

**State of the Science Report**  
**Phthalate Substance Grouping**

**1,2-Benzenedicarboxylic acid, diisononyl ester**  
**1,2-Benzenedicarboxylic acid, di-C<sub>8-10</sub>-branched alkyl**  
**esters, C<sub>9</sub>-rich**  
**(Diisononyl Phthalate; DINP)**

**Chemical Abstracts Service Registry Numbers**  
**28553-12-0 and 68515-48-0**

**Environment Canada**  
**Health Canada**

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**Canada** 

## Synopsis

The Minister of the Environment and the Minister of Health have prepared a state of the science report on 1,2-Benzenedicarboxylic acid, diisononyl ester (diisononyl phthalate or DINP). The purpose of this report is to review the currently available science on these substances so that the public has an opportunity to review, comment, and/or provide additional information for consideration, prior to proposing conclusions through the publication of a draft screening assessment. A proposed approach for considering the cumulative risk of phthalates has also been prepared for public review and comment, and will be used in the development of the draft screening assessment.

DINP is one of 14 phthalate esters (or phthalates) identified for screening assessment under the Chemicals Management Plan (CMP) Substance Grouping Initiative. Key selection considerations for this group were based on similar potential health effects of concern; potential ecological effects of concern for some phthalates; potential exposure of consumers and children; potential to leverage/align with international activity; and potential risk assessment and risk management efficiencies and effectiveness.

While many phthalate substances have common structural features and similar functional uses, differences in the potential health hazard, as well as environmental fate and behaviour, have been taken into account through the establishment of subgroups. The primary basis for the subgroups from a health hazard perspective is a structure activity relationship (SAR) analysis using studies related to important events in the mode of action for phthalate-induced androgen insufficiency during male reproductive development in the rat. The effects of phthalate esters for these important events appear to be structure-dependent and highly related to the length and nature of their alkyl chain. Further information on the approach by which the substances in the Phthalate Substance Grouping were divided into three subgroupings from a health hazard perspective is provided in Health Canada (2015a). From an ecological perspective, subgrouping was based primarily on differences in log  $K_{ow}$  and water solubility, and their resulting effects on bioaccumulation and ecotoxicity. Further information on the ecological rationale for the subgroups is provided in an appendix to the draft approach for considering the cumulative risk of phthalates (Environment Canada and Health Canada 2015a). For the purposes of the health review, DINP is included with the medium chain phthalate esters subgroup, and for the purposes of the ecological review it is considered to align more closely with the long-chain phthalate subgroup.

DINP is a complex substance that is assigned two different Chemical Abstracts Service Registry Numbers (CAS RN<sup>1</sup>). The CAS RNs, Domestic Substances List (DSL) names and the common names and acronyms for DINP are listed in the table below.

#### Names and CAS RNs for DINP

CAS RN	Domestic Substances List name	Common name
28553-12-0	1,2-Benzenedicarboxylic acid, diisononyl ester	Diisononyl phthalate (DINP)
68515-48-0	1,2-Benzenedicarboxylic acid, di-C <sub>8-10</sub> -branched alkyl esters, C <sub>9</sub> -rich	Diisononyl phthalate (DINP)

DINP is an organic substance that is primarily used as a plasticizer in a wide variety of consumer, commercial and industrial products. The substance is not naturally occurring in the environment. Based on information collected through a survey issued pursuant to section 71 of the *Canadian Environmental Protection Act* (CEPA 1999) (Canada 2013), DINP (CAS RNs 68515-48-0 and 28553-12-0) was imported, manufactured, and exported at quantities of over 10 000 000 kg, 1 000 000 to 10 000 000 kg, and 1 000 000 to 10 000 000 kg, respectively. DINP has applications in the electronics and home appliance sector, and may be used in wires and cables (e.g., insulation, sheathing), home and exterior appliances, and consumer electronics. DINP is used in the automotive industry as a coating, sealant, and plasticizer. Other applications of DINP are in the production of various types of sealants, adhesives, coatings and paints, and as a plasticizer and coating in fabrics (e.g., upholstery and artificial leather). DINP is also used in the production of various types of manufactured items; examples of these are vinyl flooring, roofing, toys, children's articles, pool liners, interior and exterior appliances, and screen printing inks.

Air and water are expected to be the primary receiving media for DINP in the environment. Based on properties of low water solubility and vapour pressure, and high partitioning potential into organic carbon, DINP released into water will distribute into sediment and the suspended particulate fraction of surface waters. DINP released into air is expected to distribute primarily into soil and sediments through wet and dry deposition processes. DINP released into soil is predicted to remain within this environmental compartment and is not expected to leach through soil into groundwater.

Based on empirical and modelled degradation data, DINP is expected to degrade rapidly in aerobic environments but may take longer to break down under low oxygen conditions such as those occurring in sub-surface sediments and soil. The substance is

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not expected to persist in the environment. DINP has been detected in all environmental media, indicating that sources of DINP into the environment result in detectable concentrations reflecting the balance of emission inputs and degradation losses.

Based on high partition coefficients and low water solubility, exposure of DINP to organisms will occur primarily through the diet. Empirical and modelled data indicate that DINP has low bioaccumulation and biomagnification potential. However, DINP has been measured in a variety of aquatic species and this confirms that the substance is bioavailable.

Results from standard laboratory tests suggest that DINP has low hazard potential in aquatic and terrestrial species, with no adverse effects on survival, growth, development or reproduction seen in acute and chronic testing at concentrations up to and exceeding the solubility and saturation limits of the substance. Results from an analysis of critical body residues (CBRs) conducted for aquatic organisms determined that the maximum tissue concentration of DINP based on the solubility limit will be much lower than levels associated with adverse acute or chronic lethality effects due to neutral narcosis. An analysis conducted for soil organisms indicated that the maximum tissue concentration calculated from the saturation limit of DINP in a 4% organic carbon (OC) soil does not exceed minimum concentrations estimated to cause narcotic effects. A similar result was determined for DINP measured directly in the tissues of Canadian aquatic biota. No suitable data were available for DINP in sediment species and a CBR analysis could not be conducted. However, results from a CBR analysis conducted for a suitable analogue substance, DIDP, suggest that tissue concentrations of DINP in sediment species are unlikely to reach levels predicted to result in acute or chronic effects due to baseline narcosis. Therefore, based on the analyses of CBRs, it is considered unlikely that internal body concentrations of DINP in exposed organisms will reach levels causing adverse narcotic effects. It should be noted that the CBR analysis does not consider the potential for adverse effects resulting from modes of action other than baseline narcosis.

With regard to human health, the main source of exposure to DINP is from food, with dust also being a contributor. Exposure from dermal contact (adults and infants) with manufactured items containing DINP or mouthing of these objects by children was also estimated.

Based principally on the weight of evidence from the available information, critical effects associated with oral exposure to DINP are carcinogenicity (hepatocellular tumours) and effects in the liver. Consideration of the available information on genotoxicity indicates that DINP is not likely to be genotoxic. With respect to non-cancer effects, several repeated-dose studies have shown that the liver and the kidney are the primary target organs in rodents and dogs following repeated exposure to this phthalate through the oral route.

Comparison of estimates of exposure from various sources (food, dust, plastic articles) as well as systemic exposure estimates calculated from biomonitoring measurements,

for all age groups of concern, with the appropriate critical effect levels, including the lowest dose associated with a significant increase in incidence of hepatocellular tumours, results in margins of exposures (MOEs) that are considered adequate to address uncertainties in the exposure and health effects databases.

DINP is also associated with developmental effects on the male reproductive system following exposure *in utero* but at higher doses compared to those inducing effects on liver and kidneys. These effects were reported to include decreased testicular and serum testosterone levels, decreased anogenital distance, areolar/nipple retention, effects in sperm, and evidence of testicular pathology indicating that DINP may be a weak antiandrogen. Although the margins of exposures are currently considered adequate on an individual basis, this does not address potential risk in a cumulative context when exposure to DINP in addition to other phthalates exhibiting a similar mode of action is aggregated.

Accordingly, a proposed cumulative risk assessment approach for certain phthalates is provided in a separate report (Environment Canada and Health Canada 2015a).

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## 1. Introduction

Pursuant to sections 68 and 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999), the Minister of the Environment and the Minister of Health conduct evaluations of substances to determine whether these substances present, or may present, a risk to the environment or to human health.

The Substance Groupings Initiative is a key element of the Government of Canada's Chemicals Management Plan (CMP). The Phthalate Substance Grouping consists of 14 substances that were identified as priorities for assessment, as they met the categorization criteria under section 73 of CEPA 1999 and/or were considered a priority based on human health concerns (Environment Canada, Health Canada 2013). Certain substances within this Substance Grouping have been identified by other jurisdictions as a concern due to potential reproductive and developmental effects in humans. There are also potential ecological effects of concern for some phthalates. A survey conducted for phase 1 of the Domestic Substances List (DSL) Inventory Update identified that a subset of phthalates have a wide range of consumer applications that could result in exposure to humans, including children (Environment Canada 2012). Addressing these substances as a group allows for consideration of cumulative risk, where warranted.

This state of the science (SOS) report provides a summary and evaluation of the current available science intended to form the basis for a draft screening assessment scheduled for publication in 2016. The Government of Canada developed a series of SOS reports for the Phthalate Substance Grouping to provide an opportunity for early public comment on a proposed cumulative assessment approach for certain phthalates (Environment Canada and Health Canada 2015a), prior to that approach being used to propose conclusions on the substances in the Phthalate Substance Grouping through publication of a draft screening assessment report.

This SOS report focuses on 1,2-Benzenedicarboxylic acid, diisononyl ester, or DINP (CAS RNs 28553-12-0 or 68515-48-0). This substance was identified in the categorization of the DSL under subsection 73(1) of CEPA 1999 as a priority for assessment. This substance also met the categorization criteria for inherent toxicity to non-human organisms, but not for persistence or bioaccumulation.

While phthalates have common structural features and similar functional uses, differences in their potential health hazard, environmental fate and behaviour have been taken into account through the establishment of subgroups. The primary basis for the subgroups from a health hazard perspective is a structure activity relationship (SAR) analysis using studies related to important events in the mode of action for phthalate-induced androgen insufficiency during male reproductive development in the rat. The effects of phthalate esters for these important events appear to be structure dependent, and highly related to the length and nature of their alkyl chain (Health Canada 2015a). From an ecological perspective, subgrouping was based primarily on differences in log  $K_{ow}$  and water solubility, and their resulting effects on bioaccumulation and ecotoxicity (Environment Canada and Health Canada 2015a).

This SOS report includes consideration of information on chemical properties, environmental fate, hazards, uses and exposure, including additional information submitted by stakeholders. Relevant data were identified up to October 2014 for the ecological portion and up to August 2014 for the health portion of the assessment. Empirical data from key studies, as well as some results from models, were used. When available and relevant, information presented in assessments from other jurisdictions was considered.

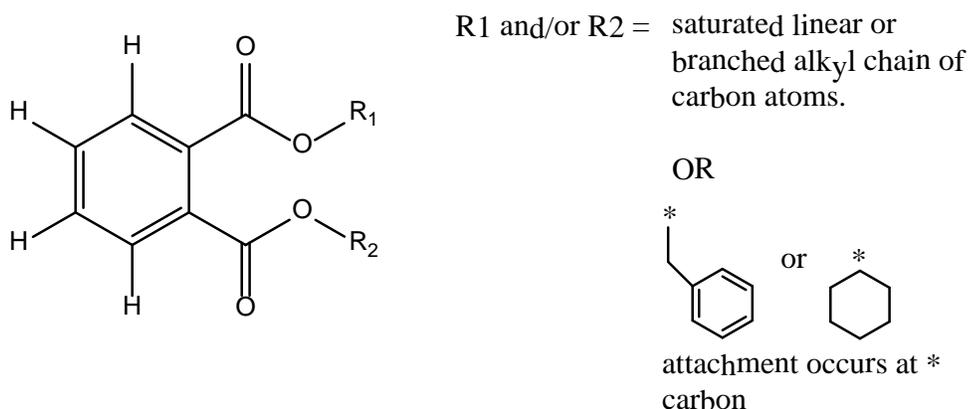
The SOS report does not represent an exhaustive or critical review of all available data. Rather, it presents the most critical and reliable studies and lines of evidence pertinent to development of a screening assessment in the future.

This SOS report was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. The ecological and human health portions of this report have undergone external written peer review and/or consultation. Comments on the technical portions relevant to the environment were received from Dr. Frank Gobas (Frank Gobas Environmental Consulting), Dr. Chris Metcalfe (Ambient Environmental Consulting, Inc.), Dr. Thomas Parkerton (ExxonMobil Biomedical Sciences, Inc.), and Dr. Charles Staples (Assessment Technologies, Inc.). Comments on the technical portions relevant to human health were received from Dr. Raymond York (York & Associates), Dr. Michael Jayjock (The Lifeline Group), and Dr. Jeanelle Martinez (Toxicology Excellence for Risk Assessment). While external comments were taken into consideration, the final content and outcome of the report remain the responsibility of Health Canada and Environment Canada.

## 2. Identity of Substances

Phthalate esters are synthesized through the esterification of phthalic anhydride (1,2-benzenedicarboxylic acid anhydride; CAS RN 85-44-9) with various alcohols (ACC 2001). The resulting phthalate esters are diesters of benzenedicarboxylic acid comprised of a benzene ring with two side chain ester groups. Phthalates have the general structure outlined in Figure 1, where R1 and R2 represent ester side chains that can vary in length and structure (ACC 2001). The ester side groups can be the same or different and the nature of the side groups determines both the identity of the particular phthalate and its physical and toxicological properties. All substances in the Phthalate Grouping are *ortho*-phthalates (*o*-phthalates), with their ester side chains situated adjacent to each other at the 1 and 2 positions of the benzene ring (refer to Figure 1; US EPA 2012).

The structural formula for phthalate esters is derived from the isomeric composition of the alcohol used in their manufacture (Parkerton and Winkelmann 2004). Dialkyl phthalates have ester groups of linear or branched alkyl chains containing from one to thirteen carbons, while benzyl phthalates generally contain a phenylmethyl group and an alkyl chain as ester side groups and cyclohexyl phthalates contain a saturated benzene ring as an ester group (Parkerton and Winkelmann 2004).



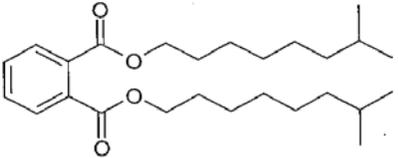
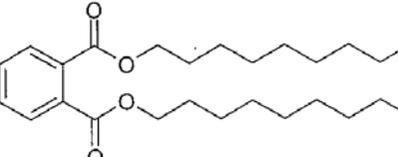
**Figure 1. General structure of *ortho*-phthalates.**

Diisononyl phthalate (DINP) is one of the 14 phthalate esters in the Phthalate Substance Grouping. Information on the chemical structure and identity of DINP is given in Table 2-1, with further details provided in Environment Canada (2015).

DINP is not a single compound, but is a complex isomeric mixture containing mainly C<sub>8</sub> and C<sub>9</sub>-branched isomers on its side chains (NICNAS 2008a). Two Chemical Abstracts Service Registry Numbers (CAS RNs) have been assigned to DINP, based on the starting alcohol and the proportions of the side chain components. DINP with CAS RN 28553-12-0 is produced from *n*-butene that is converted primarily to methyloctanols and dimethylheptanols (CERHR 2003). The resulting mixed phthalate has side chains composed of 5 to 10% methyl ethyl hexanol, 40 to 45% dimethyl heptanol, 35 to 40%

methyl octanol, and 0 to 10% *n*-nonanol (NICNAS 2008a). DINP with CAS RN 68515-48-0 is manufactured from octene that is converted to alcohol moieties of 3,4-, 4,6-, 3,6-, 3,5-, 4,5- and 5,6-dimethyl-heptanol-1, and has side chains comprised of 5 to 10% methyl ethyl hexanol, 45 to 55% dimethyl heptanol, 5 to 20% methyl octanol, 0 to 1% *n*-nonanol, and 15 to 25% isodecanol (CERHR 2003; NICNAS 2008a). ACC (2000) indicates that while DINP is a complex substance, it is not variable in composition because of the stability of the alcohol manufacturing process and it is therefore not a UVCB (i.e., substance of Unknown or Variable Composition, Complex Reaction Products or Biological Materials). While the two CAS RNs for DINP indicate different starting alcohols, the resulting isomeric phthalate mixtures share common constituents and cannot be differentiated through their physicochemical properties (ECJRC 2003). For this reason, the two CAS RNs are examined together in this SOS report.

**Table 2-1. Substance identity of DINP**

CAS RN acronym	DSL name and common name	Chemical structure and molecular formula <sup>a</sup>	Molecular weight (g/mol)
28553-12-0 DINP	1,2- Benzenedicarboxylic acid, diisononyl ester  Diisononyl phthalate	 C <sub>26</sub> H <sub>42</sub> O <sub>4</sub>	418.62 (average)
68515-48-0 DINP	1,2- Benzenedicarboxylic acid, di-C <sub>8-10</sub> - branched alkyl esters, C <sub>9</sub> -rich  Diisononyl phthalate	 C <sub>26</sub> H <sub>42</sub> O <sub>4</sub>	418.62 (average)

Abbreviations: CAS RN, Chemical Abstract Service Registry Number; DSL, Domestic Substances List.  
Source: ECJRC 2003; EPI Suite 2000-2008.

<sup>a</sup> As DINP is an isomeric mixture, the chemical structures provided in Table 2-1 are considered to be representative structures for the substance.

## 2.1 Selection of Analogues and Use of (Q)SAR Models

Guidance on the use of a read-across approach and Quantitative Structure-Activity Relationships or (Q)SAR models for filling data gaps has been prepared by various organizations such as the Organisation for Economic Co-operation and Development (OECD). These methods have been applied in various regulatory programs including the European Union's (EU) Existing Substances Programme. In this assessment, a read-across approach using data from analogues and the results of (Q)SAR models, where appropriate, have been used to inform the ecological and human health assessments. Analogues were selected that were structurally similar and/or functionally similar to substances within this subgroup (e.g., based on physical-chemical properties,

toxicokinetics), and that had relevant empirical data that could be used to read-across to substances that were data poor. The applicability of (Q)SAR models was determined on a case-by-case basis.

### 2.1.1 Selection of Analogues for Ecological Assessment

Information on analogues used to inform the ecological component of this SOS report is presented in Table 2-2 along with an indication of the read-across data used for different parameters. Further information relating to the analogue substance is provided in Appendix Table A-1.

**Table 2-2. Read-across data used to inform various parameters evaluated in this assessment**

CAS RN for analogue	Common name	Type of data used
26761-40-0	Diisodecyl phthalate (DIDP)	Critical body residue (CBR) analysis for sediment-dwelling organisms
68515-49-1		

DIDP was selected as a source of read-across data for the analysis of critical body residues (CBR) in sediment-dwelling organisms. DIDP has structural comparability of greater than 85 to 94% with DINP as determined by the OECD QSAR Toolbox software (2012; see Appendix Table A-1). In addition, comparability in their molecular dimensions (maximum diameter range 27 to 30 nm, effective diameter range 19 to 20 nm; Table A-1) and chemical properties (water solubility less than 0.0001 mg/L, log  $K_{ow}$  greater than 8 and log  $K_{oc}$  in the range of 5.5 to 6.5) suggests that they will have similar uptake and bioaccumulation characteristics, making DIDP acceptable as a source of read-across data for the CBR analysis of DINP in sediment species.

### 2.1.2 Selection of Analogues for Human Health Assessment

As there were no specific gaps in the toxicological database for DINP related to the characterization of risk to human health from exposure to DINP, no analogues were necessary.

## 3. Physical and Chemical Properties

Physical and chemical properties determine the overall characteristics of a substance and are used to determine the suitability of different substances for different types of applications. Such properties also play a critical role in determining the environmental fate of substances (including their potential for long-range transport), as well as their toxicity to humans and non-human organisms.

A summary of physical and chemical properties for DINP is presented in Table 3-1. More detailed information is available in Appendix B. Property values designated for use in modelling are identified in Appendix Tables B-1 and B-2.

**Table 3-1. Range of experimental and predicted physical and chemical properties (at standard conditions) for DINP**

Property	Value or range <sup>a</sup>	Type of data	Key references
Physical state	Liquid	Experimental	European Commission 2000
Melting point (°C)	-34 – -54	Experimental	European Commission 2000
Melting point (°C)	85 – 115	Modelled	MPBPVWIN 2010
Boiling point (°C)	331 – > 400	Experimental	ECHA 2014
Boiling point (°C)	440 – 454	Modelled	MPBPVWIN 2010
Density (kg/m <sup>3</sup> )	967 – 983	Experimental	European Commission 2000
Vapour pressure (Pa)	$6.8 \times 10^{-6}$ – $7.2 \times 10^{-5}$ , <sup>b</sup>	Experimental, calculated	Howard et al. 1985; Cousins and Mackay 2000
Vapour pressure (Pa)	$1.1 \times 10^{-3}$ – $2.9 \times 10^{-3}$	Modelled	MPBPVWIN 2010
Water solubility (mg/L)	$3.1 \times 10^{-4}$ – 0.2 <sup>b</sup>	Experimental, calculated	Howard et al. 1985; Cousins and Mackay 2000
Water solubility (mg/L)	$4.1 \times 10^{-5}$ – 0.1	Modelled	WATERNT 2010; VCCLab 2005
Henry's Law constant (Pa·m <sup>3</sup> /mol)	9.26	Calculated	Cousins and Mackay 2000
Henry's Law constant (Pa·m <sup>3</sup> /mol)	1.4 – 2.1	Modelled	HENRYWIN 2011 Bond and Group estimates
Henry's Law constant (Pa·m <sup>3</sup> /mol)	46 – 47	Modelled	HENRYWIN 2011 VP/WS estimate <sup>c</sup>
Log K <sub>ow</sub> (dimensionless)	8.6 – 9.7	Experimental, calculated	ECHA 2014; Cousins and Mackay 2000
Log K <sub>ow</sub> (dimensionless)	8.4 – 10	Modelled	ACD/Percepta c1997–2012; VCCLab 2005
Log K <sub>oc</sub> (dimensionless)	5.5 – 5.7	Modelled	KOCWIN 2010
Log K <sub>oa</sub> (dimensionless)	11	Calculated	Cousins and Mackay 2000
Log K <sub>oa</sub> (dimensionless)	12 – 13	Modelled	KOAWIN 2010

Abbreviations: K<sub>ow</sub>, octanol-water partition coefficient; K<sub>oc</sub>, organic carbon-water partition coefficient; K<sub>oa</sub>, octanol-air partition coefficient.

<sup>a</sup> All values are at 25°C unless otherwise stated.

<sup>b</sup> Includes values measured at 20 and 22°C.

<sup>c</sup> VP/WS estimate derived using empirical values for vapour pressure and water solubility (see Appendix B).

Models based on quantitative structure-activity relationships (QSARs) were used to generate data for some of the physical and chemical properties of DINP. These models are mainly based on fragment addition methods, i.e., they sum the contributions of sub-structural fragments of a molecule to make predictions for a property or endpoint. Most

of these models rely on the neutral form of a chemical as input and this is appropriate for DINP as it occurs as a neutral (non-ionized) substance in the environment.

DINP is a viscous oily liquid at room temperature. Based on experimental, modelled and calculated physicochemical property values, DINP has very low to low solubility in water, low vapour pressure, and high to very high partition coefficients ( $K_{ow}$ , octanol-water partition coefficient;  $K_{oc}$ , organic carbon-water partition coefficient;  $K_{oa}$ , octanol-air partition coefficient).

## 4. Sources

DINP is not naturally occurring in the environment.

An industry survey, issued pursuant to section 71 of CEPA 1999, was conducted in 2013 to obtain information on quantities in commerce for substances in the Phthalate Substance Grouping in Canada (Canada 2013). Based on information collected from this survey DINP (CAS numbers 68515-48-0 and 28553-12-0) was imported, manufactured, and exported at quantities of over 10 000 000 kg, 1 000 000 – 10 000 000 kg, and 1 000 000 – 10 000 000 kg, respectively, in 2012 (Environment Canada 2014a). Due to the targeted nature of the survey, reported use quantities may not fully reflect all uses in Canada.

In the United States, national aggregated production volumes of DINP were reported through Inventory Update Reporting (IUR) between 1986 and 2002 (US EPA 2014a). Based on non-confidential reporting information, DINP production volume ranged between greater than 4 540 000 to 226 796 000 kg in 2002; in 2006, the reported range was between 45 359 000 and less than 226 796 000 kg (US EPA 2014a; US EPA 2014b).

Production and use volumes of 100 000 000 to 1 000 000 000 kg per year have been reported by registrants under the European Union's REACH Initiative (ECHA 2014). DINP has also been identified as a high production volume chemical in Europe (ESIS 2014).

## 5. Uses

Based on available information, there are various manufacturers of DINP in North America and Europe (Cheminfo Services Inc. 2013a). Different forms of DINP (DINP 1, DINP 2, and DINP 3) exist due to varying methods of manufacture (Cheminfo Services Inc. 2013a), although DINP 3 seems to have been phased out in the European Union (ECHA 2013a).

DINP is a plasticizer for polyvinyl chloride (PVC) and cellulose (Ash and Ash 2003). It is also used in other polymer formulations, such as with polyalkyl methacrylate and polyvinyl butyrate and is further used in plasticized vinyl thermoplastics and plastisol (Evonik 2013; BASF 2009). DINP may also act as a substitute for some phthalates,

including DEHP and DOP, in certain applications (SCHER 2008; Cheminfo Services Inc. 2013a).

In Canada, DINP has applications in the electronics and home appliance sector, and may be used as a plasticizer in the production of wires and cables (e.g., insulation, sheathing, etc.), home and exterior appliances, consumer electronics, etc. (Environment Canada 2014a). Additionally, DINP is also used (as a plasticizer) in the production of various types of manufactured items; examples of these are vinyl flooring, roofing, toys, children's articles, pool liners, interior and exterior appliances, etc. (Environment Canada 2014a). DINP is also used as a coating, sealant, component of rubber, and plasticizer and these uses have applications in the automobile, housing, appliance sectors, etc. (Environment Canada 2014a). Other applications of DINP are as a plasticizer and coating in fabrics (e.g., upholstery, artificial leather) and in the production of screen printing inks, and rubber compounds (Environment Canada 2014a).

Globally, DINP is also used in lacquer coatings, paints, thinners, printing inks, colours, dyestuffs, varnishes, pigments, lubricants, adhesives and glues, furniture lacquers, paint thinners and removers, etc. (HSDB 2009; Evonik 2013; Ash and Ash 2003; ECHA 2014; NICNAS 2008a). It was also found in 1 of 36 perfume samples (concentration of 26 ppm) in an investigation by Greenpeace (SCCP 2007). DINP may also have commercial and industrial applications and may be used as a functional fluid, processing aid, and a viscosity adjustor (US EPA 2014b). It is also used in lubricants and greases and may have automotive applications (ECHA 2014; NICNAS 2008a).

A major use of DINP is in the production of plastic (PVC, polyurethane, polyester, etc.) manufactured items (outlined below in Table 5-1).

