studies are summarised in Table 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) (μg/cm^2/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 (eg. chewing) is involved;
- Migration rates have little direct relationship with the phthalate content of an article in the tested phthalate range of 15%-58% by weight, indicating that differences seen between different test articles may depend more on the properties of the PVC grade comprising the article;
- The amount of phthalate released into saliva through biting and chewing exceeded its expected solubility in water in all in vivo studies, indicating that migration is not merely a simple diffusion process;
- Migration rates are proportional to the amplitude of mechanical action ie. chewing results in a higher migration rate than mouthing or sucking alone.

Based on the above conclusions, it is evident that migration of phthalate plasticisers from plastic toys into saliva through biting and chewing is the combined effect of molecular diffusion and mechanical action with the latter likely to be the dominating factor. The migration rate of phthalates from articles appears largely determined by the magnitude of the mechanical force applied to an article, and the properties of the PVC grade comprising the article, and less affected by the physicochemical characteristics or concentration of a particular phthalate.

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 $\mu g/cm^2/h$ from articles with up to 54% DINP content (Chen, 1998). This migration rate is therefore applicable for a worst case exposure assessment for children from the use of DINP in toys. The mean migration rate for DINP in this study was 26.03 $\mu g/cm^2/h$ (Chen, 1998), which is similar to the highest mean migration rate of 32.6 $\mu g/cm^2/h$ (Niino, 2002), in a study using a smaller number of volunteers. The mean migration rate determined by Chen (1998) is regarded as applicable for typical exposure assessment in toys.

Glossary

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

The IPCS Risk Assessment Terminology can be accessed at:
http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf.

References

Adamsson A, Salonen V, Paranko J, Toppari J (2009) Effects of maternal exposure to di-isononylphthalate (DINP) and 1,1-dichloro-2,2-bis(p-chlorephenyl)ethylene (p,p"-DDE) on steroidogenesis in the foetal rat testis and sdrenal gland, Reproductive Toxicology, 28:66-74.

AFC (2005) Opinion of the science panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to di-isononylphthalate (DINP) for use in food contact materials, The EFSA Journal 244:1-18.

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

Anderson W, Castle L, Scotter M, Massey R, Springall C. (2011) A biomarker approach to measuring human dietary exposure to certain phthalate diesters. Food Additives Contaminants 18:1068–74.

Aristech Chemical Corporation (1994) 2-Year dietary oral toxicity study in rats with diisononyl phthalate. TSCA 8(e) Submission 8EHQ-0794-13083. CAS Number 68515-48-0. Dated July 13, 1994.

Aristech Chemical Corporation (1995) TSCA 8(e) Submission 8EHQ-0794-13083. Corroborative information in second species. Dated April 12, 1995.

Asante-Duah (2002) Public health risk assessment for human exposure to chemicals, Kluwer Academic Publishers.

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

Babich MA (2002) Updated risk assessment of oral exposure to diisononyl phthalate (DINP) in children's products. Bethesda, MD, US Consumer Product Safety Commission.

Babich MA, Chen SB, Greene MA, Kiss CT, Porter WK, Smith TP, Wind ML, Zamula WW (2004) Risk assessment of oral exposure to diisononyl phthalate from children's products. Regul Toxicol Pharmacol, 40:151-67.

Baker TK, Kalimi GH, Lington AW, Isenberg JS, Klaunig J and Nikiforov AI (1996). Gap junctional intercellular communication (GJIC) studies on 5 phthalate monoesters in hepatocytes of four species; implications for cancer risk assessment. The Toxicologist, 30 (1), Part 2, #1063.

Bannasch P (2003) Comments on R. Karbe and R. L. Kerlin (2002). Cystic Degeneration/Spongiosis Hepatis (Toxicol Pathol 30 (2), 216-227). Toxicol Pathol 31: 566-570.

Barber ED, Astill BD, Moran EJ, Schneider BF, Gray TJB, Lake BG, & Evans JG (1987) Peroxisome induction studies on seven phthalate esters. Tox Indust Health 3: 7-22.

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

Barber ED, Teetsel NM, Kolberg KF, & Guest D (1992) A comparative study of the rates of in vitro percutaneous absorption of eight chemicals using rat and human skin. Fundamental and Applied Toxicology, 19:493-497.

BASF AG (1981a) Gewerbetoxikologische Grundprufung. Akules orale Toxizitat (Ratte) (Report on the study of the acute oral toxicity of Palatinol CE 5250) by oral route in rats). Unpublished results (80/266).

BASF AG (1981a) Gewerbetoxikologische Grundprufung. Primare Hautreizwirkung (kaninchen; Draize test) (Report on the study of the irritation to the intact and abraded dorsal skin of white rabbits based on Draize of Palatinol CE 5250). Unpublished results (80/266).

BASF AG (1981b) Gewerbetoxikologische Grundprufung. Primare Schleimhautreizwirkung (Kaninchenauge; Draize-test) (Report on the study of the irritation to the eye of white rabbits based on Draize of Palatinol CE 5250). Unpublished results (80/266).

BASF AG (1995a) Study of the prental toxicity of Palatinol DN (test substance No 92/64) in rats after oral administration (gavage) performed by BASF Aktiengesellschaft Department of toxicology, FRG. Project No 10R0126/91088, Report dated 06 September 1995, Study carried out in 1992.

BASF AG (1995b). Study of the prenatal toxicity of Palatinol N (test substance N° 91/126) in rats after oral administration (gavage) performed by BASF Aktiengesellschaft Department of Toxicology, FRG. Project N° 10R0126/91088, Report dated 04 May 1995, Study carried out in 1992.