**Table 5-1. Manufactured items where DINP use has been reported**

<b>Examples of Uses</b>	<b>References</b>
Transportation products, traffic cones	US EPA 2014b;
Carpet backing, roofing membranes, hoses, , wall coverings and flooring, vinyl flooring, tiles, and sheets	NICNAS 2008a; HSDB 2009; Evonik 2013; Ash and Ash 2003; COWI, IOM and AMEC 2012; CHAP 2001
Inks for screen printing, predominantly for printed T-shirts	NICNAS 2012;
Electrical and electronic products, electrical batteries and accumulators	US EPA 2014b; ECHA 2014
Artificial leather, coated fabrics (e.g., tarps and conveyer belts), general and athletic footwear, jewelry, clothing accessories and clothing articles (including sleepwear and sportswear), gloves	US EPA 2014b; HSDB 2009; Evonik 2013; Ash and Ash 2003; ECHA 2014; CSPA Reports 2014; COWI, IOM and AMEC 2012; CHAP 2001

Toys, erasers, modelling clay, bath ducks, pajamas, balls, exercise balls, products produced from foam plastic, nursing pillows, Scented articles designed for children, slimy toys, baby products and baby equipment, baby furniture, changing tables, arts and crafts and needlework supplies, sporting articles	SCHER 2008; Peters 2003; NICNAS 2008a; Danish EPA 2006b; Danish EPA 2006c; Danish EPA 2006d; CSPA Reports 2014; CSTE 2001; Cheminfo Services Inc. 2013a; COWI, IOM and AMEC 2012; CHAP 2001
Rubber and plastic items, headsets, air mattresses, swimming pool liners and bathing equipment, adult toys, medical devices	US EPA 2014b; ECHA 2014; COWI, IOM and AMEC 2012; Danish EPA 2008a; Danish EPA 2006a

In Canada, DINP has been identified as a substance used in food packaging materials, as a plasticizer in polyvinylchloride (PVC) hose liners, with potential for direct contact with food (September 2014 emails from the Food Directorate, Health Canada to the Risk Management Bureau, Health Canada; unreferenced). DINP has been identified as a plasticizer for food-contact polymers, and is also used in food-contact coatings and plastics (Ash and Ash 2003; Cheminfo Services Inc. 2013a). Furthermore, it is also found in closures with sealing gaskets for food containers (Ash and Ash 2003).

DINP is not listed in the Drug Products Database, the Therapeutic Product Directorate's internal Non-Medicinal Ingredients Database, the Natural Health Products Ingredients Database or the Licensed Natural Health Products Database as a medicinal or non-medicinal ingredient present in final pharmaceutical products, veterinary drugs or natural health products in Canada (DPD 2014; NHPID 2014; LNHPD 2014; September 2014 email from the Therapeutic Products Directorate, Health Canada to the Risk Management Bureau, Health Canada).

DINP is not included on the List of Prohibited and Restricted Cosmetic Ingredients (more commonly referred to as the Cosmetic Ingredient Hotlist or simply the Hotlist), an administrative tool that Health Canada uses to communicate to manufacturers and others that certain substances, when present in a cosmetic, may contravene the general prohibition found in section 16 of the *Food and Drugs Act* or a provision of the *Cosmetic Regulations* (Health Canada 2011). Based on notifications submitted under the Cosmetic Regulations Health Canada, DINP was not notified to be present in cosmetics (September 2014 email from the Consumer Product Safety Directorate (CPSD), Health Canada to Existing substances Risk Assessment Bureau (ESRAB), Health Canada).

Finally, DINP was identified as used as a formulant in pest control products registered in Canada (April 2012 email from the Pest Management Regulatory Agency, Health Canada to the Risk Management Bureau, Health Canada; unreferenced).

## 6. Releases to the Environment

There are no known natural sources of DINP, and potential releases to the environment are restricted to those associated with anthropogenic activities.

Release of DINP to the Canadian environment could occur during manufacture and processing of the substance, including the transportation and storage of materials, as well as during the production, use and disposal of DINP-containing products. Releases from processing include losses from the manufacture of DINP, the compounding of plasticizers and PVC resins to make flexible PVC, the fabrication of flexible PVC into products, and the production of construction materials, plastisols, coatings, and other products containing the PVC product (Leah 1977). Losses could also occur during transportation activities, such as during the cleaning of holding containers and truck tanks. Releases of DINP from use and disposal activities include losses from products during service life, as well as during the final disposal of the products in landfills and by incineration (Leah 1977). DINP contained in products and manufactured items that are disposed of in landfills may migrate out of the products and items and could end up in landfill leachate. In 94% of large landfill sites in Canada (permitted to receive 40 000 tonnes of municipal solid waste annually), leachate is collected and treated on-site and/or off-site (sent to nearby wastewater treatment systems<sup>2</sup>) prior to being released to receiving water. However, leachate is most likely not treated in smaller landfills (Conestoga-Rovers and Associates 2009). At these sites, DINP may potentially be released to ground or surface water via leachate. Based on this, both non-dispersive and dispersive releases of DINP to the environment are possible.

Releases of DINP are expected to occur primarily to air and to water. As DINP is not chemically bound into polymer matrices during processing activities (Hakkarainen 2008), it can migrate to the surface of polymer products over time and potentially enter air through vapourization and water through leaching or abrasion. The rate of this migration is expected to be slow, however, and counteracted by chemical and physical attractive forces which work to hold DINP within polymers (personal communication, correspondence from Assessment Technologies, Inc., Keswick, VA to Ecological Assessment Division, Environment Canada dated October 2014; unreferenced). Schossler et al. (2011) calculated an area-specific emission rate of 0.22 µg/h·m<sup>2</sup> for DINP in soft-PVC samples, noting that about 50 days was required for the substance to reach a steady-state value in air. The five-month study was conducted in a controlled

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<sup>2</sup> In this assessment, the term “wastewater treatment system” refers to a system that collects domestic, commercial and/or institutional household sewage and possibly industrial wastewater (following discharge to the sewer), typically for treatment and eventual discharge to the environment. Unless otherwise stated, the term wastewater treatment system makes no distinction of ownership or operator type (municipal, provincial, federal, aboriginal, private, partnerships). Systems located at industrial operations and specifically designed to treat industrial effluents will be identified by the terms “on-site wastewater treatment systems” and/or “industrial wastewater treatment systems”.

climatic room set at 23°C, with 50% relative humidity and an air flow rate of 300 mL/min. As well, while DINP has low vapour pressure ( $6.8 \times 10^{-6}$  to  $2.9 \times 10^{-3}$  Pa at 25°C; see Table 3-1), higher temperatures associated with some processing activities and environmental conditions could enhance the volatility of the substance and result in increased release into air.

Results from a section 71 survey conducted for the year 2012 (Canada 2013) indicated that manufacturing and processing activities for DINP during that year were restricted to the industrial areas of Quebec and southern Ontario and, for this reason, potential releases during these activities are likely to be in these regions (Environment Canada 2014a). In all parts of Canada, releases are expected to primarily result from the use and disposal of products which contain DINP.

DINP is not a reportable substance under Environment Canada's National Pollutant Release Inventory (NPRI) program (Environment Canada 2014b).

## 7. Environmental Fate and Behaviour

### 7.1 Environmental Distribution

A summary of the steady-state mass distribution for DINP based on three emission scenarios to either air, water or soil is given in Table 7-1 below. Results for the individual DINP CAS RNs are provided in Environment Canada (2015). The results in Table 7-1 represent the net effect of chemical partitioning, inter-media transport, and loss by both advection (out of the modelled region) and degradation/transformation processes. The results of Level III fugacity modelling indicate that DINP can be expected to distribute primarily into soil or sediment, depending upon the compartment of release, with smaller proportions distributing into air and water.

**Table 7-1. Summary of level III fugacity modelling (EQC 2011) for DINP, showing percent partitioning into each medium for three release scenarios**

Substances released to:	Air (%)	Water (%)	Soil (%)	Sediment (%)
Air (100%)	3.5 – 8.6	2.0 – 2.9	77 – 78	11 – 16
Water (100%)	0 – 0.1	11 – 20	0.2 – 1.1	79 – 89
Soil (100%)	0	0	100	0

When released into air, DINP is predicted to distribute primarily into soil (77 to 78%; Table 7-1). High partition coefficients ( $\log K_{ow}$  8.4 to 10,  $\log K_{oc}$  5.5 to 5.7; see Table 3-1) indicate that DINP entering water from air can be expected to mainly distribute into sediment (11 to 16%), with only a small proportion (2 to 3%) remaining in the water column. A small proportion (3.5 to 8.6%) of DINP released into air is predicted to remain within this medium. The high predicted  $\log K_{oa}$  of 12 to 13 (see Table 3-1) suggests that DINP present in the atmosphere will be mainly sorbed to particulates in the air (Cousins et al. 2003). EQC (2011) predicts that about 65% of DINP released directly into air will distribute to the aerosol (particulate) fraction. These particulates may subsequently be deposited to soil through wet or dry deposition processes, thereby limiting the potential

for transport of DINP in air. As well, there is potential for DINP sorbed to air particulates to be transported some distance from the site of release; however, rapid photolytic degradation (see Abiotic degradation section below) indicates that long-range atmospheric transport of DINP is unlikely to occur.

DINP released into water is predicted to distribute primarily into the sediment compartment (79 to 89%), with a smaller proportion (11 to 20%) remaining in the water. EQC (2011) predicts that DINP in water will not distribute appreciably into air (0 to 0.1%) and this is in keeping with the low volatility of this substance (vapour pressure  $6.8 \times 10^{-6}$  to  $2.9 \times 10^{-3}$  Pa at 25°C; see Table 3-1). While moderate modelled Henry's Law constant values (1.4 to 47 Pa·m<sup>3</sup>/mol at 25°C; see Table 3-1) suggest that DINP may potentially volatilize from water, this effect will likely be mitigated by strong sorption of the substance to suspended material in the water column (Cousins et al. 2003).

Level III fugacity modelling predicts that DINP released into soil will remain within this compartment (100%). The high partition coefficients indicate that DINP will sorb strongly to organic matter in soil and, together with the low water solubility ( $4.1 \times 10^{-5}$  to 0.2 mg/L at 22 to 25°C; see Table 3-1), this suggests that DINP will have low mobility and is unlikely to leach through soil into groundwater.

## 7.2 Environmental Persistence

DINP is expected to degrade rapidly in air and water, with respective half-lives of less than two days and less than six months in these media. While the substance also biodegrades in sediment, studies demonstrate that it will biodegrade more slowly in natural sediments than would be predicted based on laboratory screening studies. This suggests that DINP may remain resident in natural sediments for longer periods, possibly in excess of one year. However, degradation data for mono-isononyl phthalate (MINP), the primary degradation product of DINP, indicate that while initial primary degradation of the parent product may be slow, MINP will degrade rapidly even in natural sediments. Based on this, DINP is not expected to persist in sediment.

No soil degradation data were found for DINP. Similar to sediment, it is likely that residence times in this medium may be longer due to sorption to soil particulates. However, DINP is not expected to persist in soil.

### 7.2.1 Abiotic Degradation

As with all phthalates, DINP can mineralize completely through a degradation pathway that occurs abiotically or through biological mechanisms and involves sequential hydrolysis of the ester linkages on the molecule (Liang et al. 2008; Otton et al. 2008). The first hydrolytic step results in the formation of the mono-alkyl phthalate ester (MPE), in this case, mono-isononyl phthalate (MINP). The MPE can then undergo further ester hydrolysis to form phthalic acid, which degrades to benzoic acid and ultimately to carbon dioxide (Otton et al. 2008). As hydrolysis reactions are important in the breakdown of DINP, the fairly slow rate of hydrolytic degradation in water (see Table 7-

2) is likely to be influenced by the very low water solubility of this substance. Table 7-2 presents key abiotic degradation data for DINP.

**Table 7-2. Summary of key abiotic degradation data for DINP**

Medium	Fate process	Degradation endpoint or prediction	Extrapolated half-life ( $t_{1/2}$ = days)	Reference
Air	Atmospheric oxidation	Half-life	0.23	AOPWIN 2010
Air	Ozone reaction	N/A	N/A	AOPWIN 2010
Water	Hydrolysis	Rate constant $1.5 \times 10^{-4} - 9.5 \times 10^{-4} \text{ d}^{-1}$	460 – 1200	Lertsirisopon et al. 2009
Water	Photolysis + hydrolysis	Rate constant $4.9 \times 10^{-3} - 2.1 \times 10^{-2} \text{ d}^{-1}$	32 – 140	Lertsirisopon et al. 2009
Water	Hydrolysis	Half-life (pH = 7)	1251 – 2808	HYDROWIN 2010
Water	Hydrolysis	Half-life (pH = 8)	125 – 281	HYDROWIN 2010

Abbreviations: N/A, not applicable (model does not provide an estimate for this type of structure).

Photodegradation through reaction with atmospheric hydroxyl radicals (indirect photolysis) is the predominant degradation process for DINP in air (Staples et al. 1997), with a predicted half-life for the reaction of 0.23 day (AOPWIN 2010; see Table 7-2). In addition, DINP contains chromophores that will absorb light at wavelengths of greater than 290 nm and therefore may be susceptible to direct photolysis by sunlight (Lyman et al. 1990). These results suggest that DINP is unlikely to remain in air for long periods of time.

Both hydrolysis and photolysis of DINP may occur in surface waters, although these abiotic processes proceed much more slowly than biodegradation (see section 7.2.2 below). An evaluation of the relative contributions of hydrolysis and photolysis determined that abiotic removal of DINP in the aqueous phase was catalyzed mainly by photolysis, with half-lives of 460 to 1200 days calculated for hydrolysis alone and 32 to 140 days calculated for the combined reactions of hydrolysis and photolysis (Lertsirisopon et al. 2009). While abiotic degradation appeared to be most active under acidic or alkaline conditions (i.e., pH 5 or 9, respectively), DINP under sunlight irradiation was also effectively degraded at neutral pH, with greater than 50% removal occurring over the 140-day test period (Lertsirisopon et al. 2009). No distinctive intermediate substances were detected over the course of the study. The long hydrolysis half-lives of about one to three years determined by Lertsirisopon et al. (2009) at ambient temperatures (range 0.4 to 27.4°C, mean 10.8°C) are consistent with those of 125 to 2808 days (4 months to 8 years) at 25°C predicted by HYDROWIN (2010; see Table 7-2).

## 7.2.2 Biodegradation

Table 7-3 and Table 7-4 summarize key primary and ultimate biodegradation data for DINP.

DINP is rapidly biodegraded to intermediate products (primary biodegradation) in aerobic aqueous environments, with 68% removal of the parent substance reported to occur within 1 day (O’Grady et al. 1985) and 90 to 100% removal of the parent in 5 to 28 days using acclimated (Sugatt et al. 1984; O’Grady et al. 1985) and non-acclimated (O’Grady et al. 1985; Furtmann 1993) microorganisms (see Table 7-3). Lertsirisopon et al. (2003) determined aerobic half-lives of 7 to 40 days for DINP in water, which is consistent with the BIOWIN sub-model 4 prediction of fast biodegradation in days to weeks (BIOWIN 2010).

**Table 7-3. Summary of key primary biodegradation data for DINP**

Medium	Fate process	Degradation endpoint or prediction	Extrapolated half-life ( $t_{1/2}$ = days)	Reference
Water	Aerobic	68% at 1 d <sup>a</sup>	N/A	O’Grady et al. 1985
Water	Aerobic	5 d to achieve $\geq$ 90% biodegradation <sup>b</sup>	N/A	O’Grady et al. 1985
Water	Aerobic	91 – 100% at 7 d <sup>a</sup>	N/A	Furtmann 1993
Water	Aerobic	>99% at 28 d <sup>c</sup>	N/A	Sugatt et al. 1984
Water	Aerobic	Half-life <sup>a</sup>	7 – 40	Lertsirisopon et al. 2003
Water	Aerobic	3.7 – 4.2 <sup>d,e</sup> “biodegrades fast”	Days to weeks	BIOWIN 2010
Water + sediment	Aerobic	1 – 2% at 28 d <sup>a</sup>	N/A	Johnson et al. 1984
Water + sediment	Aerobic	Rate constant 0.00006 d <sup>-1,a</sup>	12 000	Kickham et al. 2012
Water + sediment	Anaerobic	Half-life <sup>a,f</sup>	373 – 660	Lertsirisopon et al. 2006

Abbreviations: N/A, not applicable.

<sup>a</sup> Test used non-acclimated inoculum.

<sup>b</sup> Test used acclimated (1 d) inoculum.

<sup>c</sup> Study used acclimated (14 d) inoculum; 7.1 d lag phase observed.

<sup>d</sup> Output is a numerical score from 0 to 5.

<sup>e</sup> Sub-model 4: Expert Survey; qualitative results.

<sup>f</sup> Lag phase of 5.0 to 11.7 days observed.

Ultimate biodegradation (mineralization) of DINP proceeds more slowly than primary biodegradation, with 62 to 79% removal of the substance over a 28 or 29-day test period as measured using both acclimated and non-acclimated microorganisms (see Table 7-4). Sugatt et al. (1984) reported that a 7-day lag phase occurred for DINP prior

to the onset of biodegradation; however, once underway, 62% of the DINP was degraded over the 28-day test period and a biodegradation half-life of 5.31 days was calculated from the study. Scholz et al. (1997) determined DINP to be “readily biodegradable” under the conditions of their study, meeting ready biodegradation criteria as set out in the OECD Guidelines for the Testing of Chemicals (OECD 1992). BIOWIN (2010) and CATALOGIC (2012) predict that DINP will undergo rapid ultimate biodegradation in the environment.

**Table 7-4. Summary of key ultimate biodegradation data for DINP**

Medium	Degradation endpoint or prediction	Test method or model basis	Extrapolated half-life ( $t_{1/2}$ = days)	Reference
Water	62% at 28 d <sup>a</sup>	CO <sub>2</sub> evolution	5.31	Sugatt et al. 1984
Water	67.5% at 28 d <sup>b,c</sup>	O <sub>2</sub> consumption	N/A	Exxon Biomedical Sciences, Inc. 2004
Water	74% at 28 d <sup>b</sup>	BOD	N/A	CHRIP 2014
Water	79% at 28 d <sup>b</sup>	CO <sub>2</sub> evolution	N/A	Scholz et al. 1997
Water	56.6% at 29 d <sup>b</sup>	CO <sub>2</sub> evolution	N/A	Exxon Intermediates Technology 1996
Water	2.5 – 3.1 <sup>d</sup> “biodegrades fast”	Sub-model 3: Expert Survey (qualitative)	Weeks to months	BIOWIN 2010
Water	0.7 – 1.0 <sup>e</sup> “biodegrades fast”	Sub-model 5: MITI linear probability	Readily biodegradable	BIOWIN 2010
Water	0.7 – 0.9 <sup>e</sup> “biodegrades fast”	Sub-model 6: MITI non-linear probability	Readily biodegradable	BIOWIN 2010
Water	77 – 83 “biodegrades fast”	% BOD	11 – 13 <sup>f</sup>	CATALOGIC 2012

Abbreviations: BOD, biological oxygen demand; CO<sub>2</sub>, carbon dioxide; N/A, not applicable; O<sub>2</sub>, oxygen.

<sup>a</sup> Study used acclimated (14 d) inoculum; 7.1 d lag phase observed.

<sup>b</sup> Study used non-acclimated inoculum.

<sup>c</sup> Mean value; range was 59.5 to 74.1%.

<sup>d</sup> Output is a numerical score from 0 to 5.

<sup>e</sup> Output is a probability score.

<sup>f</sup> Based on the predicted BOD, CATALOGIC 2012 also predicts primary half-life values of 3.9 and 4.4 days for DINP.

DINP biodegrades more slowly in aerobic and anaerobic sediment:water test systems, indicating that the substance has the potential to remain for longer periods in these

environmental media. In aerobic freshwater sediment testing with several phthalates, Johnson et al. (1984) observed slower rates of primary biodegradation for phthalates having longer and/or more complex alkyl chain configurations, including DINP, as well as for all phthalates at lower chemical concentrations and lower test temperatures. Decreased biodegradability at low chemical concentrations was reported by Boethling and Alexander (1979), who hypothesized that the energy obtained from oxidizing chemicals at low concentrations may be insufficient to meet the energy demands of the microorganisms. This, in turn, limits the proliferation of the organisms to levels needed to cause appreciable loss of the chemical (Boethling and Alexander 1979). The inability of microorganisms to metabolize biodegradable molecules at low concentrations may contribute to the measured presence of trace levels of some synthetic organic chemicals in natural waters (Boethling and Alexander 1979). The results suggest that laboratory biodegradation tests conducted at chemical concentrations that are greater than those in nature may not correctly assess the rate of biodegradation of the chemical in natural ecosystems (Boethling and Alexander 1979).

Lertsirisopon et al. (2006) calculated biodegradation half-lives of 373 to 660 days (about 1 to 2 years) for DINP in three natural anaerobic freshwater sediment:water systems and attributed the slow biodegradation rates to the complexity of the DINP molecule, specifically the long alkyl chain. A lag phase of 5 to 12 days was needed prior to the onset of biodegradation, indicating the need for acclimation of the microorganisms.

Kickham et al. (2012) investigated the relationship between biodegradation rates, hydrophobicity and sorption potential of phthalates in sediment and determined that while phthalates, including DINP, have the inherent capacity to be rapidly degraded by sediment microbes, the rate of biodegradation in natural sediments is influenced by the sorption potential of the phthalate to sediment. Phthalates with high sorption potential will have slower biodegradation rates, mainly due to a reduced fraction of bioavailable, freely dissolved chemical concentration in the interstitial water (Kickham et al. 2012). DINP has high hydrophobicity ( $\log K_{ow}$  8.4 to 10,  $\log K_{oc}$  5.5 to 5.7; see Table 3-1) and therefore high sorption potential, and this is reflected in the long sediment biodegradation half-life of 12 000 days (about 33 years) calculated from the study. The study concluded that inherently biodegradable substances that are subject to a high degree of sorption, such as DINP, can be expected to exhibit long half-lives in natural sediments. The reduced bioavailability to microbial attack due to sorption also implies that the substance will be less bioavailable for uptake by benthic organisms.

Several studies indicate rapid removal of the primary degradation product of phthalates, MPEs. Scholz (2003) reported 89% removal, after a two-day lag phase, of the MPE of DINP, MINP, as determined by standard 28-day OECD ready biodegradation testing (OECD 1992). Otton et al. (2008) measured mean biodegradation half-lives of 23 and 39 hours for MINP in field-collected marine and freshwater sediments, following a lag phase of 20 to 70 hours and 4 hours, respectively. The degradation half-life of 23 hours obtained in marine sediments tested at 22°C increased to 200 hours when the test temperature was decreased to 5°C, indicating slower biodegradation at a more environmentally-relevant test temperature. Despite this slower rate, biodegradation of

MINP in the sediments was still relatively rapid when compared with sediment half-life data reported for the parent DINP. This suggests that the initial conversion of DINP to MINP may act as the rate-limiting step in the degradation of DINP in natural sediments.

No soil degradation data were found for DINP. Similar to presence in sediment, it is likely that the high sorptive potential will result in longer soil residence times than in water due to sorption to soil particulates. However, the evidence for biodegradation of the parent DINP, as well for the primary MINP degradation product, suggests that DINP is unlikely to persist in soil.

### 7.3 Potential for Bioaccumulation

An empirical fish bioconcentration factor (BCF) of less than 3 L/kg ww, and an earthworm biota-soil accumulation factor (BSAF) of 0.018, suggest that DINP has low potential to bioaccumulate in aquatic and terrestrial organisms. This is supported by modelled BCF and bioaccumulation factor (BAF) values of less than 1000 L/kg ww. However, DINP has been measured in a number of Canadian aquatic species (Mackintosh et al. 2004; McConnell 2007; Blair et al. 2009) and this confirms that the substance is bioavailable. A field-based food web magnification factor (FWMF), equivalent to a trophic magnification factor (TMF), of 0.46 indicates that the substance was not biomagnifying across trophic levels of the studied food web but was instead undergoing trophic dilution. Empirical and modelled data indicate that DINP is bioavailable but is not likely to be bioaccumulative within individual organisms or to undergo biomagnification between trophic levels.

#### 7.3.1 Bioconcentration Factor (BCF) and Bioaccumulation Factor (BAF)

No experimental bioconcentration data were found for DINP. The accurate determination of a water-based BCF is likely to be complicated by the very low water solubility and high log  $K_{ow}$  of this substance ( $4.1 \times 10^{-5}$  to 0.2 mg/L and 8.4 to 10, respectively; see Table 3-1).

ExxonMobil Biomedical Sciences, Inc. (2002) conducted a feeding study in order to determine the elimination rate constant for DINP in rainbow trout, *Oncorhynchus mykiss*. A mean measured dietary concentration of 182 mg DINP/kg feed was administered to the fish over the 14-day exposure phase, which was followed by an 8-day depuration period during which the fish were fed untreated food. An elimination rate constant of  $1.16 \text{ day}^{-1}$  was determined from the study and, based on a resulting half-life of less than one day, a BCF of less than 3 L/kg wet weight (based on 5% lipid content) was calculated for DINP in the fish (see Table 7-5).

**Table 7-5. Summary of empirically derived bioconcentration factor (BCF) for DINP**

Test organism	Experimental concentration (duration)	BCF (L/kg ww)	Reference

Rainbow trout ( <i>Oncorhynchus mykiss</i> )	182 mg/kg feed (14 days)	< 3	ExxonMobil Biomedical Sciences, Inc. 2002
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Abbreviations: ww, wet weight.

ECHA (2014) describes an unpublished 14-d acute toxicity study using earthworm, *Eisenia fetida*, and DINP at a mean measured concentration of 7571 mg/kg dw soil. The test was conducted according to OECD Test Guideline 207 (Earthworm, acute toxicity; OECD 1984a) and was assigned a reliability of 1 (reliable without restriction) by the European Chemicals Agency (ECHA) when evaluating the study for data quality. Although not a specified endpoint under the test guideline, a biota-soil accumulation factor (BSAF) of 0.018 was calculated based on DINP concentrations determined in the worm tissues and test soil. The results suggested that DINP did not bioaccumulate in earthworms under the conditions of the study.

No empirical BAF values were found for DINP. In order to provide an additional line of evidence for bioaccumulation potential, modelled BCF and BAF estimates were derived using the BCFBAF (2010) model in EPI Suite (2000-2008) and the Baseline Bioaccumulation Model with Mitigating Factors (BBM 2008). BCFBAF (2010) sub-model 1 estimates a BCF of 442 for DINP using a regression-based approach that does not include consideration of metabolism (see Table 7-6). Sub-model 2 of BCFBAF incorporates metabolism and yields lower BCF estimates of 2.4 and 6.3. BBM (2008) estimates BCFs of 661 and 851 for DINP, but cites mitigating factors of metabolism, large molecular size, and low water solubility. BAF values of 15.7 and 176 were obtained using the Arnot-Gobas mass balance approach of BCFBAF sub-model 3, which incorporates consideration of whole body primary biotransformation but does not consider gut metabolism, a process that has been recognized to be of importance for phthalates in fish (Webster 2003). Therefore, the estimates derived using BCFBAF sub-models 2 and 3 are conservative values. Considered together, the predicted BCF and BAF values and the experimental data indicate that DINP will have low bioaccumulation potential.

**Table 7-6. Summary of modelled bioaccumulation data for DINP**

Test organism	Model and model basis	Endpoint	Value (L/kg ww)	Reference
Fish	BCFBAF Sub-model 1: linear regression	BCF	442	BCFBAF 2010
Fish	BCFBAF Sub-model 2: mass balance	BCF	6.3 <sup>a</sup> , 2.4 <sup>a</sup>	BCFBAF 2010
Fish	BCF <sub>max</sub> with mitigating factors	BCF	661 <sup>b</sup> , 851 <sup>b</sup>	BBM with Mitigating Factors 2008

Fish	BCFBAF Sub-model 3: Arnot-Gobas mass balance	BAF	176 <sup>a</sup> , 15.7 <sup>a</sup>	BCFBAF 2010
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Abbreviations: BAF, bioaccumulation factor; BCF, bioconcentration factor; ww, wet weight.

<sup>a</sup> Model used an internally calculated  $K_m$  of  $0.3 \text{ d}^{-1}$  for middle trophic level fish.

<sup>b</sup> Model identified mitigating factors of metabolism, molecular size and water solubility.

The results of both models emphasize the importance of metabolism in determining the bioaccumulation potential of DINP. While no empirical metabolism data specific to DINP were found, a number of aquatic and terrestrial species have demonstrated the capacity to metabolize phthalates, including long-chain phthalates (e.g., Barron et al. 1995; Bradlee and Thomas 2003; Gobas et al. 2003), and it is expected that DINP will also be effectively metabolized. Further evidence for the metabolic potential of DINP is provided by the results of ready biodegradation testing which confirm that microorganisms can readily break down the substance (see Biodegradation section 7.2.2).

The low water solubility of DINP, as well as a tendency for the substance to form stable emulsions in water (Bradlee and Thomas 2003), is expected to limit exposure to aquatic organisms, thereby limiting the potential for uptake and accumulation. Active metabolism of the substance will further reduce the potential for bioaccumulation.

### 7.3.2 Biomagnification Factor (BMF) and Trophic Magnification Factor (TMF)

BMF values describe the process by which the concentration of a chemical in an organism reaches a level that is higher than that in the organism's diet, due to dietary absorption (Gobas and Morrison 2000). ExxonMobil Biomedical Sciences, Inc. (2002) reported a lipid-normalized BMF of less than 0.1 and a tissue elimination half-life of less than one day for DINP, based on an empirically-determined elimination rate constant of  $1.16 \text{ day}^{-1}$  in rainbow trout, *Oncorhynchus mykiss*, fed a mean measured dietary concentration of 182 mg/kg feed over 14 days. A BMF below 1 indicates that biomagnification is not likely to be occurring.

Mackintosh et al. (2004) examined the distribution of DINP and 12 other phthalates in a Canadian marine aquatic food web. Concentrations of the target substances were measured in 18 marine species, representing approximately four trophic levels, and a food web magnification factor (FWMF) was calculated for each phthalate. The FWMF provided a measure of the degree of biomagnification occurring in the food web, and was determined from the average increase in lipid-equivalent chemical concentration for each unit increase in trophic position. Based on this description, the FWMF can be considered equivalent to a TMF. DINP was detected in 13 of 18 species sampled, confirming that the substance is bioavailable to aquatic biota. The FWMF for DINP was calculated as 0.46, indicating that DINP was not biomagnifying in this aquatic food web since a FWMF below 1 indicates that biomagnification is not likely to be occurring in the food web. Rather, the substance was undergoing trophic dilution, and this was considered to be consistent with substances that are predominantly absorbed via the

diet and depurated at a rate greater than the passive elimination rate via fecal egestion and respiratory ventilation, due to metabolism (Mackintosh et al. 2004).

## 7.4 Summary of Environmental Fate

DINP may be released during industrial activities and through consumer use, with releases occurring primarily to air and to water. As DINP is not chemically bound into polymer matrices, it can slowly migrate to the surface of polymer products over time and potentially enter air through vapourization and water through leaching or abrasion. However, the rate of this migration is expected to be slow and counteracted by chemical and physical attractive forces which work to hold the phthalates within polymers (see Releases to the Environment section). DINP entering air will distribute primarily into soil and to a lesser extent into water and then sediment, while DINP released into water will distribute into sediment and the suspended particulate fraction of surface waters. DINP degrades rapidly through abiotic and biological means and is not expected to persist in the environment. However, degradation may be slower under low oxygen conditions and this may promote slower removal and higher relative concentrations of DINP in the environment. As well, high use quantities suggest that releases to the environment, and therefore organism exposure, may be continuous. Based on information relating to releases and the predicted distribution in the environment, organisms residing in soil and in the aquatic environment (water column and sediment species) have the highest exposure potential to DINP. The relatively rapid biodegradation rate of DINP suggests that exposure will be greatest for organisms inhabiting areas close to release sites, as concentrations are expected to decrease with increasing distance from points of discharge into the environment. The very low water solubility and high hydrophobicity of DINP suggest that exposure will be primarily through the diet rather than via the surrounding medium. Empirical and modelled evidence indicate that DINP has low bioaccumulation and biomagnification potential, likely as a result of reduced potential for uptake and high biotransformation capacity.

## 8. Potential to Cause Ecological Harm

### 8.1 Ecological Effects

All phthalates, including DINP, are considered to exert adverse effects through a non-specific, narcotic mode of toxic action. Parkerton and Konkel (2000) estimated critical body residues (CBRs) for parent phthalates and their metabolites to be in the range for nonpolar narcotics, suggesting that these substances exert adverse effects through baseline toxicity. Some phthalates and phthalate metabolites may also operate as polar narcotics. A more detailed discussion on the possible mode of action for substances in the Phthalates Substances Grouping is provided in the Approach document for considering cumulative risk (Environment Canada and Health Canada 2015a).

Parkerton and Konkel (2000) proposed that phthalates with high hydrophobicity (i.e., log  $K_{ow}$  greater than 5.5), such as DINP, do not cause acute or chronic toxicity in aquatic organisms because the combined effects of low water solubility and limited

bioconcentration potential prevent concentrations of the substance in the tissues of organisms from reaching levels sufficient to cause adverse effects.

Results from standard laboratory toxicity studies conducted using water column, sediment and terrestrial species found no adverse effects up to the water solubility or saturation limit of DINP. The few reported effects are associated with concentrations exceeding solubility or saturation limits and may be attributable to the presence of undissolved DINP in the test system rather than to direct chemical toxicity. It is important to note that standard toxicity tests were conducted using test concentrations that are well above those expected to occur in the environment and do not therefore represent realistic exposure conditions.

Recent novel testing conducted using transgenic medaka, as well as *in vitro* testing with porcine ovarian cells, suggests that DINP, while not estrogenic itself, may have the capacity to enhance the action of endocrine-active substances. A multigenerational medaka feeding study, however, found no evidence that chronic dietary exposure to DINP over several generations adversely affected the fish at the biochemical, individual or population level.

### 8.1.1 Water

Table 8-1 summarizes the key aquatic toxicity studies for DINP. Acute median lethality or effects data (L/EC<sub>50</sub> or acute lowest- and no-effect levels) are available for fish, invertebrates and bacteria, while endpoint values for chronic testing (EC<sub>50</sub>, lowest- and no-effect levels) were found for algae and *Daphnia*. In all studies except the chronic *Daphnia* testing of Rhodes et al. (1995), the value for the selected endpoint exceeded the highest concentration of DINP used in the study. As studies were conducted using test concentrations that approached or exceeded the water solubility limit for DINP under the conditions of the particular study (Adams et al. 1995; Rhodes et al. 1995; Exxon Biomedical Sciences, Inc. 1998; ECHA 2014), the results indicate that adverse effects are not expected to occur up to the water solubility limit of the substance.

**Table 8-1. Key aquatic toxicity studies for DINP**

Test organism	Endpoint	Value (mg/L) <sup>a</sup>	Reference
Rainbow trout, <i>Oncorhynchus mykiss</i>	96 h LC <sub>50</sub> mortality	> 0.16	Adams et al. 1995
Fathead minnow, <i>Pimephales promelas</i>	96 h LC <sub>50</sub> mortality	> 0.10, > 0.19	Adams et al. 1995
Bluegill sunfish, <i>Lepomis macrochirus</i>	96 h LC <sub>50</sub> mortality	> 0.14	Adams et al. 1995
Sheepshead minnow, <i>Cyprinodon variegatus</i>	96 h LC <sub>50</sub> mortality	> 0.52	Adams et al. 1995
Midge, <i>Paratanytarsus parthenogenetica</i>	96 h LC <sub>50</sub> mortality	> 0.08	Adams et al. 1995

Test organism	Endpoint	Value (mg/L) <sup>a</sup>	Reference
Mysid shrimp, <i>Mysidopsis bahia</i> <sup>b</sup>	96 h LC <sub>50</sub> mortality	> 0.39	Adams et al. 1995
Water flea, <i>Daphnia magna</i>	48 h EC <sub>50</sub> immobilization	> 0.06	Adams et al. 1995
Water flea, <i>Daphnia magna</i>	21 d NOEC 21 d LOEC survival, reproduction	0.034 0.089 <sup>c</sup>	Rhodes et al. 1995
Water flea, <i>Daphnia magna</i>	21 d NOEC 21 d LOEC survival, reproduction, growth	1.0 > 1.0 <sup>d</sup>	Brown et al. 1998
Water flea, <i>Daphnia magna</i>	21 d NOEC 21 d LOEC survival, reproduction	2 > 2 <sup>e</sup>	Exxon Biomedical Sciences, Inc. 1998
Water flea, <i>Daphnia magna</i>	21 d NOEC 21 d LOEC survival, reproduction	0.0036 > 0.0036	ECHA 2014
Green algae, <i>Selenastrum capricornutum</i> <sup>f</sup>	96 h EC <sub>50</sub> growth	> 1.80	Adams et al. 1995
Marine bacterium, <i>Photobacterium phosphoreum</i>	15 minute NOEC 15 minute LOEC photo-luminescence inhibition	83 > 83	ECHA 2014
Activated sludge microorganisms	30 minute EC <sub>50</sub> respiration inhibition	> 83.9	ECHA 2014

Abbreviations: d, day; EC<sub>50</sub>, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms; h, hour; LC<sub>50</sub>, the concentration of a substance that is estimated to be lethal to 50% of the test organisms; NOEC, the no observed effect concentration is the highest concentration in a toxicity test not causing a statistically significant effect in comparison to the controls; LOEC, the lowest observed effect concentration is the lowest concentration in a toxicity test that caused a statistically significant effect in comparison to the controls.

<sup>a</sup> Concentrations are reported as mean measured values unless otherwise stated.

<sup>b</sup> Now *Americamysis bahia*.

<sup>c</sup> Observed effect attributed to physical effects from entrapment of daphnids on surface at LOEC.

<sup>d</sup> Dispersant Tween 20 or Marlowet R40 used; nominal concentration reported following analytical confirmation.

<sup>e</sup> Water accommodated fraction (WAF) tested; nominal concentration reported following analytical confirmation.

<sup>f</sup> Now *Pseudokirchneriella subcapitata*.

Rhodes et al. (1995) reported reduced survival in the water flea, *Daphnia magna*, exposed over a 21-day period to a highest test concentration of 0.089 mg/L DINP. The observed mortality was attributed to physical effects associated with surface entrapment of the daphnids, rather than toxicity from exposure of the animals to dissolved aqueous-phase chemical (Rhodes et al. 1995). This entrapment may have resulted from the presence of undissolved DINP in micro droplet form or as a surface layer on the water (Knowles et al. 1987; Rhodes et al. 1995).

Brown et al. (1998) conducted 21-day *D. magna* testing using a single concentration of DINP (1 mg/L nominal; measured range was 0.77 to 1.1 mg/L) solubilized in one of two dispersants, “Tween 20” or “Marlowet R40” (castor oil-40-ethoxylate) at a proportion of 1:10 DINP: dispersant. The testing was conducted according to OECD Guideline 202 (OECD 1984b), modified by individually separating the *Daphnia* into single test vessels. Endpoints examined were survival of the parent generation, number of young produced, and mean body length of the surviving parent daphnids. A dispersant was used to enhance the solubility of DINP in order to more clearly delineate between adverse effects associated with physical entrapment and those relating to direct chemical toxicity. No adverse effects were seen in *Daphnia* survival, reproduction or growth under the conditions of the study.

Scholz (2003) conducted standard toxicity testing on the primary metabolic degradation product of DINP, MINP. The 96-hour LC<sub>50</sub> for MINP in carp, *Cyprinus carpio*, was 40 mg/L, while a 48-hour EC<sub>50</sub> of 29 mg/L was determined for *D. magna*. The 72-hour EC<sub>50</sub> for the green alga, *Desmodesmus subspicatus*, was greater than the highest test concentration of 51 mg/L. The water solubility limit for MINP is reported to be 56 mg/L (Scholz 2003), therefore all endpoint values fall below the solubility limit of the substance. Acute median lethal effect values of 29 to greater than 51 mg/L indicate that MINP does not have high toxicity to the species tested.

Patyna et al. (2006) conducted a multigenerational feeding study using Japanese medaka, *Oryzias latipes*, and DINP at a nominal concentration of 20 µg/g of food (mean measured concentration was 21.9 µg/g). A feeding study is of particular relevance as the high log K<sub>ow</sub> of DINP (8.4 to 10; see Table 3-1) indicates that dietary exposure will be the primary exposure route for this substance. The study evaluated the potential for DINP to cause reproductive and developmental effects in three generations of medaka, and examined a number of endpoints relating to biochemical, individual and population parameters. No significant effects were seen on survival, development, growth, and egg production of the DINP-treated fish relative to the controls (negative and acetone). In addition, there were no significant effects to parameters associated with evidence of endocrine modulation, such as sex ratios and gonadal-somatic index, and no effects on 7-ethoxyresorufin-o-deethylase (EROD) activity. The metabolism of testosterone was elevated in male DINP-treated fish as compared with the controls, but the effect was not seen in female fish. The relevance of this observation is unclear, as there appeared to be no adverse impacts to the individual fish and development and fecundity were not affected. Based on the results of the study, the researchers concluded that chronic dietary exposure to DINP did not adversely impact Japanese medaka at the biochemical, individual, or population level (Patyna et al. 2006).

Chen et al. (2014) examined the potential for DINP to exert acute toxicity or estrogenic activity in two species of fish. Embryos of zebrafish, *Danio rerio*, were exposed to 0.01 to 500 mg/L DINP (nominal) in methanol carrier for 72 hours. While some toxicity occurred at high concentrations, greater than 50% lethality was not observed at any exposure level and the 72-hour LC<sub>50</sub> was therefore greater than 500 mg/L. Twenty-four hour testing with estrogen-responsive transgenic medaka, *Oryzias melastigma*, was

then conducted to determine the potential for estrogenic activity. The transgenic medaka contained an estrogen-dependent liver-specific gene which exhibited fluorescence when stimulated by estrogenic activity. Eleutheroembryos, the stage of hatched fish that rely on yolk and have not started external feeding, were used in the study. DINP alone showed no estrogenic activity at exposure concentrations of 0.01 to 50 mg/L. By comparison, fish exposed to increasing concentrations of a known estrogen-active compound, estradiol (E2) exhibited a dose-dependent increase in fluorescence in the estrogen-dependent marker gene, indicating enhanced estrogenic activity. When co-exposed with E2, DINP enhanced the estrogenic activity of E2 to levels above those seen when the hormone was present on its own.

Modelled aquatic toxicity estimates were also considered in a weight-of-evidence approach to evaluating the potential for adverse effects in organisms (Environment Canada 2007). Modelled ecotoxicity values derived using the ECOSAR program (ECOSAR 2009) in EPI Suite (2000-2008) were deemed to be not reliable because the model domain of applicability for log  $K_{ow}$  was exceeded in both the ester and neutral organic (baseline toxicity) SARs.

#### **8.1.1.1 Derivation of a predicted no-effect concentration**

No evidence of chemical toxicity was seen up to the limit of water solubility in standard aquatic toxicity testing with DINP, although physical effects were sometimes observed. As noted, the very low water solubility and high hydrophobicity of DINP suggests that dietary exposure will be the major route of exposure for organisms, rather than from the surrounding medium. For this reason, endpoint values derived from water concentrations may not fully describe the potential for effects. The toxic potential of substances that are taken up primarily through the diet is better captured by examining whole-body residues (internal concentrations) of the substance in an organism. Critical body residues (CBRs) can then be calculated in order to estimate the potential for the substance to reach internal concentrations that are sufficiently high to cause effects through baseline neutral narcosis (McCarty and Mackay 1993; McCarty et al. 2013). Baseline narcosis refers to a mechanism of toxic action which, rather than resulting from chemical changes caused by exposure to a substance, results from the disruption of cellular membranes due to the physical presence of the substance in tissues (Schultz 1989; McCarty et al. 2013).

CBRs were calculated for DINP using the McCarty and Mackay (1993) equation:

$$\text{CBR} = \text{BAF} \times \text{WS} / \text{MW}$$

where:

CBR = the critical body residue (mmol/kg)

BAF = fish bioaccumulation factor (L/kg); normalized to 5% body lipid

WS = water solubility of the substance (mg/L)

MW = molecular weight of the substance (g/mol)

Input values to the equation were

- BAF 176 L/kg (highest Arnot-Gobas mass balance prediction; see Table 7-6);
- Water solubility  $6.1 \times 10^{-4}$  mg/L (Letinski et al. 2002; see Table B-2); and
- Molecular weight 418.62 g/mol (see Table 2-1).

The input parameters selected for use in the calculation of CBR represent a conservative but realistic scenario.

Based on the above input values, the calculated CBR for DINP was  $2.6 \times 10^{-4}$  mmol/kg.

McCarty and Mackay (1993) determined that CBRs associated with acutely lethal baseline neutral narcosis in small aquatic organisms typically range from about 2 to 8 mmol/kg, while those for chronic exposures range from 0.2 to 0.8 mmol/kg. The CBR value calculated for DINP is much lower than these, indicating that internal concentrations are unlikely to reach levels sufficient to elicit acute or chronic effects through a neutral narcosis mode of toxic action.

While there is evidence from novel (non-standard) toxicity testing methods that DINP may have the capacity to influence hormonal levels when in the presence of an endocrine-active substance, the results are considered to be indicators of potential effects but too preliminary to form the basis of a quantitative analysis.

### 8.1.2 Sediment

Table 8-2 summarizes the key sediment toxicity studies for DINP. No adverse effects were observed in sediment testing up to the highest concentrations of DINP tested.

**Table 8-2. Key sediment toxicity studies for DINP**

Test organism	Endpoint	Value (mg/kg dw) <sup>a</sup>	Reference
Amphipod, <i>Hyalella azteca</i>	10 d NOEC 10 d LOEC survival, growth	2900 > 2900	Call et al. 2001
Midge, <i>Chironomus tentans</i>	10 d NOEC 10 d LOEC survival, growth	2680 > 2680	Call et al. 2001
Moor frog, <i>Rana arvalis</i>	9–21 d NOEC 9–21 d LOEC egg hatching	707, 1009 <sup>b</sup> > 707, > 1009	Solyom et al. 2001
Moor frog, <i>Rana arvalis</i>	26 d NOEC 26 d LOEC larval survival, growth	707, 1009 <sup>b</sup> > 707, >1009	Solyom et al. 2001

Abbreviations: d, day; dw, dry weight; NOEC, the no observed effect concentration is the highest concentration in a toxicity test not causing a statistically significant effect in comparison to the controls; LOEC, the lowest observed effect concentration is the lowest concentration in a toxicity test that caused a statistically significant effect in comparison to the controls.

<sup>a</sup> Concentrations are reported as mean measured values.

<sup>b</sup> Endpoints were derived for two test sediments with organic carbon contents of 16 and 17.3%.

The maximum saturation of DINP in sediment can be determined using the relationship:

$$C_s = C_w \times K_{oc} \times f_{oc}$$

where:

$C_s$  = maximum saturation of DINP in sediment (mg/kg dw)

$C_w$  = water solubility of DINP (mg/L)

$K_{oc}$  = organic carbon-water partition coefficient of DINP (L/kg OC)

$f_{oc}$  = fraction of organic carbon (OC) in the sediment (kg OC/kg)

Maximum saturation reflects the amount of a substance that can dissolve into a given medium at equilibrium. It cannot be exceeded according to thermodynamic principles. In surface waters, the presence of co-solvents or surfactants can create conditions that allow for an “apparent solubility” which is slightly greater than the maximum solubility value. In solid phases, such as sediments and soils, maximum saturation is a direct function of the amount of organic carbon present in the matrix if it is assumed that only hydrophobic interactions with organic matter occur. Sediment organic carbon content can vary from location to location and often average carbon contents are used for calculating maximum saturation in sediments. The apparent solubility in water, and saturation in sediment or soil, can increase or decrease the bioavailability of a compound.

Selecting best values for water solubility and  $K_{oc}$  of  $6.1 \times 10^{-4}$  mg/L and 398 107 (log  $K_{oc}$  5.6), respectively (see Tables 3-1 and B-2), and an  $f_{oc}$  value of 0.04 (default value for average Canadian sediment OC content), the maximum saturation of DINP is calculated as 9.7 mg/kg dw of sediment. This value is much lower than the highest test concentrations used in the Call et al. (2001) study, suggesting that free DINP may have been present in the test system. However, while the saturation limit was exceeded under the conditions of the study, no adverse effects were observed in either test species (see Table 8-2).

Two test sediments were used in the Solyom et al. (2001) study, one with 16% organic carbon and the second with 17.3%. Applying the above equation, the maximum saturation of DINP in these sediments was 38.8 and 42.0 mg/kg dw, respectively. These concentrations are much lower than those used in the study, again suggesting that the maximum saturation limits of DINP were exceeded under the conditions of the study although no adverse effects were seen in the test organisms.

#### **8.1.2.1 Derivation of a predicted no-effect concentration**

No adverse effects were observed in sediment toxicity testing with DINP. As well, no measured biota-sediment accumulation factors (BSAFs) have been reported for DINP; therefore, a CBR analysis cannot be conducted for this substance.

A CBR analysis for sediment species was conducted for a structurally-similar phthalate, DIDP (CAS RNs 26761-40-0 and 68515-49-1; see Environment Canada and Health Canada 2015c), based on a BSAF of 0.6 kg/kg for the midge, *Chironomus tentans*, a sediment saturation limit of 6.2 mg/kg and a molecular weight of 446.68 g/mol. The resulting CBR of 0.008 mmol/kg is less than the minimum effect threshold ranges of 2 to 8 mmol/kg and 0.2 to 0.8 mmol/kg proposed by McCarty and Mackay (1993) for acute and chronic narcotic effects, respectively, indicating that tissue concentrations of DIDP are unlikely to reach levels predicted to result in acute or chronic effects due to baseline narcosis. Given the similarities in chemical properties and molecular dimensions between DIDP and DINP (see Appendix Table A-1), it is considered likely that the CBR for DINP in sediment-dwelling species will be comparable with that of DIDP and therefore less than minimum effect threshold limits as specified by McCarty and Mackay (1993).

### 8.1.3 Terrestrial

Terrestrial toxicity data for DINP are presented in Table 8-3.

**Table 8-3. Key soil toxicity studies for DINP**

Test organism	Endpoint	Value (mg/kg dw) <sup>a</sup>	Reference
Soil microbes	33 d NOEC 33 d LOEC inhibition of glucose utilization	9616 > 9616	Exxon Biomedical Sciences, Inc. 2002
Earthworm, <i>Eisenia fetida</i>	14 d NOEC 14 d LOEC survival	7571, 8421 <sup>b</sup> > 7571, > 8421	Exxon Biomedical Sciences, Inc. 1996a
Earthworm, <i>Eisenia fetida</i>	56 d NOEC 56 d LOEC survival, reproduction	1000 <sup>c</sup> > 1000	ExxonMobil Biomedical Sciences, Inc. 2010
Lettuce, <i>Lactuca sativa</i>	5 d NOEC 5 d LOEC seed germination	8473, 8062 <sup>b</sup> > 8473, > 8062	Exxon Biomedical Sciences, Inc. 1996b
Rye grass, <i>Lolium sp.</i>	5 d NOEC 5 d LOEC seed germination	8473, 8062 <sup>b</sup> > 8473, > 8062	Exxon Biomedical Sciences, Inc. 1996b
Lettuce, <i>Lactuca sativa</i>	5 d NOEC 5 d LOEC seed germination	1000 <sup>c</sup> 3000	Exxon Biomedical Sciences, Inc. 1996c
Lettuce, <i>Lactuca sativa</i>	28 d NOEC 28 d LOEC seed germination, growth	1387 > 1387	Exxon Biomedical Sciences, Inc. 2000

Abbreviations: d, day; dw, dry weight; NOEC, the no observed effect concentration is the highest concentration

in a toxicity test not causing a statistically significant effect in comparison to the controls; LOEC, the low observed effect concentration is the lowest concentration in a toxicity test that caused a statistically significant effect in comparison to the controls.

<sup>a</sup> Concentrations are reported as mean measured values unless otherwise stated.

<sup>b</sup> Testing was conducted using a natural and an artificial soil.

<sup>c</sup> Reported as nominal concentrations following analytical confirmation.

In acute and/or chronic testing with soil microorganisms, earthworms, and ryegrass, no adverse effects were seen at highest test concentrations ranging from 1000 to 10 000 mg/kg dw of soil. Adverse effects on seed germination were reported in lettuce at a nominal test concentration of 3000 mg/kg dw (Exxon Biomedical Sciences, Inc. 1996c). Concentrations in the study were analytically confirmed to be close to nominal; therefore, concentrations were reported in terms of nominal values. This study was conducted using two soils (natural and artificial) with organic carbon (OC) contents of 1.7 and 4.0% (Parkerton and Staples 2003). Using the procedure described in section 8.1.2 above, the maximum saturation of DINP in the test soils under the conditions of the study can be calculated from:

$$C_{\text{soil}} = C_w \times K_{\text{oc}} \times f_{\text{oc}}$$

where:

$C_{\text{soil}}$  = maximum saturation of DINP in soil (mg/kg dw)

$C_w$  = water solubility of DINP (mg/L) =  $6.1 \times 10^{-4}$  mg/L

$K_{\text{oc}}$  = organic carbon-water partition coefficient of DINP = 398 107 L/kg OC

$f_{\text{oc}}$  = fraction of organic carbon (OC) in the soil = 0.017 and 0.040 kg OC/kg

The maximum saturation of DINP under the conditions of the study was therefore 4.1 to 9.7 mg/kg dw of soil. These values are well below that of the lowest effect concentration of 3000 mg/kg dw, suggesting that physical effects resulting from the presence of undissolved DINP in the test system may have contributed to the observed toxicity.

No data were found on the potential for adverse effects to terrestrial plants through atmospheric exposure to DINP. While DINP is expected to be released into air (see Releases to the Environment section), the estimated short atmospheric half-life of 0.23 day (AOPWIN 2010; Environmental Persistence section) and low solubility and levels measured in this environmental medium (see Measured Environmental Concentrations section) suggest that organism exposure to DINP via ambient air is limited.

No information was found on potential effects of DINP in wildlife species. Laboratory studies using rodents and other mammalian species, considered as surrogates for mammals such as piscivores have been conducted in order to evaluate the potential for impacts to human health. Relevant data from these studies are presented in the Human Health Effects section of this SOS report.

Results from *in vitro* testing provide preliminary indications that DINP may have the potential to influence normal endocrine activity in mammalian species. Cultured porcine granulosa cells exposed simultaneously to  $10^{-4}$  to  $10^{-8}$  M DINP and follicle-stimulating hormone (FSH) over a 72-hour incubation period exhibited enhanced levels of

progesterone production and decreased estradiol production relative to FSH-only controls (Mlynarčíková et al. 2007). No significant change occurred in the production of either hormone when cells were exposed to DINP in the absence of FSH. The results indicate that DINP can alter the production of steroid hormones in the presence of an endocrine-active substance and under laboratory conditions.

### 8.1.3.1 Derivation of a predicted no-effect concentration

A biota-soil accumulation factor (BSAF) of 0.018 was reported in ECHA (2014) for earthworm, *Eisenia fetida*, exposed to DINP (see section 7.3.1) and a CBR analysis was conducted to determine the potential for adverse effects.

Applying the CBR relationship described in sections 8.1.1 and 8.1.2 to DINP in soil,

CBR = BSAF × C<sub>soil</sub> / MW where:

CBR = the critical body residue (mmol/kg)

BSAF = biota-soil accumulation factor (kg/kg); normalized to 5% body lipid

C<sub>soil</sub> = saturation limit of the substance in soil (mg/kg)

MW = molecular weight of the substance (g/mol)

Input values for the equation were

- BSAF 0.018 kg/kg (ECHA 2014; see section 7.3.1);
- Saturation limit of DINP in 4% OC soil 9.7 mg/kg (determined above); and
- Molecular weight 418.62 g/mol (see Table 2-1).

Using the maximum saturation in the calculation of CBR represents a conservative but realistic scenario.