Benford DJ, Patel S & Reavy HJ (1986) Species differences in the response of cultured hepatocytes to phthalate esters. Food and Chemical Toxicology, 24 (6-7): 799-800.

BIBRA (1985) A 21-day feeding study of di-isononyl phthalate to rats: Effects on the liver and liver lipids, Report No. 0495/6785.

Bio/Dynamics (1982a) One week prechronic oral feeding study. Test materials: MRD 8240, MRD 8241. Project number VO 4053, performed by Bio/Dynamics, Inc., Unpublished laboratory report submitted to Exxon Biomedical Sciences, Inc., November 19, 1982.

Bio/Dynamics (1982b) Thirteen week pre-chronic oral feeding study in Fischer 344 rats. Test material: MRD-82-41. Project VO 4154-F, performed by Bio/Dynamics, Inc., Report submitted to Exxon Biomedical Sciences, Inc., December 8, 1982.

Bio/Dynamics (1982c) Thirteen week pre-chronic oral feeding study in Sprague-Dawley rats. Test material: MRD-82-41. Project VO 4154-S, performed by Bio/Dynamics, Inc., Report submitted to Exxon Biomedical Sciences, Inc., December 8, 1982.

Bio/Dynamics (1986) A chronic toxicity carcinogenicity feeding study in rats with Santicizer 900 final report. Project no 81-2572 (BD-81-244) performed by Bio/Dynamics, Inc., unpublished laboratory report (incomplete report, appendices not available) submitted to Monsanto Company, June 20, 1986.

Boas M, Frederiksen H, Feldt-Rasmussen U, Skakkebæk NE, Hegedüs L, Hilsted L, Juul A, Main KM (2010) Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. Environ Health Perspect. 118:1458-64.

Boberg J, Christiansen S, Axelstad M, Kledal TS, Vinggaard AM, Dalgaard M, Nellemann C, Hass U (2011) Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reprod Toxicol 31:200-9.

Borch J, Ladefoged O, Haas U, & Vinggaard AM (2004) Steroidogenesis in foetal male rats is reduced by DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in foetal, prepubertal and adult male rats. Reproductive Toxicology, 18: 53-61.

Brodell RT, Torrence BP (1992) Sqwish Ball® dermatitis. J. Am. Acad. Dermatol, 26 (4): 641-2.

Butala JH, David RM, Gans G, McKee RH, Guo TL, Peachee VL, White Jr KL (2004) Phthalate treatment does not influence levels of IgE or Th2 cytokines in B6C3F1 mice. Toxicology, 201: 77-85.

Calafat AM, Slakman AR, Silva MJ, Herbert AR, Needham LL (2004) Automated solid phase extraction and quantitative analysis of human milk for 13 phthalate metabolites. J Chromatogr B Analyt Technol Biomed Life Sci 805:49–56.

Calafat AM, Brock JW, Silva MJ, Gray Jr. LE, Reidy JA, Barr DB and Needham LL (2006) Urinary and amniotic fluid levels of phthalate monoesters in rats after the oral administration of di(2-ethylhexyl) phthalate and di-n-butyl phthalate, Toxicology 217: 22–30.

Caldwell DJ et al. (1999a) Retrospective evaluation of alpha 2µ globulin accumulation in male rat kidneys following high doses of diisononyl phthalate. Toxicological Sciences, 51 (1): 153-160.

Caldwell DJ (1999b) Review of mononuclear cell leukemia in F-344 rat bioassays and its significance to human cancer risk: A case study using alkyl phthalates. Reg. Toxicol. Pharmacol., 30:45-53

Canada Gazette (2009) Phthalate Regulations. Accessed November 2009 at http://www.gazette.gc.ca/rp-pr/p1/2009/2009-06-20/html/reg3-eng.html.

CDC (2009) Fourth National Report on Human Exposure of Environmental Chemicals. Centers for Disease Control and Prevention, Department of Health and Human Services.

CERHR (2003) NTP-CERHR Monograph on the potential human reproductive and developmental effects of di-isononyl Phthalate (DINP).. The National Toxicology Program Center for the Evaluation of Risks to Human Reproduction, US Department of Health and Human Services. Accessed April 2011 at http://cerhr.niehs.nih.gov/evals/phthalates/dinp/DiNP_Monograph_Final.pdf

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

CHAP (2001) Report to the U.S. Consumer Product Safety Commission by the Chronic Hazard Advisory Panel (CHAP) on diisononyl phthalate (DINP), US Consumer Product Safety Commission, Directorate for Health Sciences.

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

Chernoff N and Rogers JM (2004) Supernumerary ribs in developmental toxicity bioassays and in human populations: Incidence and biological significance. J Toxicol Environ Health Part B, 7: 437-449

Corton JC & Lapinskas PJ (2005) Peroxisome proliferator-activated receptors: mediators of phthalate ester-induced effects in the male reproductive tract? Toxicological Sciences, 83: 4-17.

CPSC (1998) The risk of chronic toxicity associated with exposure to diisononyl phthalate (DINP) in children's products, US Consumer Product Safety Commission.

CPSC (2010) Toxicity review of diisononyl phthalate (DINP), US Consumer Product Safety Commission.

CSTEE (2001) Opinion on the results of the Risk Assessment of: 1,2-Benzenedicarboxylic acid, di-C8-10 branched alkyl esters, C9-rich and di-"isononyl" phthalate CAS No.: 68515-48-0 and CAS No.: 28553-12-0 -EINECS No.: 271-090-9 and EINECS No.: 249-079-5. Report version (Human Health Effects): Final report, May 2001, Scientific Committee on Toxicity, Ecotoxicity and the Environment, European Commission.