Based on these input values, the calculated CBR is  $4.2 \times 10^{-4}$  mmol/kg. This suggests that internal concentrations of DINP in the earthworms did not reach levels sufficient to elicit acute or chronic effects through a neutral narcosis mode of toxic action as specified by McCarty and Mackay (1993).

The mean measured test concentration of 7571 mg/kg dw reported by ECHA (2014) is equivalent to that of Exxon Biomedical Sciences, Inc. (1996a), in which no adverse effects were observed in 14-d earthworm toxicity testing (see Table 8-3). The lack of observed toxicity in this testing aligns well with the result obtained in the CBR analysis.

The results of studies conducted using rodents and other mammalian species indicate that lowest observed adverse effect levels (LOAELs) from dietary studies are higher than levels of DINP measured in the environment. For example, dietary LOAELs in the range of 1000 to 3000 ppm (1000 to 3000 mg DINP/kg food) are reported for chronic and subchronic rodent studies (see Human Health Effects section). Levels measured in the Canadian environment are low (see section 8.2.1) and this suggests that wildlife is unlikely to be exposed to concentrations approaching these LOAEL values.

While the *in vitro* testing of Mlynarčíková et al. (2007) provides preliminary evidence to suggest that DINP may have the capacity to influence normal mammalian hormonal function in the presence of an endocrine-active substance and under laboratory conditions, further supporting information is needed in order for the study results to be deemed acceptable for use in a quantitative analysis.

## 8.2 Ecological Exposure

A number of factors can contribute to variability and uncertainty in environmental monitoring data for DINP. The widespread use of DINP can lead to issues with contamination during sample collection and especially during sample preparation and analysis, where phthalates including DINP can be present in low background levels in analytical equipment, laboratory air and reagents (Lin et al. 2003; McConnell 2007). This is especially problematic when measuring environmental occurrence at concentrations approaching the method detection limit. As well, incomplete separation of commercial mixtures can complicate the accurate determination of specific phthalates (Lin et al. 2003). There are also analytical issues associated with the chemical properties of DINP; for example, the very low water solubility and high hydrophobicity can result in emulsion formation in water samples and/or sorption of DINP to glassware, both of which will influence the accuracy of analytical determinations (Staples et al. 1997; Cousins et al. 2003).

### 8.2.1 Measured Concentrations in Environmental Media and Wastewater

Data concerning the measured presence of DINP in the environment are presented in Environment Canada (2015). No Canadian monitoring data are available for air and soil. However, Canadian data are available for surface waters, sediments and biota. Canadian data are also available for wastewater systems and their effluents.

DINP was not detected (detection limit 370 ng/L) in 10 surface seawater samples collected in 1999 from False Creek, in Vancouver, British Columbia (Mackintosh et al. 2004), but was present at a mean value of 85 ng/L (estimated from graphical data) in 10 samples collected from the same inlet over the period 2004 to 2006 (Blair et al. 2009). False Creek is an urbanized marine inlet in Vancouver and has a history of contamination from various industrial and urban sources.

Mackintosh et al. (2006) analyzed for DINP in surface water and sediment samples collected in False Creek. Concentrations in total seawater samples ranged from 61 to 135 ng/L; however, much higher concentrations of 14 700 to 50 400 ng/g dry weight (dw) were determined in the suspended particulate fraction of water samples as compared with the dissolved fraction, where concentrations ranged from 29 to 64 ng/L. The results illustrate that, based on its physicochemical properties of low water solubility and high partition coefficients (see Table 3-1), DINP in water will associate to a high degree with suspended organic material present in the water column rather than occurring in the dissolved fraction. Interestingly, sediment samples collected from the

same area contained lesser quantities than those measured in suspended particulates, ranging from 259 to 900 ng/g dw. The researchers proposed that the observed decline in concentration between suspended and bottom sediments suggested that desorption and biodegradation of DINP were occurring at rates which exceeded the rate of decline in organic carbon content between suspended particulates (40% OC) and bed sediment (2.8% OC) (Mackintosh et al. 2006). A mean concentration of about 1000 ng/g dw has also been reported for False Creek sediments (McConnell 2007; Blair et al. 2009).

Concentrations of 30 to 1108 ng DINP/L were measured in effluents collected in 2002 and 2003 from eight wastewater treatment systems in Alberta (Sosiak and Hebben 2005). DINP was also detected in surface water samples collected upstream (66 ng/L) and downstream (18 to 246 ng/L) of the plants.

DINP was not detected (detection limit 90 ng/g dw) in 15 sediment samples collected in 1997 near the outflow of a wastewater treatment system in Hamilton Harbour, Ontario (McDowell and Metcalfe 2001).

Mackintosh et al. (2004) reported concentrations of 257 to 10 965 ng/g lipid weight (lw) in marine aquatic biota collected in 1999 from False Creek, in Vancouver BC. The sampling program analyzed for DINP in 18 marine species representing approximately four trophic levels of the marine aquatic food web and was designed to examine the potential for biomagnification of DINP within the food web. In general, highest concentrations were associated with lower trophic level species, including plankton, algae (*Enteromorpha intestinalis*), geoduck clams (*Panope abrupta*) and blue mussels (*Mytilus edulis*), while lower levels were found in fish (pile perch, *Rhacochilus vacca*; English sole, *Pleuronectes ventulus*) and birds (surf scoter, *Melanitta perspicillata*). DINP was below the detection limit of 1.0 ng/g lw in samples of manila clam (*Tapes philippinarum*), purple seastar (*Pisaster ochraceus*), Pacific herring (*Clupea harengus pallasii*), white-spotted greenling (*Hexagrammos stelleri*) and spiny dogfish (*Squalus acanthias*). Further details on this study are provided in the Potential for Bioaccumulation section of this SOS report.

McConnell (2007) and Blair et al. (2009) also reported the presence of DINP in aquatic biota collected from False Creek, including green algae (*Prasiola meridionalis*; 24 000 ng/g lw, 330 ng/g wet weight (ww) mean), blue mussel (*M. edulis*; 2300 ng/g lw, 10 to 12 ng/g ww mean), soft-shell clam (*Mya arenaria*; 13 000 ng/g lw, 48 ng/g ww mean), Dungeness crab (*Cancer magister*; 320 ng/g lw, 27 ng/g ww maximum), and white-spotted greenling (*H. stelleri*; 300 ng/g lw, 4.9 ng/g ww mean). The substance was not detected (detection limit 60 to 170 ng/g ww) in shiner perch (*Cymatogaster aggregata*) but was present at 2400 to 2900 ng/g lw (270 to 1600 ng/g ww) in spiny dogfish, *S. acanthius* (McConnell 2007). It is not clear why the levels for some species, in particular the dogfish, differ markedly between McConnell (2007) and Mackintosh et al. (2004). McConnell (2007) noted, but could not account for, the differences observed between the two datasets which reported DINP concentrations for the same or similar species collected from the same sampling location but in two different years (1999 for Mackintosh et al. and 2005 for McConnell).

DINP has been measured in air samples in Sweden (0.28 to 50 ng/m<sup>3</sup>; Cousins et al. 2007, 2014) and soil samples in Denmark (maximum 1500 ng/g dw; Vikelsøe et al. 1999), as well as in surface waters, precipitation, sediments, and biota collected at a number of European locations (Environment Canada 2015). It has also been detected in European urban stormwaters and wastewater treatment system sludges.

## **8.3 Characterization of Ecological Risk**

### **8.3.1 Consideration of Lines of Evidence**

This state of the science report presents information relating to the potential for DINP to cause harm to the Canadian environment and/or to human health. Lines of evidence considered in the report include those pertaining to use patterns, environmental release and distribution, potential for environmental persistence, bioaccumulation potential, toxicity and hazard potential, and the results of environmental monitoring studies.

DINP is primarily used as a plasticizer and has applications in a wide variety of consumer, commercial and industrial products. The substance is not chemically bound into the polymer matrices of products containing it and can slowly migrate to the surface of polymer products over time, potentially entering environmental media such as air or water. The rate of this migration is expected to be slow and counteracted by chemical and physical attractive forces which work to hold DINP within polymers. Based on high use quantities and widespread distribution in products, DINP is considered to have high potential to be released into the Canadian environment.

Air and water are the primary receiving media for DINP in the environment. DINP released into the environment is predicted to distribute primarily into soil and sediment. It will also associate with suspended particulates in air and in water. DINP adsorbed to air particulates will distribute into soil and surface waters through wet and dry deposition processes. Therefore, sediments, soil and suspended particulates in surface waters represent primary exposure routes for organisms to DINP in the environment.

DINP degrades rapidly under aerobic conditions, but may take longer to break down under low oxygen conditions such as those occurring in sub-surface sediments and soil. However, the substance is not expected to persist in the environment. A lack of persistence indicates that the substance will eventually be removed through degradation and continued releases will dictate the resulting environmental concentrations reported in field monitoring programs. Therefore, organisms in the environment will not be exposed to increasing quantities of the substance over time providing future emissions remain unchanged or decline.

DINP has been measured in all environmental media, indicating that ongoing sources of DINP result in detectable concentrations that reflect the balance of emission inputs and degradation losses. Given the evidence for degradation potential, it is unlikely that DINP will be transported long distances from the point of release and highest organism exposures are therefore expected to primarily occur near discharge sites. The predicted

limited distribution into air, along with a short atmospheric half-life, suggests that DINP will have little potential for long-range atmospheric transport.

The high partition coefficients and low water solubility of DINP indicate that uptake into organisms will occur primarily via the diet. Empirical and modelled data indicate that DINP will have low bioaccumulation potential and low potential to biomagnify through trophic food webs. This suggests that DINP will have little tendency to accumulate in tissues to levels that are high enough to cause adverse effects in the organism, nor will it be likely to transfer between organisms in predator-prey interactions in high enough amounts to cause toxicity. Despite this, DINP has been measured in a variety of aquatic species and this confirms that the substance is able to be taken up by organisms. Highest concentrations are usually associated with lower trophic level species, possibly due to feeding strategies which may include exposure through the ingestion of planktonic organisms and detritus having DINP adsorbed to their external surfaces and filter feeding that can include co-uptake of DINP adsorbed to particulates. Lower rates of metabolism in invertebrates may also contribute to the higher concentrations in these species. As well, lower trophic organisms commonly have reduced metabolic capacities when compared with higher trophic species such as fish. However, DINP has also been detected in some higher trophic species. Given the higher metabolic capabilities of these species, the presence of DINP in these organisms is suggestive of high exposure, possibly from local sources into the environment.

Results from standard laboratory testing indicate low hazard potential in aquatic and terrestrial species. In most cases, no adverse effects have been observed at concentrations up to and exceeding the solubility and saturation limits of DINP. There are preliminary findings which suggest that DINP may be capable of altering the production of steroid hormones in the presence of an endocrine-active substance and under laboratory conditions. However, the potential for DINP to influence normal hormonal function in organisms when in the presence of an endocrine-active substance in the environment has not been established. In light of monitoring data which indicate the potential for continuous exposure to DINP in the environment, the possible influence of DINP on endocrine-active substances requires further exploration.

Results from an analysis of critical body residues (CBRs) derived using the water solubility limit of DINP indicated that maximum tissue concentrations based on solubility limits will be much lower than levels associated with adverse acute or chronic effects in organisms due to neutral narcosis. Similar analyses conducted for DINP in soil organisms indicated that maximum tissue concentrations, calculated from the saturation limit of DINP in a 4% OC soil, do not exceed minimum concentrations estimated to cause narcotic effects. Therefore, while DINP has been measured in Canadian surface waters (no Canadian soil monitoring data are available), it is unlikely that internal body concentrations in exposed organisms will reach levels that are sufficiently high to cause adverse effects. For example, a maximum freshwater concentration of 246 ng/L was reported for DINP downstream of a wastewater treatment system (Sosiak and Hebben 2005) and this corresponds to a CBR in aquatic organisms of  $1.0 \times 10^{-4}$  mmol/kg (see CBR calculation in Ecological Effects Assessment section). As this value falls below the

ranges of 2 to 8 mmol/kg and 0.2 to 0.8 mmol/kg for acute and chronic effects, respectively, aquatic organisms exposed to this concentration in the environment are unlikely to exhibit adverse effects resulting from baseline narcosis. No BSAF data are available for DINP in sediment species and a CBR analysis could not be conducted. However, results from a CBR analysis conducted for a suitable analogue substance, DIDP, suggest that tissue concentrations of DINP in sediment species are unlikely to reach levels predicted to result in acute or chronic effects due to baseline narcosis.

Several studies report the presence of DINP in a number of Canadian aquatic species. McConnell (2007) reported a mean measured concentration of 1600 ng/g ww in spiny dogfish, *Squalus acanthias*, collected from Vancouver BC. This value was converted to CBR units in order to investigate whether tissue levels in the fish were high enough to potentially result in adverse effects attributable to baseline narcosis. The CBR for this tissue concentration is 0.004 mmol/kg (1.6 mg/kg / MW 418.62 g/mol). This value is below the ranges of 2 to 8 and 0.2 to 0.8 mmol/kg attributed to acute and chronic narcotic effects, respectively, suggesting that the fish in the study are not likely to be experiencing adverse narcotic effects due to the presence of DINP in their tissues. DINP was below the detection limit of 1.0 ng/g lw in nine dogfish samples analyzed by Mackintosh et al. (2004) in the same sample area, suggesting that levels of the substance in this species are quite variable.

It should be noted that the CBR analysis does not consider the potential for adverse effects resulting from modes of action other than baseline narcosis. The analysis does not therefore provide a measure of potential for effects from modes of action such as peroxisome proliferation or disruption to normal hormonal function. The structure and chemical properties of DINP suggest that baseline narcosis will be the primary mode of action for this substance.

Estimated total daily intake (TDI) values for DINP in two fish-eating mammalian wildlife species, mink and river otter, were calculated using Canadian monitoring data in order to compare the potential daily ingestion rates for these two species with the lowest LOAEL of 27 mg/kg bw/day reported for the rat (Bio/dynamics 1986; see Human Health Effects Assessment). The procedure was based on the methods of Sample et al. (1996) and considered average body weights for mink and otter (1.08 and 7.98 kg, respectively), as well as the fish BAF of 176 (BCFBAF 2010; see Potential for Bioaccumulation section) and a highest Canadian surface water concentration of 246 ng/L (Sosiak and Hebben 2005; see Measured Environmental Concentrations). Estimated TDIs were  $7.8 \times 10^{-3}$  and  $6.0 \times 10^{-3}$  mg/kg bw/day for mink and otter, respectively, indicating that daily intake rates of DINP would be very low and much lower than the lowest reported LOAEL. Together with the low bioaccumulation potential, this suggests that effects are unlikely to occur in either wildlife species.

### **8.3.2 Uncertainties in Evaluation of Ecological Risk**

Empirical and modelled data indicate that DINP has low bioaccumulation and biomagnification potential. However, DINP has been measured in a variety of aquatic

species, confirming that the substance can be taken up by organisms. Since dietary exposure is the main exposure route for DINP, the measured presence in organisms implies that some degree of trophic transfer is possible. Further research is needed in order to clarify the nature and extent of DINP uptake, as well as the relative roles of environmental exposure and metabolism in determining the ultimate fate of this substance in organisms.

While DINP is considered to present low hazard potential in terms of baseline narcosis based on empirical and modelled data and CBR analyses, there is uncertainty regarding the potential for effects relating to other modes of toxic action. Preliminary data suggest that DINP may be capable of influencing the activity of endocrine-active substances. Further chronic toxicity information, particularly for species with lower metabolic capabilities than fish and therefore potentially higher internal concentrations, would help to more fully address chronic hazard potential.

DINP has substantial use in Canada and additional recent Canadian monitoring data would help to inform the risk assessment on the potential for exposure to organisms in the Canadian environment. In particular, soil monitoring data could be used to further evaluate the potential for risk to organisms in this environmental compartment.

Some physical and chemical properties of DINP are not easily measured by standard laboratory methods and this introduces uncertainty into some of the property values reported in the literature. Special measurement procedures are needed to account for the characteristics of very low water solubility and vapour pressure and high partition coefficients displayed by DINP. For this reason, chemical property values obtained using techniques specifically designed for hydrophobic substances, such as the slow-stir method for determining of water solubility, have received greater weight in the assessment than those derived from procedures which may not account as well for the behaviour of these substances.

## 9. Potential to Cause Harm to Human Health

### 9.1 Exposure

#### Environment media and food

Ambient air, drinking water and soil

No Canadian data were identified for DINP in ambient air. However, DINP has been detected in air samples from Sweden (0.28 to 50 ng/m<sup>3</sup>, see section 8.2.1).

No Canadian data were identified for DINP in drinking water. DINP has been detected in seawater (suspended and dissolved fractions) in False Creek, B.C. (see section 8.2.1). DINP was also found to be present downstream, upstream, and in the effluent of wastewater treatment systems in Alberta (see section 8.2.1).

No Canadian data were identified for DINP in ambient air. DINP has been observed in soil samples in China and Denmark (Liu et al. 2010, see section 8.2.1). In sediment, DINP has been detected in samples from the False Creek and Hamilton areas and its presence in sediment, obtained from point sources in Sweden, has also been observed (see section 8.2.1).

Due to the absence of Canadian and North American background air and soil data, intakes were not estimated from these sources. Additionally, monitoring data pertaining to DINP presence in drinking water were not identified. However, due to its presence in wastewater treatment systems, potential exposure from this source cannot be precluded and is currently an uncertainty in this assessment. It should be noted, however, that the very low water solubility and high hydrophobicity of DINP and high propensity to partition to organic matter in the water column suggest that exposure via water would be unlikely.

Indoor air and dust

DINP, a medium chain phthalate (for the purposes of the health review) with relatively high molecular weight, has been monitored primarily in settled house dust (Bornehag et al. 2005; Abb et al. 2009; Kubwabo et al. 2013). Monitoring as to its presence in gaseous or particle phases was not identified. Concentrations of DINP in house dust are outlined below (Table 9-1).

DINP has applications as a plasticizer in the manufacturing of automobiles and automobile parts (NICNAS 2008a; Environment Canada 2014). For the general population, indirect exposure (e.g., off-gassing) is considered a relevant source, but no data on this exposure source has been identified, which is currently an uncertainty in the assessment.

**Table 9-1. DINP concentration in dust**

Location	Detection frequency	Concentration (µg/g)	Reference
Canada	100% of 126 homes	Median: 112 95 <sup>th</sup> percentile: 527 Range: 3.3 – 1364	Kubwabo et al. 2013
Germany <sup>a</sup>	100% of 30 homes	Median: 129	Abb et al. 2009
Sweden	50% of 346 bedrooms	Median: 0.041 Mean: 0.639 95 <sup>th</sup> percentile: 1.93 Maximum: 40.667	Bornehag et al. 2005

<sup>a</sup> Abb et al. 2009 reported median concentrations of 72 µg/g (Kersten and Reich 2003) and 80 µg/g (Nagorka et al. 2005): publication in German and not evaluated.

The median (112 µg/g) and 95th percentile (527 µg/g) concentrations in the Kubwabo et al. (2013) study were used to estimate daily intake of DINP from dust for the Canadian general population and estimates of exposure for infants 0 to 6 month-old infants (highest exposed group) are 0.57 and 2.7 µg/kg/day, respectively (see Appendix C, Table C).

#### Food, beverages and infant formula

Phthalates may be present in food and beverages through their potential use in PVC tubing and gloves, food packaging films, PVC gaskets for glass jars, and printing inks in food packaging (Fasano et al. 2012) and have been known to migrate into food and beverages (Alin et al. 2011; Barros et al. 2010; Bradley et al. 2007; Gartner et al. 2009; Page and Lacroix 1992; Fierens et al. 2012; Petersen et al. 2010; Xu et al. 2010; Xue et al. 2010).

In Canada, DINP presence in food was monitored as part of the 2013-2014 Canadian Food Inspection Agency (CFIA) Food Safety Action Plan (FSAP) (April 2014 personal communication from the Food Directorate to Existing Substances Risk Assessment Bureau). DINP was detected in 26% of 677 (LOD: 0.2 µg/g) packaged and processed foods sampled. The food groups with the highest frequency of products containing a detectable DINP concentration were frozen meals (60% of 91 samples), oils and fats (41% of 73 samples), and bread (37% of 68 samples) while the highest concentrations measured (after applying a value of zero to samples containing a DINP below the LOD) were in bread (mean: 1.2 µg/g, 95<sup>th</sup> percentile: 4.6 µg/g) and oils and fats (mean: 2.4 µg/g, 95<sup>th</sup> percentile: 11.6 µg/g).

DINP has been monitored in total diet surveys in the United Kingdom and Taiwan. Specifically, DINP was detected in low frequency (detection frequency not provided) in food groups sampled from a total diet survey conducted in the United Kingdom (Bradley et al. 2013a,b). Additionally, it was detected in all food groups (detection frequency not provided) sampled in a total diet survey conducted in Taiwan (Chang et al. 2014). Limits of detection (LOD) for DINP analysis are significantly higher than for other

phthalates, specifically Bradley et al. (2013a) reported LODs of 0.026 – 0.115 µg/g (reported DEHP LOD: 0.006 µg/g). These high LODs are associated with the challenge of measuring DINP isomers in different matrices based on analytical methodologies for DINP showing multiple peaks (Bradley et al. 2013a,b).

Using CFIA surveillance data, and US and UK data to supplement data gaps, probabilistic estimates of dietary intakes were derived for DINP and results are outlined in Table 9-2 below (for methodology for estimating probabilistic intakes, see Appendix D).

**Table 9-2. Probabilistic dietary exposure estimates from DINP presence in food (µg/kg/day)**

Age group	median	90 <sup>th</sup> percentile
< 6 months	N/A	9.15 <sup>a</sup>
6 months – 1 yr	N/A	9.62 <sup>a</sup>
1 – 3 yrs.	1.4	17.77
4 – 8 yrs.	1.33	13.95
M: 9 – 13 yrs.	1.01	11.41
F: 9 – 13 yrs.	0.83	7.72
M: 14 – 18 yrs.	0.79	7.39
F: 14 – 18 yrs.	0.58	5.74
M: 19 – 30 yrs.	0.69	6.91
F: 19 – 30 yrs.	0.44	4.81
M: 31 – 50 yrs.	0.56	6.59
F: 31 – 50 yrs.	0.43	5.69
M: 51 – 70 yrs.	0.53	8.01
F: 51 – 70 yrs.	0.47	7.05
M: > 71 yrs.	0.53	7.34
F: > 71 yrs.	0.52	8.64

<sup>a</sup> These values should be interpreted with caution, coefficients of variation > 16%.

N/A notates that coefficients of variation (> 33%) associated with intake estimates were not sufficiently low to allow for reporting the intake value.

The highest estimates of dietary intake for DINP are for the 1 – 3 year old group with median and 90<sup>th</sup> percentile intakes of 1.40 and 17.77 µg/kg/day, respectively. For adults the highest median estimates of dietary intake are for males 19 – 30 years old (0.69 µg/kg/day) while the highest 90<sup>th</sup> percentile intakes were for females greater than 71 years of age (8.64 µg/kg/day).

Food types that drive consumption of DINP, for infants and adults, are pizza and bread products. Other minor contributors for all age groups are ready to eat meals, vegetable oils, chicken, and butter.

Bradley et al. (2013b) did not report intakes for DINP primarily because they observed very low incidence of DINP in food; however, their limits of detection were significantly higher than the LODs derived for the CFIA analytical method (260 – 1150 times higher).

Chang et al. (2014) measured higher concentrations of DINP in foods, and quantified intakes for DINP from food consumption. For the 0 to 3 year old children the authors reported 50<sup>th</sup> and 95<sup>th</sup> percentile intakes of 1.46 and 7.75 µg/kg/day, respectively. For adults (19 – 65 years old) the authors reported 50<sup>th</sup> and 95<sup>th</sup> percentile intakes of 0.54 and 3.68 µg/kg/day, respectively. Comparison of intakes between the two analyses shows general agreement at the central tendency but differences at the tails of the distribution. This may be due to differences in the methods employed to derive dietary exposure estimates and/or the dietary consumption patterns of the studied populations (Chang et al. 2014 was conducted in Taiwan and likely used consumption figures representative of the Taiwanese population).

### Breast milk

Limited data, regarding presence of DINP and its metabolites in breast milk, were identified. In Canada, analyses of breast milk samples obtained from 56 women in the Plastics and Personal-care Product Use in Pregnancy (P4) cohort survey showed no detection of MINP, the monoester of DINP. Internationally, Fromme et al. (2011) did not observe DINP at levels above the detection limit in breast milk samples obtained from 78 German women while Hogberg et al. (2008) did not detect DINP in breast milk samples obtained from 42 Swedish women. Neither primary nor secondary metabolites of DINP were measured in these surveys (DINP is a high molecular weight medium chain phthalate, thus parent and metabolites would be expected to be detected in milk).

Given the limited data pertaining to breast milk potential exposure to infants via breast milk was not derived.

## Products used by consumers

### Manufactured items

Globally, DINP may also be present in a wide variety of manufactured items, including children's articles, children's toys and textiles (see Uses section: Table 5-1). These uses were confirmed in Canada where DINP was found in various types of manufactured items (e.g., vinyl, flooring, roofing, toys, children's articles, pool liners, interior and exterior appliances) (Environment Canada 2014a).

### Oral exposure

Numerous studies have examined the concentrations of DINP in childcare articles and toys (Stringer et al. 2000; Biedermann-Brem et al. 2007; ECHA 2013a; Babich et al. 2004; US CSPC 2010; Johnson et al. 2011). A summary of Health Canada market surveys of toys and childcare articles are presented in Table 9-3.

**Table 9-3. Percent content of DINP in various toys and childcare articles**

Detection frequency	% Content	Reference
27 of 42 samples	< 0.01 – 44%	Health Canada 1998
36 of 73 samples	Mean: 22.1% Range: ND <sup>a</sup> – 39.9%	Health Canada 2007
4 of 39 samples	<0.03 – 33%	Health Canada 2009
1 of 54 samples	< 0.03 – 20.2%	Health Canada 2012
ND of 117 samples	< 0.0045%	Health Canada 2014

<sup>a</sup> Detection limit not stated.

Migration of phthalates from plastic to saliva depends on many of factors such as saliva composition, duration of contact, type of contact, concentration, and molecular weight (RIVM 2001; Danish EPA 2010a). Recently, ECHA (2013a) has conducted an extensive review of DINP migration rates (*in vivo* and *in vitro*) from numerous studies (ECHA 2013a). Evaluation of these migration rates showed that there is significant variation (e.g., conditions of method, concentration of substance in toys) leading to significant uncertainty in estimates derived.

Previously, several risk assessments have evaluated mouthing exposure to DINP using various migration rates (RIVM 1998; EC 2003; US CPSC CHAP 2001; CHAP 2001). These assessments used migration rates conducted with both *in vitro* and *in vivo* methodology and rates ranged from 7 to 60 µg/cm<sup>2</sup>/hr. More recently, three jurisdictions evaluated DINP and migration rates in these evaluations are outlined below in Table 9-4.

**Table 9-4. Recent migration rates used in DINP risk assessments**

Jurisdiction	Methodology	Migration rate (µg/cm <sup>2</sup> /h)	Reference
US CPSC	NS	Mean: 4.2, 95 <sup>th</sup> percentile: 10.1	CHAP 2013
ECHA	Typical: Average of <i>in vivo</i> migration rates  Reasonable worst case: upper-bound <i>in vitro</i> migration rate	Typical: 14  Reasonable worst case: 45	ECHA 2013a

Jurisdiction	Methodology	Migration rate ( $\mu\text{g}/\text{cm}^2/\text{h}$ )	Reference
NICNAS	<i>In vivo</i>	Typical: 26 Worst case: 58	NICNAS 2012

The migration rates selected by ECHA fall in the middle of migration rates used by these three jurisdictions, and were consequently selected for use in estimating exposure from mouthing a manufactured item for 0 to 18 month old children (see Table 9-5 below). A mouthing time (toys and children's articles) of 30 and 120 min/day was used to estimate exposure, consistent with mouthing time used in estimates derived by ECHA (2013a). Note, migration rates used to estimate exposure were based on concentrations in toys (12.9 – 77%) that are higher than concentrations observed in recent Health Canada surveys (see Table 9-3).<sup>3</sup>

**Table 9-5. Daily exposure estimates from mouthing manufactured items intended for children**

Migration rate ( $\mu\text{g}/\text{cm}^2/\text{h}$ )	Exposure $\mu\text{g}/\text{kg}/\text{day}$ (mouthing time 0.5 h/day) <sup>a,b,c,d</sup>	Exposure $\mu\text{g}/\text{kg}/\text{day}$ (mouthing time 2 h/day) <sup>a,b,c,d</sup>
14 <sup>e</sup>	9.3	37.3
45 <sup>e</sup>	30	120

<sup>a</sup> Surface area of 10 cm<sup>2</sup> mouthed was used to estimate exposure (ECHA 2013a; NICNAS 2012).

<sup>b</sup> Mouthing time obtained from ECHA (2013), which thoroughly evaluated times spent mouthing toys and children's articles.

<sup>c</sup> A body weight of 7.5 kg was used for infants 0 – 6 months. For infants > 6 months to 18 months the same migration rates and mouthing time were used, but, due to higher body weight (15.5 kg), intakes will be lower than above and not presented (< 120  $\mu\text{g}/\text{kg}/\text{day}$ ).

<sup>d</sup> Algorithm: Exposure (per day) = (MR x SA x T)/BW.

<sup>e</sup> ECHA 2013a

## Dermal exposure

DINP has applications in the production of numerous articles and textiles that may come in contact with skin. Examples of these are artificial leather, sheets, general and athletic footwear, sleepwear and sportswear, etc. (see Table 5-1). These potential uses were confirmed in Canada as DINP was reported to be a plasticizer and coating in fabrics

<sup>3</sup> Currently, Canada (along with the US and EU) have regulations (0.1%) in place limiting the amount of certain phthalates (including DINP) in toys and childcare articles.

(e.g., upholstery, artificial leather) and in manufactured items such as children's articles, pool liners etc. (Environment Canada 2014a).

Two recent assessments have evaluated dermal exposure to DINP in plastic articles and provided rationale and parameters (outlined in Table 9-6 below).

**Table 9-6. Parameters to evaluate exposure following dermal contact by plastic articles by recent DINP risk assessments**

Jurisdiction/reference	Population assessed	Exposure parameters	Scenario outline
ECHA 2013a	Infants (0 – 18 months)	<ul style="list-style-type: none"> <li>- Typical surface area (SA) of contact: ½ SA of hands</li> <li>- Reasonable worst case surface area of contact: ½ SA hands + 1/3 full body or ½ SA hands + upper arms</li> <li>- Typical Exposure duration (ED): 3 h/day</li> <li>- Reasonable worst case ED: 3 h/day + 15 min</li> <li>- Migration rate: 0.024 µg/cm<sup>2</sup>/h/4% (% absorption of DINP)</li> </ul>	<ul style="list-style-type: none"> <li>- Typical scenario: child holding a PVC article</li> <li>- Reasonable worst case: child holding a PVC article + child being changed<sup>1</sup>/child playing on a play mat<sup>a</sup></li> <li>- Average DEHP dermal flux (from PVC plastic into skin) in rats used to assess migration onto skin of DINP (Deisinger et al. 1998).</li> </ul>
ECHA 2013a	Adults	<ul style="list-style-type: none"> <li>- Typical surface area of contact: SA of 2 hands (front and back) + 1/3 hands SA</li> <li>- Reasonable worst case surface area of contact: SA of 2 legs</li> <li>- Typical ED: 30 min/day + 2 h/day</li> <li>- Migration rate: 0.024 µg/cm<sup>2</sup>/h/4% (% absorption of DINP)</li> </ul>	<ul style="list-style-type: none"> <li>Typical scenario: wearing gloves all day + holding a steering wheel</li> <li>- Reasonable worst case: wearing faux leather pants 300 min/day (10 h a day, 3 weeks a month)</li> </ul>
US CPSC CHAP 2014	Infants (0 – 18 months)	<p><b>Change pad</b></p> <ul style="list-style-type: none"> <li>- Surface area: 90 cm<sup>2</sup> (buttocks and 1/3 genitals)</li> <li>- Mean ED: 0.08 h/day; upper bound (95%-ile) 0.17 h/day<sup>b</sup></li> <li>- Dermal absorption rate of DEHP: 0.24 µg/cm<sup>2</sup>/h</li> <li>- Dermal absorption rate of DIDP: 1<sup>c</sup></li> </ul>	Migration rate of DEHP (Deisinger et al. 1998).
US CPSC CHAP 2014	Infants (0 – 18 months)	<p><b>Play pen</b></p> <ul style="list-style-type: none"> <li>- Surface area: 60 cm<sup>2</sup> (1/3 hands)</li> <li>- Mean ED: 4.3 h/day; upper bound (95%-ile) 12.6 h/day<sup>b</sup></li> <li>- Dermal absorption rate of DEHP: 0.24 µg/cm<sup>2</sup>/h</li> <li>- Dermal absorption rate of DIDP: 1<sup>c</sup></li> </ul>	Migration rate of DEHP (Deisinger et al. 1998).

Jurisdiction/reference	Population assessed	Exposure parameters	Scenario outline
US CPSC CHAP 2014	Adults (women)	<b>Gloves</b> - Surface area: 900 cm <sup>2</sup> (hands) - ED: 0.011 h/day <sup>d</sup> - Dermal absorption rate of DEHP: 0.24 µg/cm <sup>2</sup> /h - Dermal absorption rate of DIDP: 1 <sup>c</sup>	Migration rate of DEHP (Deisinger et al. 1998).
US CPSC CHAP 2014	Adults (women)	<b>Sitting on a couch</b> - Surface area: 1600 cm <sup>2</sup> (exposed arms/legs) - ED: 4 h/day <sup>e</sup> - Dermal absorption rate of DEHP: 0.24 µg/cm <sup>2</sup> /h - Dermal absorption rate of DIDP: 1 <sup>c</sup>	Migration rate of DEHP (Deisinger et al. 1998).

<sup>a</sup> ECHA (2013a) states that these two scenarios would lead to approximately the same magnitude of exposure, therefore exposure for a child playing with ½ SA + ½ full body in contact with plastic was modelled.

<sup>b</sup> Exposure duration references (O'Reilly 1989; EPA 2011).

<sup>c</sup> US CPSC CHAP (2014) based on professional judgement with no data available.

<sup>d</sup> Average dish detergent use is 107 h/year (O'Reilly 1989; EPA 2011, both cited in US CPSC CHAP 2014).

<sup>e</sup> Time spent sitting while reading or watching television. The prevalence of vinyl-covered furniture is unknown. This is assumed to be an upper bound (US CPSC CHAP 2014).

A limited number of studies are available regarding migration rates of DINP in simulated sweat. Specifically, a survey conducted by the Danish EPA evaluated the presence of DINP in foam washcloths, pillow for baby feeding, baby carriers, nursing pillows, aprons, mattresses, etc. (Danish EPA 2008b). DINP showed a migration, into simulated sweat, (temperature: 37 degrees Celsius, 4-hour duration) of 0.03 µg/cm<sup>2</sup>/4h in samples taken from nursing pillows (Danish EPA 2008b). Additionally, DEHP has also been evaluated for migration, into simulated sweat, from various articles (see Table 9-7).

**Table 9-7. Migration of DEHP, into simulated sweat, from various articles**

Method	Type of Article	Concentration in article (% content)	Migration (µg/cm <sup>2</sup> )	Reference
<i>In vitro</i> , static <sup>a</sup>	Sandals	ND -- 46	ND – 1.7	Danish EPA 2010a
<i>In vitro</i> , static <sup>b</sup>	Balance balls, articles	ND -- 47	ND – 0.38	Danish EPA 2010b
<i>In vitro</i> , static <sup>c</sup>	Pencil cases	NS	0.039	Danish EPA 2007
<i>In vitro</i> , static <sup>c</sup>	School bags, toy bags	NS	0.0098 – 0.011	Danish EPA 2007

Abbreviations: ND, not detected; NS, not specified.

<sup>a</sup> 16-hour duration.

<sup>b</sup> 1-hour duration.

<sup>c</sup> 4-hour duration.

A conservative dermal exposure assessment was conducted using the specific migration rate for DINP and DEHP migration rates as a surrogate (see Table 9-8). Use of DEHP migration rates for DINP is similar to the approach used by NICNAS and ECHA, which have used DEHP dermal flux from a PVC article, as a surrogate, to estimate DINP exposure to various types of PVC articles (see Table 9-6).

Representative scenarios were developed to model exposure of infants in contact with various plastic objects for 1 hour/day with 25% of their body surface area (representative of multiple diaper changes per day on a change pad) and for 4 hours/day with 50% of their body surface area (representative of holding a plastic article and being changed on a plastic change pad multiple times a day and playing on a plastic mat).

Representative scenarios to model exposure of adults, in contact with various plastic articles were also assessed: the first for 3hours/day with 16% of their body surface area (representative of sitting on a couch and wearing plastic gloves), and the second for 3 hours/day with 50% of their body surface area (representative of various daily contacts with plastic articles including wearing plastic gloves, holding plastic steering wheel, sitting on a couch and wearing plastic clothing). Estimated intakes are outlined in Table 9-8.

**Table 9-8. Estimated daily exposures to DINP from dermal contact with plastic articles in two scenarios for infants (0 – 18 months) and adults**

Migration rate ( $\mu\text{g}/\text{cm}^2/\text{h}$ )	Infant (0 – 18 months) exposure $\mu\text{g}/\text{kg}/\text{day}^{\text{a}}$	Adult exposure $\mu\text{g}/\text{kg}/\text{day}^{\text{a}}$
0.03 <sup>b</sup>	0.15 (SA <sup>c</sup> =922 $\text{cm}^2$ ; T <sup>d</sup> =1h) 1.2 (SA=1840 $\text{cm}^2$ ; T=4h)	0.15 (SA=2912 $\text{cm}^2$ ; T=3h) 0.46 (SA=9100 $\text{cm}^2$ ; T=3h)
0.22 <sup>e</sup>	1.1 (SA=922 $\text{cm}^2$ ; T=1h) 8.6 (SA=1840 $\text{cm}^2$ ; T=4h)	1.1 (SA=2912 $\text{cm}^2$ ; T=3h) 3.4 (SA=9100 $\text{cm}^2$ ; T=3h)

<sup>a</sup> Based on the following algorithm: Daily exposure = (MR x SA x T x DA)/BW

Where:

<sup>b</sup> Danish EPA 2008

<sup>c</sup>SA = surface Area

<sup>d</sup>T = contact time

<sup>e</sup>Danish EPA 2007; Danish EPA 2010a, b

DA = dermal absorption 4%, see section 9.2.1, for approach to characterizing dermal absorption to DINP.

BW = body weight (7.5 kg for infants and 70.9 kg for adults), for infants 6 to 18 months same parameters (contact time, surface area) are assumed but body weights are > 7.5 kg.

The average migration rate of 0.22  $\mu\text{g}/\text{cm}^2/\text{h}$  was derived without correcting for experiment duration (e.g., the highest migration rate of 1.7  $\mu\text{g}/\text{cm}^2/\text{h}$  was not averaged over 16 hours). Evaluation of migration rate data show that a majority of phthalates leach out in the first 1 – 3 hours, therefore, dividing the migration rate by 16 hours would lead to underestimation of exposure. Note, this scenario assumes zero dermal lag times and does not account for plasticizer depletion; both of which are conservatisms with the scenario. A dermal absorption factor of 4% was used to estimate the systemic exposure (see section 9.2.1, for approach to characterizing dermal absorption to DINP).

Intakes for infants (0 – 18 months) range between 1.2 – 8.6 µg/kg/day while intakes from adults range between 0.46 – 3.4 µg/kg/day depending on migration rate used to quantify exposure. These intakes are comparable to the internal dermal estimates derived by NICNAS (infants 0 to 6 months: 2.6 – 7.0 µg/kg/day) and ECHA (infants 0 to 18 months: 1.0 – 2.2 µg/kg/day, adults: 1 – 2.0 µg/kg/day). Note intakes derived using the DEHP dermal flux of 0.24 µg/cm<sup>2</sup>/h (as recommended by NICNAS 2012, see Table 9-6) and the upper-end scenario developed above would lead to significantly higher intakes (92 and 253 µg/kg/day for adults and infants respectively). However, these intakes are higher than central tendency biomonitoring intakes outlined for the general population in Tables 9-11, 9-12 and 9-13.

Finally, as is evident from Table 9-6 different approaches are used in terms of surface area, exposure duration, and migration from plastic articles onto skin; all leading to increased variability and uncertainty in deriving dermal exposure estimates. Additionally, the methods used to derive scenarios and migration rates (especially relevant for this substance since limited data is available regarding migration rates into sweat) use professional judgement, thus further reinforcing the uncertainty in derived exposure estimates.

#### Adult toys

No Canadian use of DINP in adult toys was reported under the Section 71 industry survey (Environment Canada 2014a). Based on global use patterns, there is potential for use of DINP in adult toys. Danish EPA 2006e reported phthalate concentrations in 10 of 15 adult toy samples (max: 70%) while a later study reported DINP presence in 18 of 71 articles (range 6 – 77%, VWA 2013). Recently, ECHA 2013a assessed exposure to DINP from these products and derived intakes of 4.8 – 63 µg/kg/day for typical and worst-case exposure estimates (using migration rates derived from Danish EPA 2006e).

#### Medical devices

Globally, DINP is reported to be used in medical applications (ECHA 2014). Therefore, medical devices may be a potential source of DINP exposure. However, due to lack of data and uncertainty in quantifying estimates of exposure from medical devices this exposure source is not quantified and is currently an uncertainty in this assessment.

#### Biomonitoring

DINP metabolism, in humans, has been studied previously (Koch and Angerer 2007, Anderson et al. 2011) and showed good repeatability in fractional urinary excretions (FUE). The FUEs, for selected primary and secondary metabolites, are outlined below (Table 9-9).

#### **Table 9-9. Major Fractional Urinary Excretion (FUE) for DINP primary and secondary metabolites**

Metabolite	Molecular weight	FUE	Reference
Mono-iso-nonyl phthalate (MINP)	292	0.03	Anderson et al. 2011
mono-(carboxy-isoocetyl) phthalate (MCIOP)	322	0.099	Anderson et al. 2011
mono-(7-hydroxy-methyloctyl) phthalate (MHINP)	308	0.11	Anderson et al. 2011
Mono-oxo-isononyl phthalate (MOINP)	306	0.063	Anderson et al. 2011

MINP, the monoester of DINP, has been monitored in Cycle 1 (2009 – 2011) of the Canadian Health Measures Survey (CHMS: with non-detection in majority of samples) (CHMS 2010, 2012). The non-detection of this metabolite may be a function of DINP being primarily metabolized to secondary and tertiary metabolites (MCIOP, MHINP, and MOINP) (Calafat et al. 2011; Gurusankar and Murray et al. 2011; Anderson et al. 2011; Koch et al. 2007). This is corroborated by the National Health and Nutrition Examination Survey (NHANES), which has monitored MCIOP (secondary metabolite) and observed 100% detection (CDC 2014).

Secondary metabolites (MCIOP, MHINP, and MOINP) of DINP were also monitored by Health Canada in two cohort surveys: Plastics and Personal Care Product Use in Pregnancy survey (P4, n = 31 women, 542 individual spot samples, women provided multiple urine samples over two visits) and Maternal/Infant Research on Environmental Chemicals – Child Development Plus study (MIREC-CD Plus, 194 children, 2 – 3 years old, 1 spot sample per individual). Both these surveys reported high detection frequencies (> 90%) of MHINP and MOINP specifically (personal communication from Environmental Health Science and Radiation Directorate [EHSRD] to Existing Substances Risk Assessment Bureau [ESRAB], October 2013, 2014). MCIOP was not evaluated as a result of challenges with the analytical method and does not indicate an absence of MCIOP in samples (DINP is an isomer and metabolites elute as mixtures of isomers: multiple peaks were observed at the time where the calibration standard for MCIOP was observed but since retention times did not match these peaks were not quantified).

Based on the above, biomonitoring data collected by NHANES (CDC 2014), P4, and MIREC-CD Plus datasets were used to generate intake estimates (see Table 9-10 for metabolites used for intake calculations). Intakes were corrected for urine dilution using the creatinine correction method; a commonly used method for phthalate biomonitoring assessment (Fromme 2007; Christensen et al. 2014a; CHAP 2014; Frederiksen et al. 2014). Daily creatinine excretion rates, for participants, were estimated using the Mage

equation and biomonitoring intakes are presented in Tables 9-11 through to 9-13 below (see Appendix E for further information on the methodology).

**Table 9-10. Metabolites used for intake calculations in NHANES and P4 analyses**

Survey used for intake analysis	Metabolite	Total FUE
NHANES <sup>a</sup>	MINP + MCIOP	0.13
P4 <sup>a</sup> , MIREC-CD Plus <sup>b</sup>	MHINP + MOINP	0.18

<sup>a</sup> In the event of non-detects, ½ LOD was imputed in intake calculation.

<sup>b</sup> Machine readings were used for values below the detection limit.

**Table 9-11. 2009-2010 NHANES daily intakes (µg/kg/day), males (using creatinine correction)**

Age group (years)	n	Geometric mean	50th	75th	95th
6 – 11	209	4.6	4.2	8.1	25 <sup>a</sup>
12 – 19	225	3	2.6	5.1	33 <sup>a</sup>
20+	949	2.8	2.4	6.8	24

<sup>a</sup> Relative Standard Error (RSE) > 30%.

**Table 9-12. 2009-2010 NHANES daily intakes (µg/kg/day), females (using creatinine correction)**

Age group (years)	n	Geometric mean	50th	75th	95th
6 – 11	204	3.8	3.6	6.7	26 <sup>a</sup>
12 – 19	189	3.1	2.6	6.1	27 <sup>a</sup>
20+	948	2.3	1.9	4.6	23

<sup>a</sup> Relative Standard Error (RSE) > 30%

**Table 9-13. P4 pregnant women and MIREC-CD plus children (preliminary results), daily intakes (µg/kg/day)**

Age group (years)	N	Arithmetic mean	50th	75th	95th
2 – 3	197	1.4	0.74	1.3	5.2
19 +	31 <sup>a</sup>	2.0	0.62	1.4	5.6

<sup>a</sup> n = 31 women, 542 individual spot samples, women provided multiple urine samples over two visits.

The highest exposed group (all sources, NHANES) is 6 – 11 year old male children with median and 95<sup>th</sup> percentile intakes of 4.2 and 25 µg/kg/day respectively. For older populations, the highest exposed group (all sources, NHANES) is 12-19 year old males with median and 95<sup>th</sup> percentile intakes of 2.6 and 33 µg/kg/day respectively.

## 9.2 Health Effects

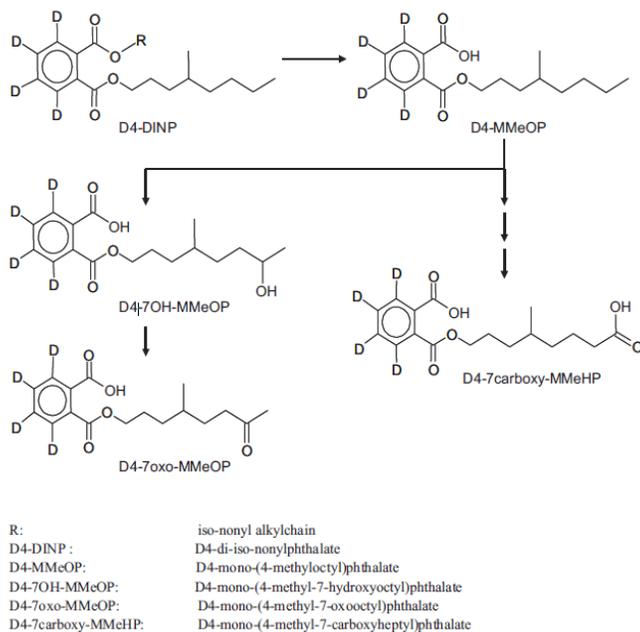
### 9.2.1 Toxicokinetics of DINP

#### 9.2.1.1 Oral route

Few studies have examined DINP kinetics in rats. Total radioactivity in feces, urine, blood, and several tissues of rats were measured in a study in which male and female rats were administered [<sup>14</sup>C]DINP orally (50, or 500 mg/kg bw/day for 1 day or 50, 150 or 500 mg/kg bw/day for 5 consecutive days). In tissues, radioactivity was mainly recovered in the GI tract, liver, and kidney. Following single dosing, it was noted that absorption decreased as dose increased (49% of the low dose of 50 mg/kg bw/day and 39% of the high dose of 500 mg/kg bw/day was eliminated in urine over 72 hours, while the proportion in feces increased slightly (50% vs. 52% of the low and high dose, respectively) (McKee et al. 2002). Clewell et al. (2013) also observed that the percentage of absorbed dose of DINP decreased at higher doses of 750 mg/kg bw/day compared to 250 mg/kg bw/day in rats administered DINP via gavage from GD 12 to 19.

McKee et al. (2002) have suggested that DINP is mainly hydrolyzed in the GI tract after oral administration. Oxidative metabolites and phthalic acid were the major urinary metabolites detected and the authors determined that of the ~40 – 50% of the administered dose excreted in urine in rats, 78 – 85% was recovered as oxidation products, and 9 – 21% as phthalic acid. The remaining ~40 – 50% was recovered in feces (46 – 67% of which as DINP, 19 – 21% as MINP) depending on repeat or single dose and time of recovery of sample between 1h and 72 hours (McKee et al. 2002).

In a study in which one human volunteer was given one dose of 1.27 mg DINP-2/kg-bw, it was noted that similar to rats, DINP is rapidly distributed and eliminated in humans (Koch and Angerer 2007). Approximately 44% of the administered dose was recovered in urine over 48 hours in the form of the following metabolites: 20.2% as OH-MINP (MHINP; based on measured standard of 7OH-MMeOP), 10.7% as carboxy-MINP (MCIOP; based on measured standard of 7-carboxy-MMeHP), 10.6% as oxo-MINP (MOINP; based on measured standard of 7oxo-MMeOP) and only 2.2% as MINP (Koch and Angerer 2007). (see Figure 9–1 below for postulated metabolic pathway).



**Figure 9-1. Suggested DINP metabolism in humans postulated by Koch and Angerer (2007).** This figure show the metabolism of DINP labelled four times with deuterium in the benzene ring position as orally dosed in the study.

Anderson et al. (2011) also studied the kinetics of DINP (CAS not identified; labelled with deuterium) in 10 male and 10 female Caucasian adult volunteers after oral exposure. Two dose levels were used: 0.78 mg (0.010 mg/kg-bw for males and 0.011 mg/kg-bw for females) and 7.3 mg (0.090 mg/kg-bw for males and 0.107 mg/kg-bw for females). A recovery of 33 +/- 6.4% of the labelled DINP was calculated in urine measurements of four metabolites over 48 hours (the same metabolites as in Koch and Angerer). Metabolite half-lives were estimated to be 4 – 8 hours with over 90% excreted in the first 24 hours of urine collection (Anderson et al. 2011; ECHA 2013a).

**Table 9-14. Summary of oral absorption percentages for DINP**

Species	Dose <sup>a</sup>	Basis	Absorption (% of dose)	Reference
Human	1.28 mg/kg	Urine	At least 44% over 48 h	Koch and Angerer 2007)
Human	0.78 and 7.3 mg	Urine	33 +/- 6.4% in 48 h	Anderson et al. 2011
Rat	50 mg/kg	Urine	49% over 72 h	McKee et al. 2002
	500 mg/kg	Urine	39% over 72 h	
	50-500 mg/kg	Estimated urine+bile	75% over 72 h	
	Daily 50, 150 or 500 mg/kg	Urine	56-62% over 24 h,	
		Estimated urine+bile	62-64% over 72 h	
			90% over 72 h	

<sup>a</sup>For humans, doses provided were converted in mg/kg to allow comparison with other species. The body weight used (not provided in the studies) was arbitrarily set to 50 kg.

Silva et al. (2006) characterized the different oxidative metabolites found in urine after administration of a single dose of 300 mg DINP/kg-bw to non-pregnant rats. Similar to DEHP, oxidative metabolism appears to be primarily the product of  $\omega$ - and  $\omega$ -1-oxidation on the side chain esters. MCIOP was the major metabolite recovered while MINP and DINP were not found in significant amounts in the urine (Silva et al. 2006). Clewell et al. (2013) determined that MCIOP was also the most abundant metabolite (76 – 81% of the urine metabolites) in rats exposed via gavage to up to 750 mg DINP/kg-bw per day during GD 12 – 19 while MINP and its glucuronidated form (MINP-Gluc) were almost negligible. Since all metabolites were present in maternal plasma and MINP was present at similar concentrations as MCIOP, it was suggested that urinary clearance of both MINP and MINP-Gluc is limited and that these urine metabolites were poor predictors of plasma and tissue disposition for DINP (Clewell et al. 2013).

Clewell et al. (2013) also characterized the metabolite disposition of DINP in the fetus and it was revealed that MINP and its oxidative metabolites along with its glucuronidated form were all present in the fetal plasma, testes and amniotic fluid. MINP-Gluc was present at higher concentrations in the fetal plasma than the maternal plasma (in contradiction with what was observed with the other metabolites), indicating potential placental transfer of this form or, more likely, that conjugation could occur in the fetus (Clewell et al. 2013).

See Table 9-15 for a summary of the different metabolites found in rats and humans urine exposed orally to DINP.

**Table 9-15. Metabolites found in rats and humans urine after oral administration of DINP**

Metabolites	Abbreviation	Reference (species)
Monoisobutyl phthalate	MINP	Anderson et al. 2011 (human) Suzuki et al. 2012 (human) <sup>a</sup> Koch and Angerer (2007) (human) Calafat et al. 2006 (rat)
Glucuronidated form	MINP-Gluc	Clewell et al. 2013 (rat)
[mono-(4-methyl-7-carboxyheptyl) phthalate] representing: Mono(carboxyisooctyl) phthalate	[D4-7carboxy-MMeHP] CO <sub>2</sub> -MINP; MCIOP	Anderson et al. 2011 (human) Koch and Angerer (2007) (human)
[D4-mono-(4-methyl-7-hydroxyoctyl) phthalate] representing: Mono(hydroxyisononyl)	[7OH-MMeOP] for OH-MINP; MHINP	Anderson et al. 2011 (human) Schulz et al. 2012 (human)

phthalate		Koch and Angerer (2007) (human) Silva et al. 2006 (rat)
[D4-mono-(4-methyl-7-oxooctyl)phthalate] representing: Mono(oxoisooctyl) phthalate	[7oxo-MMeOP] For Oxo-MINP; MOINP	Anderson et al. 2011 (human) Schulz et al. 2012 (human) Koch and Angerer (2007) (human) Silva et al. 2006 (rat)
monocarboxylisononyl phthalate	cx-MINP	Schulz et al. 2012 (human)
Mono-carboxy-isooctyl phthalate	MCiOP	Silva et al. 2006 (rat)
Mono(carboxy-isoheptyl) phthalate	MCiHpP	Silva et al. 2006 (rat)
Mono-(3-carboxypropyl) phthalate	MCP	Calafat et al. 2006 (rat)
Mono-n-octyl phthalate	MnOP	Calafat et al. 2006 (rat)
Phthalic Acid	PA	McKee et al. 2002 (rat)

<sup>a</sup> Measurements of metabolites in humans are from an epidemiological study measuring phthalate metabolites in urine, not after specific administration, but shows that these metabolites are found in humans as well.

### 9.2.1.2 Inhalation route

No data were identified in the literature for DINP.