David R, Moore M, Finney D, & Guest D (2000a) Chronic toxicity of di(2-ethylhexyl)phthalate in rats. Toxicological Sciences, 55(2): 433-443.

David R, Moore M, Finney D, & Guest D (2000b) Chronic toxicity of di(2-ethylhexyl)phthalate in mice. Toxicological Sciences, 58(2): 377-385.

David RM (2006) Proposed mode of action for in utero effects of some phthalate esters on the developing male reproductive tract. Toxicologic Pathology, 34: 209-219.

Deisinger P, Perry L, & Guest D (1998) In vivo percutanous absorption of ^{14}C-DEHP from ^{14}C-DEHP plasticized polyvinyl chloride film in Male Fischer 344 rats. Food and Chemical Toxicology, 36: 521-527.

Derelanko MJ (2000) Toxicologist's pocket handbook, CRC Press.

DTI (2002) Research into the mouthing behaviour of children up to 5 years old, commissioned by the Consumer and Competition Policy Directorate, London, UK, Department of Trade and Industry.

Duty SM, Silva MJ, Barr DB, Brock JW, Ryan L, Chen Z, Herrick RF, Christiani DC & Hauser R (2003b) Phthalate exposure and human semen parameters, Epidemiology 14: 269–277.

Duty SM, Singh NP, Silva MJ, Barr DB, Brock JW, Ryan L, Herrick RF, Christiani DC & Hauser R (2003a) The relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral comet assay, Environ. Health Perspect. 111: 1164–1169.

Eastman (2002) Eastman plasticisers: selection chart. Eastman Chemical Company. Accessed May 2010 at http://www.eastman.com/Literature_Center/L/L174.pdf.

EC (2009) ESIS: European Chemical Substances Information System. European Commission Joint Research Centre, Institute for Health and Consumer Protection,

ECB (2003) European Union Risk Assessment Report on 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich and di-"isononyl" phthalate (DINP). European Chemicals Bureau.

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

ECPI (1997) Existing substances risk assessment for diisononyl phthalate (DINP) – CAS No. 68515-48-0; 28553-12-0, European Council for Plasticisers and Intermediates (ECPI).

Elsisi AE, Carter DE and Glenn Sipes I (1989) Dermal absorption of phthalate diesters in rats. Fundamental and Applied Toxicology, 12: 70-77.

EPL (Experimental Pathology Laboratories) (1999) Histopathological peer review and pathology working group review of selected lesions of the liver and spleen in male and female F344 rats exposed to di(isononyl)phthalate. Pathology Working Group Review. Pathology Report, Experimental Pathology Laboratories Inc., Research Triangle Park NC 27709.

European Chemicals Agency (ECHA) (2010) Evaluation of new scientific evidence concerning the restrictions contained in Annex XVII to Regulation (EC) No. 1907/2006 (REACH). Review of new available information for di-isonolyl phthalate (DINP).

Exponent, Inc. (2007) Review and risk analysis of child exposure to di-isononyl phthalate in toys. Prepared for Toy Industry Association Inc. Accessed 2008 at http://www.toyassociation.org/DINPStudy.pdf.

Exxon Biomedical Sciences (1986) Chronic toxicity/oncogenicity study in F-344 rats. Test material: MRD-83-260. Project No. 326075 performed at Exxon Biomedical Sciences, Inc. Unpublished laboratory report, January 13, 1986.

Exxon Biomedical Sciences (1992) Dermal sensitisation test in the guinea pig (Buehler method). Project performed at Exxon Biomedical Sciences, Inc. Report submitted to Exxon Chemical International, Inc., October 8, 1992.

Exxon Biomedical Sciences (1994) Developmental toxicity study in rats with diisononyl phthalate (DINP; MRD-92-455). Project No 145534 performed for Exxon Chemical Company and Exxon Chemical International, Inc., unpublished laboratory report from Exxon Biomedical Sciences, Inc., November 30, 1994.

Exxon Biomedical Sciences (1996a) Primary dermal irritation study in the rabbit. Performed at Exxon Biomedical Sciences, Inc. for Exxon Chemical Europe, January 26, 1996.

Exxon Biomedical Sciences (1996b) Microbiological mutagenesis in Salmonella mammalian microsome plate incorporation assay (MRD 95-389). Project number 138925, performed by Exxon Biomedical Sciences, Inc. for Exxon Chemical Europe, March 8, 1996.

Exxon Biomedical Sciences (1996c) In vitro chromosomal aberration assay in Chinese Hamster Ovary (CHO) cells (MRD 95-389). Project number 138932, performed by Exxon Biomedical Sciences, Inc. for Exxon Chemical Europe, March 8, 1996.

Exxon Biomedical Sciences (1996d) Reproduction toxicity study in rats with diisononyl phthalate (DINP; MRD-92-455). Project number 145535 from Exxon Biomedical Sciences, Inc. submitted to Exxon Chemical Company and Exxon Chemical Europe, unpublished laboratory report, March 8, 1996.

Exxon Biomedical Sciences (1996e) Two generation reproduction toxicity study in rats with diisononyl phthalate (DINP; MRD-92-455). Project from Exxon Biomedical Sciences Inc submitted to Exxon Chemical Company and Exxon Chemical Europe, unpublished laboratory report, February 29, 1996.