### 9.2.1.3 Dermal route

Data obtained from *in vivo* and *in vitro* studies have shown that absorption of phthalates through rat and human skin decreases as the length of the alkyl chain increases (Scott et al. 1987; Elsisi et al. 1989; Mint and Hotchkiss 1993; Mint et al. 1994).

DINP is not absorbed well through skin. In McKee et al. (2002), rats were exposed dermally to DINP (topical administration of <sup>14</sup>C-DINP; 1.2 ml/kg; skin not washed after exposure) and radioactivity recovered from the application site was ≥92%. Dermal absorption was estimated to be 2 – 4% over 7 days based on amount of applied-dose recovered in urine, faeces and tissues. Furthermore, the study showed that radioactivity increased with time in skin (0.12%, 0.26% and 0.27% of the applied-dose 1, 3, and 7 days after exposure, respectively). The levels recovered in other tissues, 7 days after exposure, were as follows: GI tract (0.1%) > fat (0.05%) > muscle (0.024%) > other organs. The fractions of applied-dose recovered in tissues were higher at a lower dose (0.6 ml/kg; skin (0.78%) > GI tract (0.13%) > muscle (0.09%) > fat (0.015%) > other organs (McKee et al. 2002). However, the total percent absorbed at the low dose (3%) did not differ significantly from the high dose (4%), indicating absence of the overloading effect on the “mismeasure of dermal absorption” as described by Kissel (2011).

Based on this study, it is expected that no greater than 4% is dermally absorbed in rats. While there is no data on the dermal absorption of DINP in humans, it was recognised by various agencies (Danish EPA, ECHA, NICNAS) that absorption of phthalates is lower in human skin than through rat skin. This is specifically based on data from *in vitro* migration studies conducted with DEHP and other phthalates (Scott et al. 1987; Barber et al. 1992; Mint and Hotchkiss 1993). Therefore, it is also considered appropriate to consider a dermal absorption of 4% for humans. Similar values were chosen by NICNAS and ECHA in their assessment report on DINP (NICNAS 2008a; ECHA 2013a).

## 9.2.2 Health Effects of DINP

### 9.2.2.1 Reproductive and developmental effects in males

Based on its structure, physicochemical and toxicological properties, DINP is considered to be part of the medium chain phthalates (for the purposes of the health review) (Health Canada 2015a). A critical effect of medium-chain phthalates consists of adverse effects on the development of the male reproductive system following exposure. Exposure to these phthalates during the critical development window of gestation have been shown to result in disturbances in androgen-mediated development of the reproductive system in male rats, with the biological pathways leading to common effects or adverse outcomes in reproduction. The effects detected in early postnatal life include altered feminization parameters such as decreased anogenital distance (AGD) and areolar/nipple retention (NR) in juveniles (Gray et al. 2000).<sup>4</sup> Other effects observed include reproductive tract malformations (cryptorchidism [CRY], hypospadias [(HYP], and testicular pathological changes) and effects on sperm counts, motility, and quality at adulthood (Gray et al. 2006). This spectrum of effects on male reproductive development has been described as the “rat phthalate syndrome” (RPS) and although primarily studied in rats, it has also been demonstrated in other species (reviewed in NAS 2008).

Conceptually, the effects associated with RPS can be divided into three subsets with different mode of action considerations. The first subset of effects is related to androgen insufficiency (decreased testicular testosterone production) in the fetal rat and is caused by altered functioning of Leydig cells. The second subset of phthalate syndrome effects has also been attributed to altered functioning of Leydig cells; however, the effects are separate from the role that testosterone plays in development. *Ins13* gene expression is

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<sup>4</sup>AGD in newborn male rats is a biomarker of androgen exposure during development. Alterations in AGD result from the disruption of testosterone signalling during fetal development. Nipple regression in male rats is also androgen-dependent (Kratochwil 1977; Imperato-McGinley et al. 1986) and retention of these nipples/areolae in males further demonstrates disruption in androgen-mediated development.

reduced and is attributed to a second proposed mode of action for cryptorchidism (McKinnell et al. 2005; Wilson et al. 2004). Finally, the third subset of phthalate syndrome effects has been linked to altered functioning of Sertoli cells in the fetal testes. Certain phthalates can also affect Sertoli cell *in utero* and may result in altered Sertoli-germ cell interactions leading to multi-nucleated gonocytes (MNG) (Kleyменова 2005). The long-term biological significance of fetal MNGs is not clearly understood (Clewell et al. 2013). For a more detailed consideration of the current knowledge of the mode of action of phthalate-induced toxicity is described in the Category Approach Document (Health Canada 2015a).

This section is structured to present the evaluation of developmental and reproductive toxicity studies at three different life stages (gestational exposure [GD0-21], (pre)pubertal-pubertal [PND1-55], and adult [PND55+]) with particular focus on the male gender.<sup>5</sup> This will inform identification of the most sensitive life stage of DINP toxicity for risk characterization, as appropriate. Descriptions of effects within each life stage are structured to present a summary of effects starting from the lowest doses at which these effects were observed. Adverse effects observed subsequent to *in utero* exposure to DINP (section 9.2.2.1) are further organized and presented as follows: 1) changes in hormone levels (serum or testicular); 2) feminization effects; 3) reproductive tract malformations and/or effects on fertility; and 4) other developmental effects.

The potential reproductive developmental effects of DINP in female animals were also assessed in a similar manner in considering life stage and species sensitivity.

#### **9.2.2.1.1 Early development: *in utero* exposure**

A literature search identified 10 studies examining the effects of DINP when administered during gestation in pregnant rats during the foetal masculinization programming window (gestational days [GD] 15 – 17) (see Table 9–1). Both types of DINP identified in the grouping have been evaluated (DINP-1 with CAS RN 68515-48-0 and DINP-2 with CAS RN 28553-12-0). No studies examining the potential effects of gestational exposure to DINP were identified in any other species. Summaries of the studies are described in Table 9-16 below.

*In utero* oral exposure to DINP in rats has been reported to cause some effects in the developing male foetus related to RPS and depending on the endpoint, at higher doses compared to more potent anti-androgenic phthalates such as DEHP and further, not all effects related to this syndrome are observed. These effects include decreased testicular and serum testosterone levels, decreased AGD, NR, effects in sperm, and evidence of testicular pathology.

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<sup>5</sup> The evaluation of all toxicological information currently available was not limited to RPS endpoints in males alone, but also included review of all potential effects of phthalate exposure in both sexes at all life stages.

Perturbations of steroidogenesis have been reported as decreases in serum and testicular testosterone levels when measured post birth after *in utero* exposure to DINP. Both forms of DINP (1 and 2) were shown to reduce foetal testicular testosterone levels and/or production at close to the end of gestation, in some cases as much as 50%, immediately after cessation of exposure to DINP at 250 mg/kg bw/day and above (Borch 2004; Boberg et al. 2011 [no-dose response, *ex vivo*<sup>6</sup>]; Hannas et al. 2011 [*ex vivo*], 2012; Clewell et al. 2011a in ECHA 2013a; Furr et al. 2014 [*ex vivo*]). Studies in which hormone levels were measured 24 hrs or several days after cessation of exposure did not show significant perturbations in testicular testosterone levels and when measured, serum testosterone levels in treated animals were similar to controls (Lee et al. 2006; Adamsson et al. 2009; Clewell et al. 2011b in ECHA 2013a; Clewell et al. 2013). Borch and colleagues (2004) reported a non-significant decrease in serum testosterone levels at 750 mg/kg bw/day of DINP-2 in newborn male rats while others found that serum hormone levels were not distinguishable between exposed and control newborn males using both DINP-1 or DINP-2 (Gray et al. 2000; Boberg et al. 2011).

Several recent studies have also used the rat model to examine the effects of gestational exposure to DINP on steroidogenesis with focus on important genes involved in cholesterol transport and the transformation of cholesterol to testosterone. Hannas et al. (2011, 2012) treated pregnant Sprague-Dawley rats via gavage daily during GD 14-18 with 0 to 1500 mg/kg bw/day of DINP. Testes were isolated on GD 18 and gene expression was determined through RT-PCR. DINP reduced expression of the relevant genes in the steroid biosynthesis pathway in a dose-dependent manner at the lowest dose tested (500 mg/kg bw/day and above). No effects on expression of similar genes were reported in a previous study, but measurements were made 2 days after the last dose, limiting the interpretation of the results (Adamsson et al. 2009).

Effects related to feminization indicative of RPS were examined in a number of studies and results are varied depending on method and timing of measurement (see column 3, “feminization parameters” in Table 9-16 below). There were no observed effects on AGD in male pups immediately post-birth (PND2-4) after gestational exposure to DINP-1 (Gray et al. 2000; Clewell et al. 2011a in ECHA 2013a; Clewell et al. 2013), but decreased AGD was reported when measured at PND14 at the highest dose tested (750 mg/kg bw/day, Clewell et al. 2013). According to Boberg et al. (2011), DINP-2 also causes a decrease in measured AGD in newborn males, at a dose of 900 mg/kg bw/day. One study reported statistically significant decreases in AGD at all dose levels (~2 to 1000 mg/kg bw/day) of males measured on PND1 after gestational exposure to

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<sup>6</sup> In typical *ex vivo* testicular testosterone production assays, the fetal testes of offspring are collected on GD18 or 19 and incubated in media (*ex vivo*) for a period of two to three hours. Testosterone is then measured in the media either through radioimmunoassay (RIA) or turbulent flow liquid chromatography coupled with tandem mass spectrometry (TFC-MS/MS).

DINP-2 in diet (Lee et al. 2006). There were limitations in this study based on the analysis of AGD being calculated on a per pup basis versus per litter basis which would potentially mask a litter effect (i.e., all pups with decreased AGD being from one or two litters) and the decrease in distances were very minor (ECHA 2013a). Further, this study does not appear to coincide with other studies examining this endpoint at similar doses at the same time point.

Other results related to feminization indicative of RPS are increased nipple retention (NR) and delay in preputial separation (PPS). Two studies reported either no effect or non-significant increased incidences of males with retained nipples at the highest doses tested (up to 750 mg/kg bw/day, Clewell et al. 2011b in ECHA 2013a; Clewell et al. 2013) while two other studies found increased NR or areolas with/without nipples at and above 750 mg/kg bw/day (Gray et al. 2000; Boberg et al. 2011). DINP also did not appear to cause delays in PPS with only one study finding 1 male with delayed PPS at the highest dose tested (750 mg/kg bw/d, Clewell et al. 2013) while others did not observe any delays in this developmental parameter (Gray et al. 2000 at PND 28; Masutomi et al. 2003).

Reproductive tract malformations such as undescended testes (CRY) and hypospadias (HYP) were either not observed or observed, but not consistently, after gestational exposure to DINP-1 at relatively high dose ranges (750 mg/kg bw/d, Gray et al. 2000; Clewell et al. 2013). No studies were identified that measured these parameters with DINP-2. See Column 4, “reproductive tract malformations” in Table 9-16 below.

Examinations of the testes in males exposed to either form of DINP *in utero* revealed no permanent, significant changes in reproductive organ weights (ROW) (see Column 4, Table 9-15 below), but histopathological effects such as increased number of multinucleated gonocytes (MNGs) starting as low as 250 mg/kg bw/day and above were observed (Boberg et al. 2011; Clewell 2011b in ECHA 2013a; Clewell et al. 2013) as well as increased number of large Leydig cell aggregates at this dose and above (Clewell 2011b in ECHA 2013a; Clewell et al. 2013). These effects increased in severity with dose compared to controls. At higher doses of 750 mg/kg bw/day there were observations of testicular atrophy (Gray et al. 2000), males with small testes and epididymides (Boberg et al. 2011), and evidence of degeneration of Sertoli cells at PND77 at the highest dose tested (1164 – 2657 mg/kg bw/d; Masutomi et al. 2003).

The effect of gestational exposure to DINP on fertility, whether measured by sperm parameters at a young age or by reproductive success as adult males, was examined in a limited number of studies (see Column 4, Table 9-16). Although no studies were performed to determine the effects of DINP-1 on sperm parameters, effects in spermatogenesis (hypospermatogenesis) were observed in one male at 750 mg/kg bw/day (Gray et al. 2000). In a two-generation study, Waterman et al. (2000) did show that the reproductive success of both the adult F0 and F1 males was not affected by gestational and continued life time exposure to DINP-1 at doses up to 1100 mg/kg bw/day in mating tests. A study using DINP-2 revealed that gestational exposure (GD7-PND17) at doses of 600 mg/kg bw/day and above caused decreased percentages of

motile sperm in males examined on PND90 (Boberg et al. 2011). In the same study, there was a statistically significant increase in sperm count at the highest dose tested (900 mg/kg bw/d). Masutomi et al. (2003) reported degeneration of meiotic spermatocytes at stage XIV on PND77 at relatively high doses of 1164 – 2657 mg/kg bw/day.

Effects not necessarily associated directly with RPS, but indicative of adverse effects related to gestational exposure to DINP, included decreased pup body weight (BW), foetal viability (FV), and skeletal variation (ESV) (see Table 9-16). Pup weights were significantly decreased at birth and at various time points up to PND21 after cessation of exposure to DINP-1 in the diet at doses of 159-359 mg/kg bw/day and above (Waterman et al. 2000; Masutomi et al. 2003; Clewell et al. 2013). In the more recent study by Clewell et al. (2013), authors attributed the reduced pup weight in PND14 pups to reduced palatability of milk. In the same study, measured plasma levels of MINP and MHINP in pups on PND2 was “low” compared to foetal concentrations and authors suggested that the pup-dose of DINP from suckling is likely to be very low. This could indicate reduced palatability of the milk. In both studies, slight maternal toxicity (decreased body weight and/or food consumption) was observed only at the highest doses tested. When administered via gavage, no effects in pup weight were observed at doses up to and including 750 mg/kg bw/day in Clewell et al. (2011b), but noted at 750 mg/kg bw/day by Grey et al. (2000) with modest reductions in maternal weight gain (body weights were not significantly reduced at any time).

Evidence of increased incidence of skeletal variations was observed in studies that exposed maternal rats to DINP during early gestation (GD6 – 15) at 500 mg/kg bw/day and above (NOAEL = 100 mg/kg bw/d; Waterman et al. 1999; NTP-CERHR 2003).

**Table 9-16. Effects from gestational exposure to DINP in male offspring (mg/kg bw/day)**

<b>Strain and species; Dose (mg/kg bw/day); Route; Duration (reference) CAS RN</b>	<b>Testosterone levels<sup>a</sup> (T, S)</b>	<b>Feminization parameters<sup>b</sup></b>	<b>Reproductive tract malformations and/or fertility<sup>c</sup></b>	<b>Other developmental parameters<sup>d</sup></b>	<b>Maternal effects</b>
SD Rats; 0, 0.2, 0.4, 0.8%, est. F0 (gestation): 0, 133 – 153, 271 – 307, 543 – 577 (postpartum): 0, 159 – 395, 347 – 758, 673 –	NM	NP(AGD) NM (NR) NP (PPS)	NP (CRY) NP (HYP) NE (TP) NE (FER-mating test)	159 – 395 <sup>e</sup> (10%, BW-PND21) NP (ROW) NE (FV) NM (EMB) NP (ESV)	LOEL= 159 – 395 <sup>e</sup> (↑ kidney wt; liver wt @ 347 – 750); 673 –

<b>Strain and species; Dose (mg/kg bw/day); Route; Duration (reference) CAS RN</b>	<b>Testosterone levels<sup>a</sup> (T, S)</b>	<b>Feminization parameters<sup>b</sup></b>	<b>Reproductive tract malformations and/or fertility<sup>c</sup></b>	<b>Other developmental parameters<sup>d</sup></b>	<b>Maternal effects</b>
1541 by EURAR (2003); diet; 10 wks to prior to mating – PND 21 (Waterman et al. 2000) DINP-1 68515-48-0					1541 (↓ BW on PND14, 21)
SD Rats; 0, 0.2, 0.4, 0.8%, est. F1 (gestation): 0, 133 – 153, 271 – 307, 543 – 577 (postpartum): 0, 159 – 395, 347 – 758, 673 – 1541 by EURAR (2003); diet; 10 wks prior to mating – PND 21 (Waterman et al. 2000) DINP-1 68515-48-0	NM	NP(AGD) NM (NR) NP (PPS)	NP (CRY) NP (HYP) NE (TP) NE (FER-mating test)	347 – 758 (BW – PND7, 14, 21) NP (ROW) 347 – 758 <sup>NDR</sup> (FV - PND7) NM (EMB) NP (ESV)	LOEL= 673 – 1541 (↑ liver wt, ↓ BW)
SD Rats; 0, 50, 250, 750; gavage; GD12 – 19 (Clewell et al. 2011a in ECHA 2013a) DINP-1 68515-48-0	250 (T-GD19: 2 hrs after), NE (, T-24 hrs) NM (S)	NE (AGD-PND1) NM (NR) NM (PPS)	NM (CRY) NM (HYP) 250 (TP-MNGs) NM (FER)	NE (BW-GD19) NP (ROW) NM (FV) NM (EMB) NM (ESV)	LOEL= 250 (↑ liver wt)

<b>Strain and species; Dose (mg/kg bw/day); Route; Duration (reference) CAS RN</b>	<b>Testosterone levels<sup>a</sup> (T, S)</b>	<b>Feminization parameters<sup>b</sup></b>	<b>Reproductive tract malformations and/or fertility<sup>c</sup></b>	<b>Other developmental parameters<sup>d</sup></b>	<b>Maternal effects</b>
SD Rats; 0, 760, 3800, 11400 ppm, est. 0, 50, 250, 750; diet; GD12 – PND14 (Clewell et al. 2013) DINP-1 68515-48-0	NE (T-PND49, large variation) NM (S)	NE (AGD, PND2); 750 (PND14); NE (PND49) 750 <sup>NS</sup> (NR-increasing trend) 750 <sup>NS</sup> (PPS-one animal)	750 <sup>NS</sup> (CRY- 2 males at high dose) 750 <sup>NS</sup> (HYP- 2 animals at PND49, 1 male from same litter at 56 and 288) 250 (TP-MNGs, LC aggregates) NM (FER)	250 <sup>f</sup> (10%, BW) (@PND14; 750@PND2) NE (ROW) NE (FV) NM (EMB) NM (ESV)	LOEL= 750 (↓BW, ↓food consumption) @250 (↓Food consumption, but not BW PND2 – 14)
SD Rats; 0, 0.5, 1, 1.5%, est. 0, 360-923, 734 – 1731, 1087 – 2246 by EU RAR (2003) ; diet; 10 wks prior to mating – PND21 (Waterman et al. 2000) DINP-1 68515-48-0	NM	NM	NM (CRY) NM (HYP) NM (TP) NE (FER-mating test)	390 <sup>e</sup> (BW-PND0, PND14, PND21) NP (ROW) 1100 (FV - PND4) NM (EMB) NP (ESV)	LOEL= 390 <sup>e</sup> (↑ liver wt, ↓ BW@760 on PND10 – 21)
Wistar Rats; 0, 750; Gavage; GD14 – PND3 (Gray et al. 2000) DINP-1 68515-48-0	NM (T) NE (S-PND3)	NE(AGD-PND2) 750 <sup>NS</sup> (NR-2/52 adult); 750 (areolas with or without nipples) NE (PPS-PND28)	NE (CRY) NE (HYP) 750 (TP- small and atrophic testes, fluid filled testes lacking sperm) NM (FER)	750 (BW) NE (ROW) NE (FV) NM (EMB) NM (ESV)	LOEL= 750 (↓ BW gain until GD21 only)

Strain and species; Dose (mg/kg bw/day); Route; Duration (reference) CAS RN	Testosterone levels <sup>a</sup> (T, S)	Feminization parameters <sup>b</sup>	Reproductive tract malformations and/or fertility <sup>c</sup>	Other developmental parameters <sup>d</sup>	Maternal effects
SD Rats; 0, 500, 750, 1000, 1500; gavage; GD14 – 18 (Hannas et al. 2011) DINP-2 28553-12-0	500 (↓T- <i>ex vivo</i> ) NM (S)	NM	NM	NM	NE
Wistar Rats; 0, 300, 600, 750, 900; Gavage; GD7 – PND17 (Boberg et al. 2011) DINP-2 28553-12-0	600 <sup>NDR</sup> , 900 (↓T- <i>ex vivo</i> GD21) NE (S)	900 (AGD-PND1) 600 <sup>NS</sup> , 750 (NR,PND13) NP (PPS)	NP (CRY) NM (HYP) 300 <sup>NS</sup> , 600 (TP- MNGs GD21, NE PND90) 600 (FER PND90- sperm motility; ↑ count)	900 (BW) NE (ROW-PND90) NE (FV) NE (EMB) NE (ESV)	NE
Harlan SD Rats; 0, 750; GD14 – 18 (Furr et al. 2014)	750 (T) NM (S)	NM	NM	NM (BW) NM (ROW) NE (FV) NM (EMB) NM (ESV)	NE
Wistar Rats; 0, 750; Gavage; GD7 – PND17 (Borch et al. 2004) DINP-2 28553-12-0	750 <sup>e</sup> (T) 750 <sup>NS</sup> (S)	NM	NM	NM	NM
SD Rats; 0, 250, 750; Gavage; GD13.5 – 17.5 (Adamsson et al. 2009 <sup>9</sup> ) CAS not defined	750 <sup>NS</sup> (T) NE (S)	NM	NM (CRY) NM (HYP) NE (TP) NM (FER)	NE (BW) NM (ROW) NM (FV) NM (EMB) NM (ESV)	NE

Strain and species; Dose (mg/kg bw/day); Route; Duration (reference) CAS RN	Testosterone levels <sup>a</sup> (T, S)	Feminization parameters <sup>b</sup>	Reproductive tract malformations and/or fertility <sup>c</sup>	Other developmental parameters <sup>d</sup>	Maternal effects
SD Rats; 0, 400, 4000, 20000 ppm, est. 30 – 66, 307 – 657, 1164 – 2657; Diet; GD15 – PND10 (Masutomi et al. 2003) DINP-2 28553-12-0	NM	NE (AGD-PND2 absolute values only) NM (NR) NE (PPS)	NM (CRY) NM (HYP) 1164-2657 (TP-degeneration of Sertoli cells at PND77) 1164 – 2657 (FER-degeneration of stage XIV meiotic spermatocytes, at PND77)	NE, 1164-2657 <sup>h</sup> (BW-PND2, PND21) 1164 – 2657, NE (ROW-PND27, PND77) 1164 – 2657 <sup>NS</sup> (FV) NM (EMB) NM (ESV)	LOEL= 1164 – 2657 (↓BW, ↓ food consumption.)
Wistsar-Imamichi Rats; 0, 40, 400, 4000, 20000, est. 0, 2, 20, 200, 1000 (HC 1994); Diet; GD15 – PND21 (Lee et al. 2006) DINP-2 28553-12-0	NM (T) NE (S-PND7, PNW20)	2 <sup>e,i</sup> (AGD) NM (NR) NM (PPS)	NM	2 <sup>e,i</sup> (BW) NM (ROW) NM (FV) NM (EMB) NM (ESV)	NM

Abbreviations: NP, results not recorded (but measurement was stated in the methods and materials); NM, not measured; NE, no effect observed at the dose range tested. When NE is presented alone in the first 4 columns of effects, all parameters in the footnote description were measured and no statistically significant effects were observed in the endpoints at the dose range administered; NS, not statistically significant; NDR, No dose relationship; MNG, multinucleated gonocytes

<sup>a</sup> Testosterone levels measured (can include quantity/production) at varying days post-birth. T=Testicular testosterone; S=Serum testosterone.

<sup>b</sup> Feminization parameters can include anogenital distance (AGD), nipple retention (NR), preputial separation (PPS).

<sup>c</sup> Malformations include: cryptorchidism (CRY), hypospadias (HYP), testicular pathology (TP), and/or reproductive effects such as fertility (FER) in offspring (sperm number, motility) or reproductive success at adult stage after *in utero* exposure. TTM = transabdominal testicular migration.

<sup>d</sup> Other developmental effects include: decreases in overall foetal body weight at PND 1 (BW), decreases in reproductive organ weight (ROW), foetal viability (FV), embryotoxicity (EMB), or on the incidence of external, skeletal or visceral malformations (ESV).

<sup>e</sup> Lowest dose tested in the study.

<sup>f</sup> Clewell et al. (2013): Reduced pup weight is attributed to reduced palatability of milk and feed of the PND 14 pups. Authors concluded that in this study, there was no evidence of the rat phthalate syndrome with DINP at doses up to 11,400 ppm (~750 mg/kg-day).

<sup>g</sup> Treatment of DINP from GDs 13.5 to 17.5, and offspring were necropsied at GD 19.5. Authors suggest that, based on the lack of response in expression of steroidogenic genes, steroidogenesis may have recovered during two-day treatment delay prior to necropsy.

<sup>h</sup> Masutomi et al. (2003): At PND27, body weights of male pups were reduced at the low and high, but not mid-dose groups.

<sup>i</sup> Lee et al. 2006: At this dose (2 mg/kg bw/day or 40 ppm, there was a reduction in AGD after adjustment at this dose and above although the magnitude of effect did not appear to increase with dose when looking at the table), copulatory behaviour was also reduced in this group (but not at other, higher-dose groups).

Overall, the lowest oral lowest-observed-adverse effect level (LOAEL) for toxicity of DINP was 159 to 395 mg/kg bw/day based on decreased pup weight after birth (10%) in two diet studies (No NOAEL; Waterman et al. 2000; Clewell et al. 2013). There is some uncertainty whether the decrease in pup weight could potentially be attributed to reduced palatability of milk and therefore, reduced consumption. Because actual milk consumption of pups was not measured in either study, this critical effect was considered a conservative selection. As previously mentioned, slight maternal toxicity (decreased body weight and/or food consumption) was only observed at the highest doses tested in both studies (0.8% or ~590 mg/kg bw/day; Waterman et al. 2000). This endpoint was also selected by NICNAS (2012) in their assessment.

Within the same critical effect level range stated above, at 250 mg/kg bw/day, other effects included significantly reduced foetal testicular testosterone levels and evidence of testicular pathology (MNGs) (NOAEL of 50 mg/kg bw/d; Clewell 2011a in ECHA 2013a; Clewell et al. 2013). As summarized above, *in utero* oral exposure to DINP in rats has been reported to cause other effects in the developing male foetus related to RPS, but these effects occur at higher doses compared to more potent anti-androgenic phthalates and further, not all effects related to this syndrome are observed. These effects include decreased serum testosterone levels, decreased AGD, NR, effects in sperm, and other histopathological effects in the testes. Therefore, an overall NOAEL for developmental toxicity of DINP is 50 mg/kg bw/day. This effect level was also established by multiple agencies based on the studies described above (ECHA 2013a; NICNAS 2012; US CPSC CHAP 2014).

No developmental studies were identified examining gestational exposure to DINP using other species via any route for this life stage.

#### **9.2.2.1.2 Exposure at prepubertal-pubertal life stage**

Results from two repeated-dose oral exposure studies in sexually immature rats (PND1 – PND55) support the hypothesis that DINP is a weak anti-androgen. In the pubertal rat (PND~39 – 55), short-term exposure to DINP at this life stage appeared to cause effects such as decreased accessory organ weights as well as serum hormone levels. Summaries of the studies are described in Table 9-17 below.

An OECD guideline Hershberger assay was conducted by Lee and Koo (2007) where 0, 20, 100, and 500 mg/kg bw/day of DINP (in corn oil) was administered via gavage to

testosterone propionate (TP)-treated (0.4 mg/kg bw/d) castrated pubertal Sprague-Dawley rats for 10 days. There were no significant changes in body weight or organ weights (liver, kidney, or adrenals). Seminal vesicle weight was significantly decreased at the lowest dose tested and above (but not in a dose-responsive manner) and a significant decrease in levator ani/bulbocavernosus muscle (LABC) weights was reported at the highest dose tested (500 mg/kg bw/d). At this dose, serum testosterone levels were decreasing and leutinizing hormone (LH) levels were statistically significantly increased compared to controls when measured 24 hours after the last dose.