Fabjan E, Hulzebos E, Mennes W, Piersma AH (2006) A category approach for reproductive effects of phthalates. Crit Rev Toxicol, 36:695-726.

Fiala F, Steiner I, Kubesch K (1998) Migration of di-(2-ethylhexyl)phthalate (DEHP) and diisononyl phthalate (DINP) from PVC articles. Consumer Council, Austrian Standards Institute. Accessed at http://www.verbraucherrat.at/download/phthalates2.pdf.

Foster PM (2005) Mode of action: impaired fetal leydig cell function – Effects on male reproductive development produced by certain phthalate esters. Crit Rev Toxicol, 35:713-719.

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

Fromme H, Bolte G, Koch HM, Angerer J, Boehmer S, Drexlet H, Mayer R and Liebl B (2007) Occurrence and daily variation of phthalate metabolites in the urine of an adult population. Int J Hyg Environ Health, 210: 21-33.

Ge RS, Chen GR, Dong Q, Akingbemi B, Sottas CM, Santos M, Sealfon SC Bernard DJ and Hardy MP (2007) Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. Journal of Andrology, 28 (4): 513-520.

General Motors Research Laboratories (1981) Toxicity and fate of diisodecyl phthalate following inhalation exposure in rats. EPA Document N° 878210881, Fiche No OTS206189, 1983.

Ghisari M & Bonefeld-Jorgensen EC (2009) Effects of plasticisers and their mixtures on oestrogen receptor and thyroid hormone functions. Toxicology Letters, 189:67-77.

Gottschalck TE & McEwen GN (2006) International cosmetic ingredient dictionary and handbook, 11th Edition, The Cosmetic, Toiletry, and Fragrance Association.

Gray Jr. LE, Ostby J, Furr J, Price M, Veeramachaneni DNR, & Parks L (2000) Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 58:350-65.

Greene MA (2002) Mouthing times among young children from observational data. Bethesda, MD, US Consumer Product Safety Commission.

Groot ME, Lekkerkerk MC, and Steenbekkers (1998) Mouthing behaviour of young children; an observational study. Annex 3 to ‘ RIVM Report 613320 002: Phthalate release from soft PVC baby toys: Report from the Dutch Consensus Group’ edited by WH

Hall M, Matthews A, Webley L, Harling R (1999) Effects of di-isononyl phthalate (DINP) on peroxisomal markers in the marmoset—DINP is not a peroxisomal proliferator. J Toxicol Sci, 24:237–44.

Hannas BR, Lambright CS, Furr J, Howdeshell KL, Wilson VS, Gray LE Jr. (2011a) Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicol Sci, 123:206-16.

Hannas BR, Lambright CS, Furr J, Evans N, Foster PM, Gray LE, & Wilson VS (2011b) Genomic biomarkers of phthalate-induced male reproductive developmental toxicity: A targeted rtPCR array approach for defining relative potency. Toxicol Sci, Nov 22 (in press).

Harris CA, Henttu P, Parker MG, & Sumpter JP (1997) The oestrogenic activity of phthalate esters in vitro. Environ. Health Perspect., 105: 802-811.

Hazleton (1968a) Acute dermal application – rabbits. MRD 68-27, MRD 68-28, MRD 68-29, MRD 68-30. Unpublished laboratory report from Hazleton Laboratories, Inc. submitted to Esso Research and Engineering Company, May 20, 1968.

Hazleton (1968b) Acute eye application – rabbits. MRD 68-27, MRD 68-28, MRD 68-29, MRD 68-30. Unpublished laboratory report from Hazleton Laboratories, Inc. submitted to Esso Research and Engineering Company, May 20, 1968.

Hazleton (1968c) Acute oral application – rabbits. MRD 68-27, MRD 68-28, MRD 68-29, MRD 68-30. Unpublished laboratory report from Hazleton Laboratories, Inc. submitted to Esso Research and Engineering Company, May 20, 1968.

Hazleton (1969) Repeated dermal application – rabbits. MRD 69-4. Final report from Hazleton Laboratories, Inc. submitted to Esso Research and Engineering Company. Unpublished results, summary, August 1, 1969, Linden, New Jersey.

Hazleton (1971) Thirteen week dietary administration – Dogs. MRD-70-46 (diisononyl phthalate). Unpublished laboratory report from Hazleton Laboratories, Inc. submitted to Esso Research and Engineering Company. January 28, 1971.

Hazleton (1972) Metabolism study of ^{14}C phthalate ester in rats. Unpublished laboratory report from Hazleton Laboratories submitted to Esso Research and Engineering Company. Final report, September 25, 1972.

Hazleton (1980a) Acute inhalation toxicity study in rats DINP. Final report from Hazleton Laboratories America, Inc. submitted to Nissan Chemical Industries, Ltd., Tokyo, Japan, Unpublished laboratory report, December 18, 1980.

Hazleton (1980b) Acute oral toxicity study in rats DINP. Final report, Project No 2096-101 from Hazleton Laboratories America, Inc. submitted to Nissan Chemical Industries, Ltd., Tokyo, Japan, Unpublished laboratory report, August 29, 1980.

Hazleton (1981) Teratology study in rats DINP. Project No 2096-103 from Hazleton Laboratories America, Inc. submitted to Nissan Chemical Industries, Ltd., Tokyo, Japan, final report, March 25, 1981.

Hazleton (1986) Four final mutagenicity reports regarding diisononyl phthalate, di(heptyl, nonyl, undecyl) phthalates, diisodecyl phthalate and diundecyl phthalate. Mutagenicity of 1J in a mouse lymphoma assay. HBC unpublished laboratory report from Hazleton Biotechnologies Company submitted to the Chemical Manufacturers Association, Project No. 20989, Genetics assay No. 7158, Final report, June 1986.

Hazleton (1991a) A subchronic (13-week) dietary oral toxicity study of di(isononyl) phthalate in Fischer 344 rats with attachments and cover letter 0822291. Unpublished laboratory report from Hazleton Laboratories submitted to Aristech Chem. Corporation.

Hazleton (1991b) A subchronic (4-week) dietary oral toxicity study of di(isononyl) phthalate in B6C3F1 mice (final report) with cover sheet dated 052991. Unpublished laboratory report from Hazleton Laboratories submitted to Aristech Chem. Corporation. HWA study No 2598-100, study completion date: April 22, 1991.

Hazleton (1992) A 13-week subchronic dietary oral toxicity study in mice with di(isononyl) phthalate including ancillary hepatocellular proliferation and biochemical analyses. Hazleton project HWA 2598-103, 1992.

Health Canada (1998) Risk assessment on diisononyl phthalate in vinyl children's products. Health Canada, Ottawa, Canada.

Health Canada (2009) List of prohibited and restricted cosmetic ingredients (the Cosmetic Ingredient Hotlist). Accessed May 2010 at http://www.hc-sc.gc.ca/cps-spc/person/cosmet/info-ind-prof/_hot-list-critique/hotlist-change-liste-eng.php.

Hellwig J, Freudenberg H, Jäckh R (1997) Differential prenatal toxicity of branched phthalate esters in rats. Food and Chemical Toxicology, 35: 501-512.

Heudorf U, Mersch-Sundermann V & Angerer J (2007) Phthalates: Toxicology and exposure, Int. J. Hyg. Environ. Health 210:623-634.

Hill Top Research (1995) Evaluation of primary irritation potential in humans (single 24-hour application). Performed by Hill Top Research, Inc. for Exxon Biomedical Sciences, Inc. July 20, 1995.

Hubinger JC & Havery DC (2006) Analysis of consumer cosmetic products for phthalate esters. Journal of the Society of Cosmetic Chemists, 57: 127-137.

Hüls AG (1985a) Akute orale Toxizität von Vestinol R(9) für Ratten. Bericht Nr. 0436.

Hüls AG (1985b) Prufung der akuten Hautreizwirkung von Vestinol (R) 9 am Kaninchen. Bericht Nr. 0437. Unpublished results.

Hüls AG (1985c) Prufung der akuten Augen- und Schleimhautreizwirkung von Vestinol (R) 9 am Kaninchen. Bericht Nr. 0438. Unpublished results.

Hüls AG (1992) A 14-days oral toxicity study with three different types of diisononyl phthalates in female Fischer 344 rats; Final report SA-92/0062. Enzyme activities in liver fractions from female Fischer 344 rats treated with three isomeric diisononyl phthalates (14-day oral gavage study): Final report BT-92/0062. Dodecanoic acid 12-hydroxylase activity in liver microsomes from female Fischer 344 rats treated with three isomeric diisononyl phthalates (14-day oral gavage study) – results of individual animals and statistical evaluation; Final report BT-92/0062-1.

Huntingdon Life Sciences (1998) DINP: Toxicity study by oral gavage administration to marmosets for 13 weeks. Report no. 98 3532, October 1998.

IFCS (Intergovernmental Forum on Chemical Safety) (2006) Toys and chemical safety: a thought starter. Document 03-TS, Agenda Item 10, Forum V - Fifth Session of the Intergovernmental Forum on Chemical Safety, Budapest, Hungary, 15-29 September 2006 [IFCS/FORUM-V/03-TS]. Geneva, World Health Organisation. Accessed October 2006 at http://www.who.int/ifcs/documents/forums/forum5/03_ts_en.pdf .

Imai Y, Kondo A, Iizuka H, Maruyama T and Kurohane K (2006) Effects of phthalate-esters on the sensitisation phase of contact hypersensitivity induced by fluorescein isothiocyanate. Clinical and Experimental Allergy, 36:1462-1468.

IPCS (International Programme on Chemical Safety) (1994) Assessing human health risks of chemicals: Derivation of guidance values for health-based exposure limits. World Health Organization, Geneva. Accessed at http://www.inchem.org/documents/ehc/ehc/ehc170.htm#SubSectionNumber:5.3.4

Johnson S, Saikia N & Sahu R (2011) Phthalates in toys available in Indian market. Bulletin of Environmental Contamination and Toxicology, Online Article Accessed 5 May 2011.

Juberg D, Alfano K, Coughlin R & Thompson K (2001) An observational study of object mouthing behaviour by young children. Pediatrics, 107:135-142.

Kalimi GH, Lington AW, Nikiforov AI & Klaunig J (1995) Gap junction assay in rodent hepatocytes: a good predictor for liver cancer in rodents for phthalate esters. The Toxicologist, 15(1): 1469.

Kamrin MA (2009) Phthalate risks, phthalate regulation, and public health: A review, J Toxicology and Environmental Health, Part B, 12:157-174.

Karbe E & Kerlin RL (2002) Cystic degeneration/Spongiosis hepatic in rats, Toxicol. Pathol. 30(2): 216-227.

Kiss CT (2001) A mouthing observation study of children under 6 years of age. Washington DC, Division of Human Factors, US Consumer Product Safety Commission (USCPSC).

Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM, Deluca JG, Lai DY, McKee RH, Peters JM, Roberts RA & Fenner-Crisp PA (2003) PPAR alpha agonist-induced rodent tumours: modes of action and human relevance. Crit. Rev. Toxicol., 33: 655-780

Koch HM and Angerer J (2007a) Di-isononylphthalate (DINP) metabolites in human urine after a single oral dose of deuterium-labelled DINP. Int. J. Hyg. Environ. Health, 210: 9-19.

Koch HM, Muller J and Angerer J (2007b) Determination of secondary, oxidised di-isononylphthalate (DINP) metabolites in human urine representative for the exposure to commercial DINP plasticisers. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci., 847: 114-125.

Koike E, Yanagisawa R, Sadakane K, Inoue K, Ichinose T and Takano H (2010) Effects of diidononyl phthalate on atopic dermatitis in vivo and immunologic responses in vitro. Environmental Health Perspectives 118(4):472-478.

Kolle SN, Kamp HG, H HA, Knickel J, Verlohner A, Woitkowiak C, Landsiedel R van Ravenzwaay B (2010) In house validation of recombinant yeast oestrogen and androgen receptor agonist and antagonist screening assays, Toxicology in Vitro 24:2030-2040.

Kruger T, Long M & Bonefeld-Jøgensen EC (2008) Plastic components affect the activation of the aryl hydrocarbon and the androgen receptor, Toxicology 246: 112-123.

Larsen ST, Lund RM, Nielsen GD, Thygesen P and Poulsen OM (2002) Adjuvant effect of di-n-butyl-, di-n-octyl-, di-iso-nonyl- and di-iso-decyl phthalate in a subcutaneous injection model using BALB/c Mice. Pharmacology & Toxicology 91:264-272.

Latini G, Wittassek M, Del Vecchio A, Presta G, De Felice C and Angerer J (2009) Lactational exposure to phthalates in Southern Italy, Environment International 35:236-239.

Lee BM & Koo HJ (2007) Hershberger assay for antiandrogenic effects of phthalates, J. Toxicology and Environ. Health, Part A 70: 1365-1370.

Lee HC, Yamamnouchi K & Nishinara (2006) Effects of perinatal exposure to phthalate/adipate esters on hupothalamic gene expression and sexual behavior in rats, J. Reprod. Dev 52(3): 352-352.

Lee HL, Park J, Chung SW, Kang BY, Kim SH and Kim TS (2004) Enhancement of Interleukin-4 production in activated CD4+ T Cells by diphthalate plasticisers via increased NF-AT binding activity, Int Arch Allergy Immunol 134:213-222.

LGC (1998) Laboratory-based agitation methods for the determination of phthalate plasticiser migration from PVC toy and child care articles: LGC Technical Report No.: LGC/1998/DTI/009. Teddington, Middlesex, UK, Laboratory of the Government Chemist.

Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997) Chronic toxicity a carcinogenic evaluation of diisononyl phthalate in rats. Fundam Appl Toxicol 36:79-89.

Litton Bionetics (1981) Evaluation of R-1218 in the primary rat hepatocyte unscheduled DNA synthesis assay. Unpublished laboratory report from Litton Bionetics submitted to Tenneco Chemicals Inc. LBI project no 20991, final report, February 1981.

Main KM, Mortensen GK, Kaleva MM, Boisen KA, Damgaard IN, Chellakooty M, Schmidt IM, Soumi AM, Virtanen HA, Petersen JH, Andersson AM, Toppari J and Skakkebaek NE (2006) Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. Environmental Health Perspectives 114 (2):270-276.

Masutomi N, Shibutani M, Takagi H, Uneyama C, Takahashi N, & Hirose M (2003) Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period

on the development of the rat endocrine/reproductive systems in later life. Toxicology, 192:149-70.

McKee RH, El-Hawari M, Stoltz M, Pallas F, & Lington AW (2002) Absorption, disposition and metabolism of di-isononyl phthalate (DINP) in F-344 rats. Journal of Applied Toxicology, 22:293-302.

Meek ME, Boobis AR, Crofton KM, Heinemeyer G, Raaij MV and Vickers C (2011) Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. Regulatory Toxicology and Pharmacology, 60:S1-S14.

Microbiological Associates (1981a) Activity of T1646 in the in vitro cytogenetics assay in rodents. Unpublished laboratory report from Microbiological Associates submitted to Tenneco Chemicals Company, MA study no T1646.112.

Microbiological Associates (1981b) Activity of T1677 in the in vitro mammalian cell transformation assay in the absence of exogenous metabolic activation. Unpublished laboratory report from Microbiological Associates submitted to Tenneco Chemicals Company, MA project no T1677.108.

Midwest Research Institute (1981) Acute oral toxicity study in rats of TCI compounds: R-1268, R-1272, R-1286 and R-1287, with cover letters and index. Unpublished laboratory report from Midwest Research Institute prepared for Tenneco Chemicals, Inc., MRI Project No 7180-B(1), June 2, 1981.

Midwest Research Institute (1983a) Single and repeated oral dose pharmacokinetics of 14 C-labelled diisononyl phthalate with cover letter. Unpublished laboratory report from Midwest Research Institute prepared for Exxon Corporation, MRI Project No 7282-B, December 19, 1983.

Midwest Research Institute (1983b) Dermal disposition of ^{14}C-diisononyl phthalate in rats. Unpublished laboratory report from Midwest Research Institute prepared for Exxon Corporation, MRI Project No 7572-E, August 4, 1983.

Miynarcikova A, Fickova M & Scsukova S (2007) The effects of selected phenol and phthalate derivatives on steroid hormone production by cultured porcine granulosa cells, 2005 SSCT-EST Conference Proceedings, ATLA 35: 71-77.