A limited study by Kwack et al. (2009) observed a decrease in sperm count (~25%) in prepubertal male SD rats after 4 weeks of exposure to 500 mg/kg bw/day DINP-2 (no other doses tested). Sperm counts and sperm motility (straight-line velocity and curvilinear velocity) of epididymal sperm were also affected at this dose. Liver weights were significantly increased at this dose level, but testis weights were unchanged. There were no changes in haematological parameters, but glutamate oxaloacetate transaminase (GOT), alkaline phosphatase (ALP) and triglycerides were increased along with changed urinary protein levels (data not shown) which could be indicators of overall health of animals and potentially confound interpretation of effects in sperm (Kwack et al. 2009).

Two limited repeat-dose oral studies were identified in young non-human primates. A short, 14-day oral exposure to DINP-1 in 4 pubertal cynomolgus monkeys did not reveal any significant adverse effects in weight or pathology, urinalysis, hematology, or clinical chemistry at 500 mg/kg bw/day (Pugh et al. 2000). When young Marmosets (4 per group) were exposed to 0 to 2500 mg/kg-d DINP-2 via gavage for 13 weeks, decreased body weight gain was reported at the highest dose of 2500 mg/kg bw/day with no changes in biochemical parameters, hormone levels, organ weight changes or histopathology (Huntington Life 1998 in EU RAR 2003). There were wide variations in liver weights that were not considered biologically relevant (EURAR 2003).

**Table 9-17. Effects from exposure to DINP in prepubertal-pubertal males (mg/kg bw/day)**

<b>Strain and species; Dose (mg/kg bw/day); Route; Duration (reference) CAS RN</b>	<b>Life stage at the start of dosing (age)</b>	<b>Hormone levels<sup>a</sup> (T, S, LH)</b>	<b>Fertility<sup>b</sup></b>	<b>Reproductive tract pathology<sup>c</sup></b>	<b>Other effects<sup>d</sup></b>
SD Rats <sup>f</sup> ; 0, 20, 100, 500; Gavage; 10 days (Lee & Koo 2007) CAS no defined	Pubertal (PND 49)	NM (T) 500 (S) 500 (LH)	NM	NM	NE (BW) 20 <sup>e</sup> (ROW-seminal vesicle), 500 (2/5 organs affected = positive)

Strain and species; Dose (mg/kg bw/day); Route; Duration (reference) CAS RN	Life stage at the start of dosing (age)	Hormone levels <sup>a</sup> (T, S, LH)	Fertility <sup>b</sup>	Reproductive tract pathology <sup>c</sup>	Other effects <sup>d</sup>
					NE (ST)
SD Rats; 0, 500; Gavage; 28 days (Kwack et al. 2009) DINP-2 28553-12-0	Prepubertal (PND 35)	NM	500 <sup>e</sup> (25% ↓sperm count, velocity)	NM	500 <sup>e</sup> (BW) NE (ROW) 500 <sup>e</sup> (ST- ↑ liver wt, serum chem, urine protein)
F344 Rats; 0, 0.3, 0.6, 1.2, 2.5%, est. 0, 600, 1200, 2200; diet; 21 days (Lington et al. 1993) CAS not defined	Not specified (“young”)	NM	NM	NE	NM (BW) NE (ROW) 2200 (ST- ↓ food consumption)
Cynomolgus monkey; 0, 500; gavage; 14 days (Pugh et al. 2000) DINP-1 68515-48-0	Pubertal (24 mos)	NM	NM	NE	NE (BW) 500 <sup>NS</sup> (ROW) 500 <sup>NS</sup> (↓ lymphocyte and ↑ neutrophil count).
Marmoset; 0, 100, 500, 2500; gavage; 13 wks (Huntington Life 1998 in EU RAR 2003) DINP-2 28553-12-0	Pubertal (16-25 mos)	NM (T) NE (S) NE (LH)	NM	NE	2500 (BW) NE (ROW) 100 <sup>NS</sup> (↑ liver wt)

Abbreviations: NR, results not recorded (but measurement was stated in the methods and materials); NM, not measured; NE, no effect observed at the dose range tested. When NE is presented alone, all parameters in the footnote description were measured and no statistically significant effects were observed in the endpoints at the dose range administered; NDR, no dose relationship; MNG, multinucleated gonocytes

<sup>a</sup> Hormone level can include quantity/production of testicular testosterone (T), serum testosterone (S), or luteinizing hormone (LH).

<sup>b</sup> Fertility parameters include sperm number, motility, morphology, viability, stages of spermatogenesis, or reproductive success at adult stage after *in utero* exposure.

<sup>c</sup> Reproductive tract pathology includes: any observations based on histopathological examination of the testes such as, but not limited to, multinucleated gonocytes (MNGs), necrosis, hyperplasia, clustering of small Leydig cells, vacuolisation of Sertoli cells, decrease in Leydig cell number, an increase in Leydig cell size, focal dysgenesis, and/or seminiferous tubule atrophy.

<sup>d</sup> Other effects include: decreased overall body weight (BW), decreased reproductive organ weight (ROW) and systemic toxicity (ST).

<sup>e</sup> Lowest dose tested in the study

<sup>f</sup> According to the OECD test guideline, a result in this assay is positive for anti-androgenicity if at least two of the 5 sex accessory organs measured show a decrease in absolute weight.

Overall, the highest oral no-observed-effect-level (NOEL) for the reproductive toxicity of DINP at the pubertal life stage was 100 mg/kg bw/day based on decreased seminal vesicle and LABC weights at the next dose tested<sup>7</sup> (LOEL of 500 mg/kg bw/d; Lee and Koo 2007). Mild systemic effects such as increased liver weight and changes in serum chemistry were observed in prepubertal rats at 500 mg/kg bw/day (Kwack et al. 2009).

#### **9.2.2.1.3 Oral exposure at the mature male adult stage**

Twelve repeated-dose studies were conducted on sexually mature adult male rats (PND55+) using the previously identified forms of DINP via the oral route of exposure. Studies are described in Table 9-18 below.

The effects on the reproductive system of the male rat following exposure to DINP were mostly reduced reproductive organ weight (testis) at doses ranging from 307 – 1676 mg/kg bw/day with no histopathological findings (see Table 9-18); however, the majority of these studies did not assess fertility potential. Systemic toxicity in the adult animals included reduced body weight gain, increased liver and kidney weights, as well as liver and kidney lesions in males (LOAEL of 60 mg/kg bw/d; see section 9.2.2.5 for details).

In a 2-year diet study, Fischer 344 rats (110/sex) were administered dietary concentrations of 0 – 0.03 - 0.3 – 0.6% (w/w) DINP. The mean daily intakes of DINP over 2 years (Lington et al. 1997) were 15, 152 and 307 mg/kg bw/day for male rats. A statistically significant increase in relative testis weights was observed at the high dose associated with a slight, not statistically significant, increase of 13% in absolute testis weight (6.22 g vs. 5.48 g in the control group). Systemic effects included statistically significant, dose-related decrease in body weight (4 to 7% reduction in body weight compared to the control group) at the highest dose and increase in relative and absolute kidney and liver weights in males in the mid-dose group (20%). Those changes in organ weights were correlated to histopathological findings (systemic NOAEL of 15 mg/kg-bw/d) (Exxon Biochemical 1986; Hazleton et al. 1986a; Lington et al. 1987; Lington et al. 1997).

In the two-generational toxicity study described in previous sections (Waterman et al. 2000), information was extracted to determine the effects of DINP on the adult male (PND55+). DINP was mostly associated with slight systemic toxicity in the parental animals. In P1 males, absolute liver weights were increased at the high dose, kidney weights were increased at the mid- and high doses, while testis, epididymis, and prostate and seminal vesicle weights were unaffected. No histological effects were observed in the male reproductive organs. Overall, there were no statistically significant

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<sup>7</sup> Selection of the LOEL was based on the OECD guidance on the Hershberger Assay where a statistically significant increase ( $p \leq 0.05$ ) in any two or more of the five target androgen-dependent tissue weights should be considered a positive androgen agonist result.

adverse effects on mating, fertility, or reproductive organs. The only effect on reproduction was an increase in litter size in DINP-treated animals. Liver eosinophilia was reported in the livers of all DINP-treated parents. The reproductive NOAEL was determined to be 477 – 852 mg/kg bw/day. A LOAEL was not established for reproductive effects. A LOAEL of 118 – 215 mg/kg bw/day for systemic toxicity was identified based on microscopic liver changes observed in the P1 males.

In another 2-year study, Bio/dynamics (1986) administered 500, 5000, and 10 000 ppm (equivalent to 27, 271 and 553 mg/kg bw/d) of Santicizer 900 (DINP, CAS number not provided) to adult SD rats (70/sex/dose) via diet. Increased incidence of testicular interstitial cell hyperplasia in high-dose males was reported when compared to controls under study and outside historical range. No more details were provided. A higher incidence of small foci of hepatocellular necrosis, frequently hemorrhagic, and spongiosis hepatitis, in all treated male were observed (LOAEL of 27 mg/kg bw/d; see more detail in section 9.2.2.5).

There are four studies examining the potential toxicity of DINP in adult male mice. Two of these studies showed immature and abnormal sperm form in epididymis after exposition to DINP for thirteen weeks at doses of 2365 and 5770 mg/kg bw/day (see Table 9-18 for study details).

In a chronic study, B6C3F1 Mice were fed 0, 500, 1500, 4000, 8000 ppm (est. 0, 90, 276, 742, 1560 mg/kg bw/d) of DINP-1 in the diet for 104 weeks (Moore 1998b). Testicular effects observed in the adult male mouse included reduced absolute and relative testis weight at 742 mg/kg bw/day. However, the liver and kidney effects were observed at a lower dose level than the effect on testis weight (see section 9.2.2.5 below for more study details).

**Table 9-18. Effects from exposure to DINP in adult males (mg/kg bw/d)**

<b>Strain and species; Dose (mg/kg bw/day); Route; Duration (reference) CAS RN</b>	<b>Life stage at the start of dosing (age)</b>	<b>Hormone levels<sup>a</sup> (T, S, LH)</b>	<b>Fertility<sup>b</sup></b>	<b>Reproductive tract pathology<sup>c</sup></b>	<b>Other effects<sup>d</sup></b>
SD male Rats: 0, 60, 600, 1200 (diet) 3 wks Cited in REACH Dossier (in ECHA 2013b)	11 wks	NM	NM	1200 (minimal testicular degeneration)	600 (BW) 742 (ROW) 600(ST- ↑ liver wts)
F344 Rats; 0, 0.03, 0.3, 0.6%, est. 0, 15, 152, 307; Diet; 2 years	6 weeks	NM	NM	NR	307 (BW) 307 (ROW-rel testis wt only) 152 (ST- ↑

<b>Strain and species; Dose (mg/kg bw/day); Route; Duration (reference) CAS RN</b>	<b>Life stage at the start of dosing (age)</b>	<b>Hormone levels<sup>a</sup> (T, S, LH)</b>	<b>Fertility<sup>b</sup></b>	<b>Reproductive tract pathology<sup>c</sup></b>	<b>Other effects<sup>d</sup></b>
(Exxon Biochemical 1986; Hazleton et al. 1986a; Lington et al. 1987; Lington et al. 1977 in EU RAR 2003) DINP-1 68515-48-0					kidney & liver wt and pathology)
SD Rats; 0, 500, 5000, 10000 ppm, est. 0, 27, 271, 553; Diet; 2 years (Bio/dynamics 1986a in EU RAR 2003) DINP-A CAS not defined but reported as 71549-78-5 as never produced commercially by Babich (1998)	Not specified	NM	NM	553 (testicular interstitial cell hyperplasia)	NE (BW) NR (ROW) 271 (ST- liver lesions)
Not specified Rats; 0, 50, 150, 500; diet; 12 wks (Hazleton et al. 1971b in EU RAR 2003) DINP-1 68515-48-0	Not specified	NM	NM	NE	NE (BW- but trending decrease) NE (ROW) 500 (ST- ↑ liver wt and pathology)
F344 Rats; 0, 0.1, 0.3, 0.6, 1.0, 2.0%, est. 0, 77, 227, 460, 767, 1554; diet; 13 wks (Bio/dynamics 1982b in EU RAR 2003) DINP-1 68515-48-0	Not specified	NM	NM	NM	1554 (BW) 1554 (ROW) 227 (ST- ↑ liver wt)
SD Rats; 0, 0.3, 1.0%, est. 0, 201, 690; diet; 13 wks	Not specified	NM	NM	NM	201 (BW) 690 (ROW) 201 (ST- ↑

<b>Strain and species; Dose (mg/kg bw/day); Route; Duration (reference) CAS RN</b>	<b>Life stage at the start of dosing (age)</b>	<b>Hormone levels<sup>a</sup> (T, S, LH)</b>	<b>Fertility<sup>b</sup></b>	<b>Reproductive tract pathology<sup>c</sup></b>	<b>Other effects<sup>d</sup></b>
(Bio/dynamics 1982c in EU RAR 2003) DINP-1 68515-48-0					liver & kidney wt )
Wistar Rats; 0, 3000, 10 000, 30 000 ppm, est. 0, 152 – 333, 512 – 1101, 1543 – 3074; diet; 13 wks (BASF 1987f in EU RAR 2003) DINP-2 28553-12-0	Not specified	NM	NM	NE	152 -- 333 (BW) 1543 – 3074 <sup>NS</sup> (ROW- may not be due to treatment but ↓ body wt) 512 – 1101 (ST- ↑ liver, kidney wt and pathology)
SD Rats; 0, 1000, 3000, 10 000 ppm, est. 0, 60, 180, 600; diet; 13 wks (Hazleton et al. 1981a in EU RAR 2003) DINP-2 28553-12-0	Not specified	NM	NM	NE	NE (BW) NE (ROW) 60 (ST- ↑ kidney wt and pathology)
F344 Rats; 0, 0.6, 1.2, 2.5%, est. 0, 639, 1192, 2195; diet; 21 days (BIBRA 1985 in EU RAR 2003) DINP-1 68515-48-0	PND 41-44	NM	NM	NE	1192 (BW) 2195 (ROW- may not be due to treatment but ↓ body wt) 639 (ST- ↑ liver & kidney wt)

Strain and species; Dose (mg/kg bw/day); Route; Duration (reference) CAS RN	Life stage at the start of dosing (age)	Hormone levels <sup>a</sup> (T, S, LH)	Fertility <sup>b</sup>	Reproductive tract pathology <sup>c</sup>	Other effects <sup>d</sup>
F344 Rats; 0, 0.2, 0.67, 2.0%, est. 0, 150, 500, 1500; diet; 28 days (Shellenberg et al. 1983, Midwest Research Institute, 1981a in EU RAR 2003) DINP-2 28553-12-0	Not specified	NM	NM	NE	NE (BW) 1500 (ROW) 500 (ST- ↑ liver wt)
SD Rats; 0, 0.2, 0.4, 0.8%, est. F0: 0, 118 – 215, 236 – 426, 477 – 852; diet; 10 weeks to prior to mating – PND 21 (Waterman et al. 2000) DINP-1 68515-48-0	Not specified	NM	NE	NE	NE (BW) NE (ROW) 118 – 215 (ST- liver lesions)
SD Rats; 0, 0.5, 1, 1.5%, est. 0, 301 – 591, 622 – 1157, 966 – 1676 ; diet; 10 weeks prior to mating – PND21 (Waterman et al. 2000) DINP-1 68515-48-0	Not specified	NM	NE	NM	622 – 1157 (BW) 966 – 1676 (ROW- absolute testes wt) 301 – 591 (ST- ↑ absolute kidney and liver wt)
B6C3F1 Mice; 0, 500, 1500, 4000, 8000 ppm, est. 0, 90, 276, 742, 1560; diet; 104 wks (Moore 1998b) DINP-1 68515-48-0	Not specified	NM	NM	NR	742 (BW) 742 (ROW- ↓ abs & rel testis wt) 276(ST- ↑ kidney, liver wts)

<b>Strain and species; Dose (mg/kg bw/day); Route; Duration (reference) CAS RN</b>	<b>Life stage at the start of dosing (age)</b>	<b>Hormone levels<sup>a</sup> (T, S, LH)</b>	<b>Fertility<sup>b</sup></b>	<b>Reproductive tract pathology<sup>c</sup></b>	<b>Other effects<sup>d</sup></b>
B6C3F1 Mice; 0, 0.15, 0.4, 1.0, 2.0%, est. 0, 340, 904, 2365, 5472; diet; 13 wks (Bankston et al. 1992, Moore et al. 2000 in CPSC 2010) CAS not defined	Not Specified	NM	2365 (small number of immature / abnormal sperm)	NE	NR (BW) 2365 (ROW) 972 (ST- ↓ kidney wt, 2365, liver effects)
B6C3F1 Mice; 0, 1500, 4000, 10 000, 20 000 ppm, est. 0, 365, 972, 2600, 5770 (EU); diet; 13 wks (Hazleton et al. 1992 in EU RAR 2003) DINP-2 28553-12-0	Not specified	NM	5770 (immature / abnormal sperm forms in epididymis)	NR	5770 (BW) 2600 (ROW) 972 (ST- liver effects, ↓ liver kidney wt)
B6C3F1 Mice; 0, 3000, 6000, 12 500, 25 000 ppm, est. 0, 635, 1377, 2689, 6518; diet; 4 wks (Hazleton et al. 1991b in EU RAR 2003) DINP-2 28553-12-0	Not specified	NM	NM	6518 (cellular debris)	6518 (BW) 1377 (ROW) 635 (ST- ↑ liver wt and pathology)
Beagle Dog; 0, 0.125, 0.5, 2% (4.% after wk 9), est. 0, 37, 160, 2000; diet; 13 wks (Hazleton 1971a cited in EURAR 2003) DINP-2 28553-12-0	Age?	NM	NM	NE	160 (BW in 2/4) NE (ROW) 160 (↑ liver wt) 2000 (mult. Organ wt and liver & kidney path)

Abbreviations: NR, results not recorded (but measurement was stated in the methods and materials); NM, not measured; NE, no effect observed at the dose range tested. When NE is presented alone, all parameters in the

footnote description were measured and no statistically significant effects were observed in the endpoints at the dose range administered.

<sup>a</sup> Hormone level can include quantity/production of testicular testosterone (T), serum testosterone (S), or luteinizing hormone (LH).

<sup>b</sup> Fertility parameters include: sperm number, motility, morphology, viability, stages of spermatogenesis, or reproductive success at adult stage after *in utero* exposure.

<sup>c</sup> Reproductive tract pathology includes: any observations based on histopathological examination of the testes such as, but not limited to, multinucleated gonocytes (MNGs), necrosis, hyperplasia, clustering of small Leydig cells, vacuolisation of Sertoli cells, decrease in Leydig cell number, an increase in Leydig cell size, focal dysgenesis, and/or seminiferous tubule atrophy.

<sup>d</sup> Other effects include: decreased overall body weight (BW), decreased reproductive organ weight (ROW) and systemic toxicity (ST).

<sup>e</sup> Lowest dose tested in the study.

Overall, the highest NOEL for reproductive toxicity identified for DINP was 276 mg/kg bw/day based on reduced relative and absolute reproductive organ weights at the next dose (LOEL = 742 mg/kg bw/d; Moore 1998b) in adult male mice. This endpoint was selected in other international assessments (EURAR 2003; ECHA 2013a). In a two year study, testicular interstitial cell hyperplasia was observed at the highest dose of 553 mg/kg bw/day in male rats (Bio/dynamics 1986a). However, DINP CAS number was not provided in the study and was reported later as 71549-78-5 by Babich (1998). The lowest LOAEL for systemic toxicity was 60 mg/kg bw/day, based on an increased frequency of kidney lesions in all exposed male rats in a 13-week study (Hazleton 1981a).

### 9.2.2.2 Oral exposure in females

A total of 23 oral studies on the reproductive and developmental effects of DINP in females were identified. They include one two-generation reproductive toxicity study.

Several studies related to the reproductive and developmental effects of DINP in females have been published. Half of the reproductive studies did not report any reproductive effects in females. The remaining studies reported adverse effects on fertility and pregnancy outcomes, on reproductive related organ weights (ovaries and uterus) and on reproductive development (absence of *corpora lutea* and endometrial hyperplasia) at doses of 600 mg/kg bw/day and above.

The developmental LOAELs identified in eight studies ranged from 100 to 1164 – 2657 mg/kg bw/day. The critical effects referred to growth alterations and mild teratogenicity; malformations and variations, and were obtained at doses lower or similar to those inducing maternal toxicity. At exceptionally high doses in mice (5770 – 6920 mg/kg bw/d), decreased absolute and relative uterine weight, accompanied with hypoplasia, absence of corpora lutea (normal follicles present but no luteinisation), endometria devoid of glands were observed after repeated dose exposure to DINP (Hazleton 1991b, 1992). See section 9.2.2.5 for more study details.

The reviewed data indicated that DINP is not estrogenic, but may lead to altered sexual behaviour in mature females (Lee et al. 2006), without affecting the endocrine system of the hypothalamic pituitary-gonadal axis although this needs further validation. Behavioural changes as well as a reduction in progesterone receptor expression in the

hypothalamic medial preoptic area were also observed along with uterine adenocarcinoma after chronic exposure.

Overall, data indicate that DINP is a developmental and reproductive toxicant at high doses (600 mg/kg bw/day and above) in females. Developmental toxicity occurred at doses lower or similar to maternally toxic doses.

### 9.2.2.3 Endocrine studies

DINP was tested for agonist/antagonist aryl hydrocarbon receptor (AhR) and androgen receptor (AR) activity using the luciferase reporter gene expression bioassay in mouse Hepa1.12cR cells (AhR-CALUX) and in transient transfected Chinese Hamster Ovary(CHO-K1) cells (AR-CALUX; Krüger et al. 2008). No significant effect was observed neither on AhR activity or AR activity ( $1 \times 10^{-10}$  to  $1 \times 10^{-4}$  M). The result for AR is consistent with the results of studies by others (Roy et al. 2004; Takeuchi et al. 2005). Takeuchi and colleagues (2005) assessed human ER $\alpha$ , human ER $\beta$  and human androgen receptor (AR) activity using a reporter gene assay in CHO cells and found that DINP did not show any oestrogenic/anti-oestrogenic or androgenic/anti-androgenic activity up to concentrations of  $10^{-5}$  M.

### 9.2.2.4 Reproductive and developmental toxicity: evidence in humans

Available information on the potential effects of phthalates on humans was evaluated. The published literature was searched and human studies with an epidemiological focus were identified for further consideration. The evaluation included cross-sectional, case-control and cohort studies that encompassed 14 phthalate parent compounds and their metabolites. Given the large number of studies available in humans and the diverse outcomes identified for this substance grouping, all studies collected were scored for quality using a consistent evaluation metric<sup>8</sup> (Downs and Black 1988). This allowed for a reliable, objective assessment tool that captured the dimensions of study quality across various study designs. Statistically significant exposure-response associations were evaluated for each health outcome. A conclusion as to the level of evidence of association of a phthalate and each health outcome was based on the strength and consistency of the relationship as well as the quality of the epidemiology studies, as determined by the Downs and Black scores. Based on the overall score obtained from the evaluation approach, the level of evidence for association was designated as sufficient, limited, inadequate, or evidence suggesting no association. Studies that were rated in the lowest quartile (Quartile 1) based on the evaluation were not included in this report. This evaluation did not consider the biological plausibility of the relationship, meaning that no causal inference was established. More detail is provided in Health Canada (2015b) available upon request.

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<sup>8</sup> A more detailed description of the Downs and Black scoring system appears in Appendix F.

Available epidemiological studies examining the potential relationship between any observed reproductive and/or developmental effects and exposure to DINP in humans were reviewed (Appendix F; Health Canada 2015b). Overall, there was limited evidence for associations of MINP with sex hormones in infants and adult males (Main et al. 2006; Joensen et al. 2012). There was inadequate evidence for associations with DINP metabolites and female puberty (Frederiksen et al. 2012). No associations were found for birth measures (Philippat et al. 2012), gestational age (Meeker et al. 2009), or reproductive endpoints such as gynecomastia and time to pregnancy (Mieritz et al. 2012; Buck Louis et al. 2014). There was inadequate evidence of an association for MINP and semen parameters (sperm volume, sperm motility, etc.) (Joensen et al. 2012). No association was observed between MINP and male infant genitalia defects (Main et al. 2006).

More recent studies have found associations between DINP and various endpoints, but these have not yet been assessed using the Downs and Black evaluation approach. Some DINP metabolites were reported to be associated with changes in reproductive hormones and/or semen parameters while other metabolites studied showed no such association (Meeker and Ferguson 2014; Specht et al. 2014). Bornehag et al. (2014) observed associations between anoscrotal distance (AGDas) and metabolites of DINP (OH-MMeOP and oxo-MMeOP), although these were small. No significant associations were found between DINP or its metabolites and child psychomotor development (Polanska et al. 2014), and placenta gene expression (LaRocca et al. 2014).

#### **9.2.2.5 Other systemic effects<sup>9</sup>**

##### Consideration of human relevance of liver effects

It is well documented that phthalates can induce peroxisome proliferation in the liver as well as increase liver weight in rats and mice. In some cases, liver cancer was also observed following longer-term oral administration of high doses of phthalates. It is well established that the peroxisome proliferator-activated receptor (PPAR)  $\alpha$  plays a role in peroxisome proliferation-induced liver effects (Corton and Lapinskas 2005). However, the relevance of the hepatotoxic effects of phthalates observed in rodents is difficult to establish due to the species-specific differences in the peroxisomal proliferation response (rodents being significantly more sensitive than humans to PPAR $\alpha$ -mediated induction of peroxisome proliferation) (ECB 2008, NICNAS 2010, US CPSC 2010a). Several recent studies have suggested that the mechanisms of liver toxicity of peroxisome proliferators have not been entirely elucidated and that multiple pathways may exist, some that are likely PPAR $\alpha$ -independent (Ito et al. 2007, Yang et al. 2007,

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<sup>9</sup> This section presents studies examining effects other than reproductive effects.

Eveillard et al. 2009, Ren et al. 2010, IARC 2012). Based on this, liver effects cannot be precluded as an effect potentially relevant to humans and should be included in the characterization of health effects of phthalates (More detailed information on the mode of action of liver carcinogenicity in rodents with peroxisome proliferators is available in Health Canada (2015c).

#### Repeated-dose studies

In repeated-dose studies identified in the literature for DINP, the main effects observed following oral exposure were increased liver and kidney weights with correlating histological changes. Changes in other organ weights, such as testes, were reported as well in a few studies. No systemic effects were reported in a dermal study and no inhalation studies were identified. The most relevant studies are summarized below. NOAELs and LOAELs from the available studies are presented in Table 9-19.

In a 7-day feeding study in male F344 rats, increases in liver, kidney and testes weights with slight liver congestion, as well as slight to moderate suppression of cholesterol levels, slight to severe triglycerides suppression, and slight decrease in alkaline phosphatase were noted in exposed animals. Only one dose level, 1700 mg/kg bw/day, was used in this study (Bio/dynamics 1982c). In a 14-day gavage study in which female F344 rats were given 0, 25, 75, 150, or 1500 mg DINP mg/kg bw/day, increase in liver enzyme activity was observed for the different existing forms of DINP, from 25 mg/kg bw/day for DINP-1 (CAS 68515-48-0) and from 75 mg/kg bw/day for DINP-2 (CAS 28553-12-0) and DINP-3 (CAS 28553-12-0). Increase in absolute and relative liver weight and a slight decrease in cholesterol and triglyceride were also observed at the highest dose in this study. A significant increase in kidney weight was also observed at this dose level in animals treated with DINP-3 (Huls AG 1992). In another 14-day gavage study in which male Kunming mice were given 0, 0.2, 20, or 200 mg DINP mg/kg bw/day, histopathological changes in liver cells (central vein dilation, congestion, oedema, narrowing sinusoidal with loose cytoplasm) and kidney cells (large reduction in tubular space and extreme oedema of epithelial cells in the glomeruli) were observed in animals exposed at the highest dose (Ma et al. 2014). However, when monkeys were exposed to 500 mg DINP/kg bw/d for the same duration (14 days) and by the same route of exposure, no treatment-related effect were reported in the liver and kidneys of exposed animals (no increase in weight, no histopathological changes and no effects on liver enzymes/hepatic markers for peroxisomal proliferation such as peroxysomal beta-oxidation or replicative DNA synthesis). Also, no effect on body weight, urinalysis, hematology, or clinical chemistry was noted (Pugh et al. 2000).