Moore MR (1998b) Oncogenicity study in mice with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses. Covance 2598-105, Volume 1 of 6. Vienna, VA: Aristech Chemical Corporation.

Moore MRCL (1998a) Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses. Covance 2598-104 Volume 1 of 6. Vienna, VA: Aristech Chemical Corporation.

Mortensen GK, Main KM, Andersson AM, Leffers H and Skakkebaek NE (2005) Determination of phthalate monoesters in human milk, consumer milk, and infant formula by tandem mass spectrometry (LC-MS-MS), Anal. Bioanal. Chem. 382: 1084–1092.

National Transport Commission (2007) Australian code for the transport of dangerous goods by road & rail, 7th edition, National Transport Commission, Commonwealth of Australian.

NDPSC (2009) Standard for the uniform scheduling of drugs and poisons, No 24, National Drugs and Poisons Schedule Committee, Commonwealth of Australia.

NICNAS (2006) Declaration of certain phthalate chemicals used in toys, child care articles and cosmetics as priority existing chemicals, Commonwealth of Australia.

NICNAS (2008a) Phthalate hazard compendium: a summary of physicochemical and human health hazard data for 24 ortho-phthalate chemicals. Sydney, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia.

NICNAS (2008b) Diisononyl Phthalate, Existing Chemical Assessment Report, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia.

NICNAS (2008c) NICNAS cosmetics guidelines 2007, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia.

NICNAS (2010) Priority Existing Chemical Assessment Reprot No. 32: Diethylhexyl phthalate (DEHP). National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia.

NICNAS (2011) Priority Existing Chemical Assessment Reprot No. 33: Diethyl phthalate (DEP). National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia.

Niino T, Ishibashi T, Itho T, Sakai S, Ishiwata H, Yamada T, and Onodera S (2001) Monoester formation by hydrolysis of dialkyl phthalate migrating from PVC products in human saliva. Journal of Health Science, 47(3):318-322.

Niino T, Ishibashi T, Itho T, Sakai S, Ishiwata H, Yamada T, Onodera S. (2002) Simultaneous determination of phthalate di- and monoesters in poly(vinylchloride) products and human saliva by gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2002 Nov 15;780(1):35-44

Nishihara T, Nishikawa J, Kanayama T, Dakeyama F, Saito K, Imagawa M, Takatori S, Kitagawa Y, Hori S, & Utsumi H (2000) Oestrogenic activities of 517 chemicals by yeast two-hybrid assay. Journal of Health Science, 46(4): 282-298.

O'Brien ML, Spear BT, Glauert HP (2005) Role of oxidative stress in peroxisome proliferator – mediated carcinogenesis. Crit. Rev. Toxicol., 35:61-88.

OECD (2004) Screening Information Data Set (SIDS) Initial Assessment Profile (SIAP) for high molecular weight phthalate esters (HMWPE). SIAM 19. 19-22 October 2004.

OECD (2005) Manual for Investigation of HPV Chemicals, OECD. December 2005.

Ostby JS, Hotchkiss AK, Furr JR, & Gray Jr. LE (2001) Investigation of the ability of diisononyl phthalate (DINP) to alter androgen-dependent tissue development in Sprague-Dawley rats. Presented at Triangle Consortium for Reproductive Biology, RTP, NC, January 27, 2001.

Patyna PJ, Brown RP, Davi RA, Letinski DJ, Thomas PE, Cooper KR & Parkerton (2006) Hazard evaluation of diisononyl phthalate and diisodecyl phthalate in Japanese medaka multigenerational assay, Ecotoxicology and Environmental Safety 65: 36-47.

Peters JM, Cattley R C and Gonzalez FJ (1997). Role of PPAR alpha in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643. Carcinogenesis 18, 2029–2033.

Peters RJB (2005) Phthalates and artificial musks in perfumes. TNO Report R&I-A R 2005/011. Apeldorn, The Netherlands, Nederlanse Organisatie voor toegepast-natuurwetenschappelijk onderzoek (Netherlands Organisation for Applied Scientific Research). Accessed April 2008 at http://www.greenpeace.org/raw/content/international/press/reports/phthalates-and-artificial-musk.pdf.

Phthalate Esters Panel HPV Testing Group of the American Chemical Council (2006) High Production Volume (HPV) Chemical Challenge Programme Test Plan for the Phthalate Esters Category. Revision to Test Plan dated December 10, 2001. Prepared by ExxonMobil Biomedical Sciences, Inc.

Pugh G, Isenberg JS, Kamendulis LM, Ackley DC, Clare LJ, Brown R, Lington AW, Smith JH, Klaunig JE (2000) Effects of diisononyl phthalate, di-2-ethylhexyl phthalate and clofibrate in Cynomolgus monkeys. Toxicol. Sci., 56: 181-188

Rastogi S, Jensen G & Worsoe I (2002) Analytical chemical control of phthalates in toys: analytical chemical control of chemical substances and products: NERI Technical Report No. 404. National Environmental Research Institute, Ministry of the Environment, Denmark.

Rastogi S & Worsoe I (2001) Analytical chemical control of phthalates in toys: analytical chemical control of chemical substances and products: NERI Technical Report No. 373. National Environmental Research Institute, Ministry of the Environment, Denmark.

RIVM (1998) Phthalate release from soft PVC baby toys, Report from the Dutch Consensus Group, National Institute of Public Health and the Environment.

Ryan, L (1992) The use of generalized estimating equations for risk assessment in developmental toxicity, Risk Analysis 12:439-447.

Sarigiannis DA, Hansen U, Karakitsios SP (2010). Methodologies for quantifying health effects of exposure by multiple routes and the effects of mixtures in the light of the case studies. In: Sarigiannis DA, ed. Sixth Framework Programme – Methodologies for quantifying health effects of exposure by multiple routes and the effects of mixtures in the light of the case studies, including a report on suitable indices of exposure, p5-42.

SCCP (Scientific Committee on Cosmetic Products) (2007) Opinion on phthalates in cosmetic products. Adopted by the SCCP at its 11th plenary meeting of 21 March 2007.

Scott RC, Dugard PH, Ramsey JD and Rhodes C (1987) In vitro absorption of some o-phthalate diesters through human and rat skin. Environmental Health Perspectives, 74: 223-227 Plus Errata.

Silva MJ, Reidy JA, Preau JL, Samandar E, Needham LL and Calafat AM (2006a) Urinary biomarkers of di-isononyl phthalate in rats. Toxicology, 223: 101-112.

Silva MJ, Reidy JA, Preau JL, Needham LL and Calafat AM (2006b) Oxidative metabolites of diisononyl phthalate as biomarkers for human exposure assessment. Environmental Health Perspectives, 114(8): 1158-1161.

Sjöberg P, Bondesson U, Kjellen L, Lindquist N.-G, Mentin G., & Plöen L. (1985c) Kinetics of di-(2-ethylhexyl) phthalate in immature and mature rats and effect on testis. Acta Pharmacol. Toxicol., 56: 30-37.

Smith JH, Isenberg JS, Pugh Jr. G, Kamendulis LM, Ackley D, Lington AW and Klaunig JE (2000) Comparative in Vivo Hepatic Effects of Di-isononyl Phthalate (DINP) and Related C7–C11 Dialkyl Phthalates on Gap Junctional Intercellular Communication (GJIC), Peroxisomal Beta-Oxidation (PBOX), and DNA Synthesis in Rat and Mouse Liver, Toxicological Sciences 54: 312-321.

Stringer R., Labunska I., Santillo D., Johnston P., Siddorn J. and Stephenson A. (2000) Concentrations of phthalate esters and identification of other additives in PVC children's toys. Environ. Sci. & Pollut. Res., 7:1-10.

Stroebel P, Mayer F, Zerban H & Babbasch P (1995) Spongiotic pericytoma: A benign neoplasm deriving from the perisinusoidal (Ito) cells in rat liver, Am J Pathol. 146: 903-913.

SUSMP (2010) Standard for the uniform scheduling of medicines and poisons, No. 1. Australian Government. Department of Health and Ageing. National Drugs and Poisons Schedule Committee.

Takeuchi S, Iida M, Kobayashi S, Jin K, Matsuda T and Kojima H (2005) Differential effects of phthalate esters on transcriptional activities via human oestrogen receptors α and β, and androgen receptor. Toxicology, 210: 223-233.

The Personal Care and Products Council (2010) International Cosmetic Ingredient Dictionary and Handbook.

Tomonari Y, Kurata Y, Kawasuso T, David R, Gans G, Tsuchitani M,et al. (2003) Testicular toxicity study of di(2-ethylhexyl) phthalate (DEHP) in juvenile common marmoset. The Toxicologist, 72:385.

US EPA (2006) Child-specific exposure factors handbook (External Review Draft). U.S. Environmental Protection Agency. Accessed at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=56747.

US EPA (2006) Integrated Risk Information System. 2006. Accessed at http://www.epa.gov.iris/index.html.

US FDA (2008) Phthalates and cosmetic products. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Cosmetics and Colors, April 19, 2001; Updated March 31, 2005 and February 7, 2008. Accessed June 2008 at http://www.cfsan.fda.gov/~dms/cos-phth.html

Varga F & Csaky TZ (1976) Changes in the blood supply of the gastrointestinal tract in rats with age. Pflugers Arch. Jul 30; 364:129-343.

Waterman SJ Ambroso JL, Keller LH, Trimmer GW, Nikiforov AI & Harris SB (1999) Developmental toxicity of diisodecyl and diisononyl phthalates in rats. Repro Tox, 13: 131-136.

Waterman SJ, Keller LH, Trimmer GW, Freeman JJ, Nikiforov AI, Harris SB, Nicolich MJ, & McKee RH (2000) Two-generation reproduction study in rats given DINP in the diet. Reproductive Toxicology, 14: 21-36.

WHO (World Health Organisation) (1999) Environmental Health Criteria 210. Principles for the Assessment of Risks to Human Health from Exposure to Chemicals. World Health Organisation, Geneva.

Wilkinson CF & Lam JC (1999) The potential health effects of phthalate esters in children's toy: A review and risk assessment. Regulatory Toxicology and Pharmacology, 30:140-155.

Wittassek M & Angerer J (2008) Phthalates: metabolism and exposure. International Journal of Andrology, 31:131-138.

Wolfe G & Layton K (2003) Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet. Gov't Reports Annoucements, TherImmune Research Corp., Gaithersburg, MD.12.

Younoszai MK & Ranshaw J (1973) Gastrointestinal growth in the fetus and suckling rat pups: effects of maternal dietary protein. J Nutr., 103(3): 454-61.

Zacharewski TR, Clemons JH, Meek MD, Wu ZF, Fielden MR, & Matthews JB (1998) Examination of the in vitro and in vivo oestrogenic activities of eight commercial phthalate esters. Toxicological Sciences, 46 (2): 282-293.