When F344 rats were exposed for 3 weeks to 0, 0.6, 1.2, 2.5% DINP (males: 0, 639, 1192, 2195 mg/kg bw/d; females: 0, 607, 1198, 2289 mg/kg bw/d) through the diet, a LOAEL of 607- 639 mg/kg bw/day was identified based on increased absolute and relative liver weight, increased liver enzyme activity, and hypolipidemic effects on both sexes at all doses tested accompanied by cytoplasmic basophilia in hepatocytes at 1.2% and eosinophilia at 2.5% (Barber et al. 1987).

In a 4-week feeding study in B6C3F1 mice exposed to 0, 3000, 6000, 12 500, and 25 000 ppm DINP (males: 0, 635, 1377, 2689, 6518 mg/kg bw/d; females: 0, 780, 1761, 3287, 6920 mg/kg bw/d), histopathological findings in the liver (enlarged and discoloured livers) were observed in both sexes at all doses tested. Absolute and relative liver weight was also increased in both sexes from 6000 ppm. Hepatocytomegaly (enlarged hepatocytes) was observed in males from the lowest dose and in females from 6000 ppm. Necrosis and focal chronic inflammation were observed in males exposed at the highest dose and in females from 12 500 ppm. Instances of tubular necrosis were observed in high-dose animals. Kidney and testes weights (absolute and relative) were decreased from 6000 ppm in males. Ovaries appeared smaller in section in high-dose females, and there was a complete absence of corpora lutea (normal follicles present but no luteinisation), uteri were smaller than control and the endometrium was devoid of glands. Other findings included splenic atrophy at 25 000 ppm, thymic necrosis and depletion of lymphocytes from 12 500 ppm (although this was also observed in controls). Altered haematological parameters were observed at 25 000 ppm (reduced white blood cells in males, increased lymphocytes in females). Males at 25 000 ppm had increased aminotransferase and blood urea nitrogen levels (Hazleton 1991b). In this study, the LOAEL was 635-780 mg/kg bw/day based on histopathological findings in the liver in exposed males and females.

In other feeding studies with short-term exposure to DINP, increase in liver weight and increase in hepatic enzyme activity was noted at dose levels ranging from 125 to 546 mg/kg bw/day in male and/or female rats and mice (Shellenberg et al. 1983; Smith et al. 2000; Kaufmann et al. 2002; Kwack et al. 2009). In one of these studies, other increases in organ weight (kidney, testes, ovary, and spleen) were noted in rats at 1300 mg/kg bw/day, the highest dose tested (Shellenberg et al. 1983).

In a subchronic diet study, F344 rats were exposed to 0, 0.1, 0.3, 0.6, 1.0, 2.0% DINP-1 in feed (approximately 0, 77, 227, 460, 767, 1554 mg/kg bw/d) for 13 weeks. A dose-dependent increase in kidney and liver weight and decreased in cholesterol level was noted in males and females exposed to 0.3% DINP. These effects were accompanied with organ discoloration (brown mottling in liver at the two highest doses and dose-dependent dark brown discoloration in kidney from 0.6%). Also, biochemical parameters indicative of liver changes were noted; triglyceride decreased in both sexes from 0.6%, increase in alkaline phosphatase from 1% in males, and ALT in males at 2%. Decreased body weight gain was observed at the highest dose tested (Bio/dynamics 1982a). In this study, the NOAEL was 77 mg/kg bw/day and a LOAEL of 227 mg/kg bw/day was identified based on liver and kidney effects.

In another 13-week study in F344 rats, increased liver and kidney weights were also observed in both sexes from 2500 ppm (176 – 218 mg/kg bw/d), the lowest dose tested. Again, these increases were accompanied with histopathological changes such as hepatocellular enlargement (periportal in males, centrilobular in females) and dark liver (observed in 2 males) at 20 000 ppm (the highest dose tested) and a dose-related increase in granular casts and regenerative/basophilic tubules in the kidneys of males from 5000 ppm. Vesiculation and increased severity of acute inflammation were also

present in the stratified squamous epithelium of the non-glandular stomach in male and female rats at 10 000 ppm and higher. Clinical chemistry revealed signs of anemia (decreased RBC count, hematocrit, and haemoglobin) from 5000 ppm in males and 10 000 ppm in females. Globulin concentration was also reduced from 5000 ppm in males and 10 000 ppm in females, and albumin and A/G ratios were increased from 10 000 ppm in both sexes. Body weight gain was significantly depressed in both sexes at the highest dose tested. Finally, absolute and relative uterine weight was observed at 20 000 ppm, and testis/epididymal weight was increased from 10 000 ppm. The LOAEL was established at 176 – 218 mg/kg bw/day based once more on liver and kidney effects.

When Wistar rats were exposed at doses ranging from 3000 to 30 000 ppm for 91 days in a OECD guideline study, liver and clinical chemistry changes (trend toward reduced triglyceride level and decreased peripheral fat deposits in hepatocytes) were observed at the lowest dose and higher in both sexes. Absolute and relative liver weight was significantly increased in both sexes only from 10 000 ppm (1101 – 1543 mg/kg bw/d). The increase was pronounced in animals treated at the highest dose, and hepatic tissue was darker than control. Hypertrophy was observed in 10/20 animals at 10 000 ppm and 17/20 at 30 000 ppm. Centrolobular degenerative fatty infiltration of hepatocytes with pyknosis was observed in males from 10 000 ppm. Slight, significant increase in ALT was observed from 10 000 ppm. ALP was significantly increased in females from 10 000 ppm and males at 30 000 ppm. Increased bilirubin, significant at 30 000 ppm, was noted in females. Kidney effects were also observed in males from 10 000 ppm. At this dose, increased total protein and increased albumin were noted in males only. At 30 000 ppm, urea and creatinine values were increased, indicating a disturbance in the kidney function. Relative kidney weight was significantly increased in both sexes from 10 000 ppm; absolute weight was increased in males at 10 000 ppm. In the renal cortex, tubular epithelium was damaged in all males at 30 000 ppm (BASF AG 1987f). The LOAEL was identified at 152 – 200 mg/kg bw/day based on evidences of hepatotoxicity at all doses in rats of both sexes.

In a 13-week feeding study in Sprague-Dawley rats exposed at doses of DINP up to 10 000 ppm, a LOAEL of 1000 ppm (60 mg/kg bw/d) was identified based on an increased frequency of kidney lesions (focal mononuclear cell infiltration and mineralization) in exposed males. No such lesions were noted in females. At higher doses, increase in weight was noted for some organs. Relative liver weight was increased in females at 3000 ppm while absolute and relative weights were increased in both sexes at 10 000 ppm. Absolute and relative kidney weight was increased at 10 000 ppm (Hazleton 1981a).

In mice given DINP through the diet for 13 weeks, liver and kidney effects, as well as other organ weight changes, were also observed, but at higher dose than in similar studies in rats. An increase in absolute and relative liver weight was observed in both sexes exposed to 4000 ppm (972 mg/kg bw/d) DINP. These increases were accompanied with hepatic enlargement in males and females (only from 10 000 ppm) and histopathological changes at the two highest doses tested. Among the

histopathological changes observed, pale areas were noted in males from 10 000 ppm and females at the highest dose. At 10 000 ppm, centrilobular to midzonal hepatocellular enlargement was reported in both sexes. Diffuse hepatocellular enlargement, pigmentation in kupffer cells and bile canaliculi, and minimal to slight liver degeneration/necrosis was observed in animals treated with the highest dose. Absolute testis/epididymis weights were reduced at the two highest doses and immature/abnormal sperm were noted at 20 000 ppm. Absolute and relative uterine weight was decreased in females at the highest dose and this decrease was accompanied with hypoplasia in the uterus and an absence of corpora lutea in the ovaries. At the highest dose, histopathological changes in the kidney (granular/pitted/rough kidneys and tubular nephrosis) were observed. Increased ALT and AST were observed in males and urinary parameters were altered (increased volume, lower specific gravity, decreased sodium, chloride and creatinine) in both sexes. Dose-dependent decreases in body weight gain were seen and hunched posture, ataxia, hypoactivity, tremors, dyspnea, polypnea, sores and low body temperature were noted (Hazleton 1992).

In dogs exposed to up to 2% (2000 mg/kg bw/d) DINP in feed, liver effects were also observed, indicating that these effects are not limited only to rodents. Absolute and relative liver weights were increased from the mid-dose (0.5%; 160 mg/kg bw/d) in males and at the highest dose in females. Hepatocyte hypertrophy and histopathological changes in the liver were noted at the highest dose in both sexes. Absolute and relative kidney weights were increased in few animals at the highest dose as was hypertrophy of the tubular epithelium. Discoloration was observed in females (Hazleton Laboratories 1971b). However, no liver or kidney effects (no organ weights, biological parameters or hormonal concentrations changes, no histological findings) were noted in monkeys administered 0, 100, 500, and 2500 mg/kg bw/day DINP by gavage for 13 weeks and only a decrease in body weight and body weight gain was noted at the highest dose tested (Huntingdon Life Sciences 1998).

Finally, in a dermal study in which rabbits were exposed to up to 2500 mg DINP/kg-bw per day, 24 hours per day, five days per week for six weeks, slight or moderate erythema and slight desquamation was observed on abraded skin. No treatment-related histopathological findings were observed in the liver and kidneys. No other information was provided (Hazleton 1969).

**Table 9-19. Short-term and subchronic studies in animals**

<b>Strain and species; Doses [mg/kg bw/day]; Route; Duration (Reference)</b>	<b>NOAEL (mg/kg bw/d)</b>	<b>LOAEL (mg/kg bw/d)</b>
Fischer 344 rats (males); 0 or 2.0%; est. 0 or 1700; Diet; 7 days (Bio/dynamics 1982c) DINP-1 68515-48-0		1700 (increased liver and kidney weights with slight liver congestion accompanied with slight to moderate suppression of cholesterol levels, slightly to severe suppression of triglycerides, and slight

Strain and species; Doses [mg/kg bw/day]; Route; Duration (Reference)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)
		decrease in alkaline phosphatase)
Fischer 344 rats (females); 0, 25, 75, 150, 1500; Gavage; 14 days (Huls AG 1992) Jayflex DINP-1 68515-48-0 Palatinol N DINP-2 28553- 12-0 Vestinol 9 DINP-3 28553- 12-0		LOEL: 25 – 75 (increased DOS (dodecanoic acid 12-hydroxylase) activity at 25 mg/kg-bw/day for DINP1 and at 75 mg/kg-bw/day for DINP2 and DINP3
Fischer 344 rats (males); 0, 1000, 12 000; Diet; 2 or 4 weeks (Smith et al. 2000) DINP-1 68515-48-0 DINP-A 71549-78-5	NOEL: 1000 ppm	LOEL: 12 000 ppm (increase in relative liver weight with both DINP-1 and DINP-A, increase in PBOX (peroxisomal beta-oxidation), inhibition of GJIC (gap junction intracellular communication) by both DINP-1 and DINP-A at 2 weeks and at 4 weeks in the DINP-1 group. Periportal DNA synthesis and centrilobular DNA synthesis were also increased at 2 weeks with DINP-1 and DINP-A, and at 4 weeks for DINP-A)
Fischer 344 rats; 0, 0.6, 1.2, 2.5%; est. 0, 639, 1192, 2195 (males); 0, 607, 1198, 2289 (females); Diet; 3 weeks (Barber et al. 1987) DINP-1 68515-48-0	-	607 – 639 (increase in absolute and relative liver weights accompanied with increased 11- and 12-hydroxylase activity, hypolipidemic effects, cytoplasmic basophilia in hepatocytes at 1.2%, and eosinophilia at the highest dose) (males/females)
Fischer 344 rats; 0, 0.2, 0.67, 2%; est. 0, 150, 500, 1500 (males); 0, 125, 420, 1300 (females); Diet; 28 days (Shellenberg et al. 1983) DINP-2 28553-12-0		LOEL: 125 – 150 (increase in hepatic enzyme activity) (males/females)
Sprague-Dawley rats (males); 0 or 500; Gavage; 28 days (Kwack et al. 2009) DINP-2 28553-12-0		500 (significant decrease in body weight and increase in relative liver weights accompanied by higher levels of glutamate oxaloacetate transaminase (GOT), alkaline phosphatase and triglycerides)

<b>Strain and species; Doses [mg/kg bw/day]; Route; Duration (Reference)</b>	<b>NOAEL (mg/kg bw/d)</b>	<b>LOAEL (mg/kg bw/d)</b>
Rats; 0, 50, 150, 500; Diet; 3 months (Hazleton Laboratories 1971a) DINP-2 28553-12-0	NOEL: 150 (males/ females)	LOEL: 500 (increased kidney weight, liver weight, and hepatocyte hypertrophy) (males/females)
Fischer 344 rats; 0, 0.1, 0.3, 0.6, 1.0, 2.0%; est. 0, 77, 227, 460, 767, 1554; Diet; 13 weeks (Bio/dynamics 1982a) DINP-1 68515-48-0	77 (males/ females)	227 (increase in kidney and liver weights accompanied with histopathological changes in the liver at the two highest doses and in kidneys from 0.6%) (males/females)
Fischer 344 rats; 0, 2500, 5000, 10 000, 20 000 ppm; est. 0, 176, 354, 719, 1545 (males); 0, 218, 438, 823, 1687; Diet; 13 weeks (Hazleton 1991a) DINP-2 28553-12-0		176 – 218 (increase in kidney and liver weights accompanied with histopathological changes in the liver at the highest doses and in kidneys from 5000 ppm) (males/females)
Fischer 344 rats; 0, 0.3, 1.0%; est. 0, 201, 690 (males); 0, 251, 880 (females); Diet; 13 weeks (Bio/dynamics 1982b) DINP-1 68515-48-0		LOEL: 201 – 251 (increase in relative kidney and liver weights accompanied by a dose- dependant decrease in triglycerides and altered urine chemistry) (males/females)
Wistar rats; 0, 3000, 10 000, 30 000 ppm: est. 0, 333, 1101, 3074 at day 7; 0, 152, 512, 1543 at day 91 (males); 0, 379, 1214, 3224 at day 7; 0, 200, 666, 2049 at day 91 (females); Diet; 13 weeks (BASF AG 1987f) DINP-2 28553-12-0		152 – 200 (clinical chemistry and liver changes related to hepatotoxicity (trend toward reduced triglyceride level and decreased peripheral fat deposits in hepatocytes) (males/females)
Sprague-Dawley rats; 0, 1000, 3000, 10 000 ppm; est. 0, 60, 180, 600; Diet; 13 weeks (Hazleton 1981a) DINP-2 28553-12-0	(males) 600 (females)	60 (increase in kidney lesions) (males)  LOEL: 180 (increase in relative liver weight (females)
B6C3F1 mice; 0, 500, 1500, 4000, 8000 ppm; est.	NOEL: 117 –	LOEL: 350 – 546 (increase in liver weights, peroxisomal volume, and peroxisomal

<b>Strain and species; Doses [mg/kg bw/day]; Route; Duration (Reference)</b>	<b>NOAEL (mg/kg bw/d)</b>	<b>LOAEL (mg/kg bw/d)</b>
0, 117, 350, 913, 1860 (males); 0, 167, 546, 1272, 2806 (females); Diet; 1 or 4 weeks (Kaufmann et al. 2002) DINP-1 68515-48-0	167 (males/females)	enzyme activity) (males/females)
Kunming mice (males); 0, 0.2, 2, 20, 200; Gavage; 14 days (Ma et al. 2014) CAS not defined	20	200 (histopathological changes in the liver and kidney)
B6C3F1 mice (males); 0, 500, 6000 ppm; Diet; 2 or 4 weeks (Smith et al. 2000) DINP-1 68515-48-0	NOEL: 500 ppm	LOEL: 6000 ppm (increase in liver weights, increase in PBOX activity and GJIC inhibition)
B6C3F1 mice; 0, 3000, 6000, 12 500, 25 000 ppm; est. 0, 635, 1377, 2689, 6518 (males); 0, 780, 1761, 3287, 6920 (females); Diet; 4 weeks (Hazleton 1991b) DINP-2 28553-12-0	-	635 – 780 (histopathological changes in the liver at all doses tested) (males/females)
B6C3F1 mice; 0, 1500, 4000, 10 000, 20 000 ppm; est. 0, 365, 972, 2600, 5770; Diet; 13 weeks (Hazleton 1992) DINP-2 28553-12-0	365 (males/females)	972 (increase in absolute and relative liver weights accompanied with hepatic enlargement and histopathological changes at the two highest doses tested) (males/females)
Macaca fascicularis monkeys (males); 0, 500; Gavage; 14 days (Pugh et al. 2000) DINP-1 68515-48-0	NOEL: 500	
Marmoset monkeys; 0, 100, 500, 2500; Gavage; 13 weeks (Huntingdon Life Sciences 1998) DINP-2 28553-12-0	500 (males/females)	2500 (decrease in body weight and body weight gain) (males/females)
Dog; 0, 0.125, 0.5, 2.0%:	37	160 (increase in absolute and relative liver

<b>Strain and species; Doses [mg/kg bw/day]; Route; Duration (Reference)</b>	<b>NOAEL (mg/kg bw/d)</b>	<b>LOAEL (mg/kg bw/d)</b>
est. 0, 37, 160, 2000; Diet; 13 weeks (Hazleton Laboratories 1971b) DINP-2 28553-12-0	(males) 160 (females)	weights accompanied with histopathological changes at the highest dose tested) (males) 2000 (increase in absolute and relative liver and kidney weights accompanied with histopathological changes in both organs) (females)
New Zealand White rabbit; 0, 0.5, 2.5 ml/kg: est. 0, 500, 2500; Dermal; 6 weeks (24h application to intact and abraded skin, 5 times per week (Hazleton 1969) DINP-1 68515-48-0	2500 (males/fe males)	

Overall, the lowest LOAEL for short-term oral exposure identified for DINP was 200 mg/kg bw/day, based on histopathological changes in the liver and kidney of mice exposed for 14 days (Ma et al. 2014). As noted earlier, no systemic effects were noted in rats exposed to DINP in one dermal study (6-week duration) (Hazleton 1969). The lowest LOAEL for subchronic oral exposure was 60 mg/kg bw/day, based on an increased frequency of kidney lesions in all exposed males in a 13-week study in rats (Hazleton 1981a). In dogs, the NOAEL for subchronic exposure was 37 and 160 mg/kg bw/day based on increase in liver and/or kidney weights accompanied with histopathological changes in males and females at 160 and 2000 mg/kg bw/day, respectively, in a 13-week study (Hazleton Laboratories 1971b).

The NOAELs of 500 mg/kg bw/day identified from a short-term and a subchronic study in monkeys (Pugh et al. 2000; Huntingdon Life Sciences 1998) may indicate that monkeys and maybe humans are less sensitive than rodents and dogs to liver effects, which is consistent with the hypothesis that species differences in the activation of PPAR $\alpha$  or its signaling network by peroxisome proliferation may exist.

### Carcinogenicity

Recently, the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency (EPA) has published a document on the evidence on the carcinogenicity of DINP in which members of the Carcinogen Identification Committee (CIC) conclude that DINP has been clearly shown, through scientifically valid testing according to generally accepted principles, to cause cancer and should be listed under Proposition 65 as a carcinogen (OEHHA 2013a). Accordingly, DINP has been listed under Proposition 65 at the end of 2013 (OEHHA

2013b). DINP has not been classified for its potential carcinogenicity by other international agencies.

DINP has been tested in a number of carcinogenicity studies in rats and mice. Statistically significant increases in many tumour types were observed, such as increase in hepatocellular tumours, mononuclear cell leukemia of the spleen, and renal tubular cell carcinomas. In addition, other tumour types considered rare and/or uncommon were noted in DINP-treated animals: renal transitional cell carcinoma, pancreatic islet cell carcinoma, testicular interstitial (Leydig) cell carcinoma, and uterine adenocarcinoma. The most relevant studies are summarized below and presented in Table 9-20.

In a 2-year oral carcinogenicity study in which rats were fed diets containing 0, 500, 1500, 6000, and 12 000 ppm DINP (equivalent to 0, 29, 88, 359, 733 mg/kg bw/day for males and 0, 36, 109, 442, 885 mg/kg bw/day for females), a dose-related increase in incidence of mononuclear cell leukemia (MNCL) was observed in both sexes from 6000 ppm. An increased incidence of hepatocellular neoplasia was observed in animals treated with the highest dose. There was a significant increase in hepatocellular carcinoma in males but not in females and significant increase incidence of carcinoma or adenoma in both sexes. In the recovery studies in which rats were exposed to 0 or 12 000 ppm DINP for 78 weeks, followed by 26 weeks of recovery, MNCL was also significantly increased in both sexes and there was a significant increase in renal tubular carcinomas in male rats. No significant increases in liver tumours were observed in both males and females, indicating that neoplasms developed during the last 26 weeks of the study. Non-neoplastic effects included liver and kidney effects (increased in absolute and relative organ weight accompanied with increased serum ALT and AST, and histopathological findings) at the two highest doses. Histopathological changes in the liver included enlargement, and/or granular/pitted/rough changes in both sexes and evidence of spongiosis hepatitis in males. Histopathological changes in the kidney included dark coloration and mineralization of renal papilla in males only, and increase tubule cell pigmentation in both sexes. Some of the histological findings in males were consistent with alpha<sub>2</sub> globulin nephropathy (specific to male rats). Alpha<sub>2</sub> globulin accumulation was confirmed by Caldwell (1999) in immunohistochemical analysis of archived tissue samples. A reduced body weight gain and a significant and dose-related decrease in survival were noted at the highest dose (Moore 1998a).

In another chronic lifetime study in rats exposed though the diet to 0, 0.03, 0.3, and 0.6% DINP (equivalent to 0, 15, 152, 307 mg/kg bw/day for males and 0, 18, 184, 375 mg/kg bw/day for females), an increase in MNCL was noted in males and females at the two highest doses. However, no treatment-related increase in liver tumours or other neoplastic lesions were reported this time. It was noted by the authors that the spontaneous incidence of MNCL in F344 rats is high and variable. Non-neoplastic effects observed included an increase in absolute and relative liver and kidney weights and increased occurrence of histopathological changes at the two highest doses. Non-neoplastic lesions were observed in the livers of high-dose animals (centrilobular and

midzonal hepatocellular enlargement) and an increase in spongiosis hepatitis was observed in mid and high-dose males. Focal necrosis was increased in both sexes from 0.3%, but only significant in high-dose males. In the kidneys, increased tubular cell pigment was noted in high-dose males. Chronic progressive nephropathy was observed, but severity grade was not related to treatment. Caldwell et al. (1999) used immunohistochemical methods on retained kidney tissue, and demonstrated a dose-dependent accumulation of alpha-2-u-globulin in regions of male kidney tissue where proliferation was observed. Food consumption was reduced in high-dose males in the second 12 months of treatment. There was a dose-dependent decrease in male body weight beginning at 12 months and persisting to study termination. At study termination, reduced RBC count, haemoglobin and hematocrit were significantly reduced in males treated with the highest dose. As well, high-dose animals exhibited increased frequency of nucleated RBC, polychromatic red cells and reticulocytes. Serum chemistry revealed weak increases in ALP, SGOT and SGPT in mid and high-dose males (Lington et al. 1997).

In a third carcinogenicity study in rats, animals were exposed to 0, 500, 5000, and 10 000 ppm DINP-A (equivalent to 0, 27, 271, 553 mg/kg bw/day for males and 0, 33, 331, 672 mg/kg bw/day for females) for 2 years. An increase in incidence of hepatocellular carcinoma was noted in females treated with the two highest doses. An increased incidence of neoplastic liver nodules was also observed in treated rats at all doses; however, the observed incidence was not significant. Testicular interstitial hyperplasia was increased in males treated with the highest dose; the incidence of testicular interstitial cell carcinoma was not significantly increased but was outside the historical range. A slight increase in pancreatic islet cell carcinoma and parathyroid gland hyperplasia was noted in males treated with the highest dose. In high-dose females, endometrial hyperplasia and adenocarcinoma were slightly increased. These findings have been described as having uncertain significance. Non-cancer effects reported in this study included increased incidence of focal hepatocellular necrosis in treated males (significant in males treated at the lowest and highest dose). Absolute and relative liver and kidney weights were also increased in males at 10 000 ppm and in females from 5000 ppm. Pathological examination revealed a higher incidence of hepatic spongiosis in males from 5000 ppm and females at 10 000 ppm. Clinical chemistry revealed elevated SGOT, SGPT and ALP at various dose levels and time points; although not statistically significant in most instances, these effects were attributed to treatment (Bio/dynamics 1986).

Finally, a carcinogenicity study with exposure to DINP has also been conducted in mice. Moore 1998b exposed mice for two years to 0, 500, 1500, 4000, and 8000 ppm DINP (equivalent to 0, 90, 276, 742, 1560 mg/kg bw/day for males and 0, 112, 336, 910, 1888 mg/kg bw/day for females) through the diet. An increase incidence of total liver neoplasms was noted in females from 1500 ppm (276-336 mg/kg bw/d) and in males at the two highest doses and the incidence of carcinoma was significantly elevated at the highest dose in males and the two highest doses in females. In a recovery study in which mice were exposed to 0 or 8000 ppm DINP for 78 weeks, followed by 26 weeks of recovery, increase incidence of carcinoma was reported only in females. Non-

neoplastic effects observed included an increase in absolute liver weight, decreased body weight gain in females, increased incidence of liver masses in males, and decreased absolute kidney weight in males were observed at 1500 ppm. In males, absolute and relative liver weights were increased at the highest dose tested. These effects were accompanied by an increased incidence of hepatocellular neoplasia (in females from 1500 ppm and in males from 4000 ppm) and by non-neoplastic hepatic effects at the highest dose (diffuse hepatocellular enlargement, cytoplasmic eosinophilia, pigmentation). At that dose level, palmitoyl-CoA oxidase was significantly elevated in both sexes, suggesting carcinogenicity in mice was related to peroxisomal proliferation. Granular/pitted/rough kidneys (corresponding to treatment-related nephropathy) were noted in females at the highest dose. Survival was decreased significantly in males dosed at the highest dose. In life observations included symptoms of poor health in high-dose animals (hunched posture, hypoactivity, few feces, urine staining), as well as swelling in the ventral-abdominal region in males from 4000 ppm and females at 8000 ppm. Male body weight gain was significantly decreased at the two highest doses (Moore 1998b).

It has been proposed that DINP, like some other phthalates, induce liver tumours in rodents by a mode of action (MOA) directly linked to peroxisome proliferation. More detailed information on the MOA of liver carcinogenicity in rodents with peroxisome proliferators is available in Health Canada (2015d). Information on mode of action considerations for the other types of tumours observed in DINP-treated animals (MNCL, renal tubular cell carcinomas and other tumour types considered uncommon) is also available in this document.

**Table 9-20. Carcinogenicity studies in rodents**

<b>Strain and species; Doses [mg/kg bw/day]; Route; Duration (Reference)</b>	<b>Result</b>
Fischer 344 rats; 0, 500, 1500, 6000, and 12 000 ppm; est. 0, 29, 88, 359, 733 (males); 0, 36, 109, 442, 885 (females); Diet; 2 years  Recovery study; 0, 12 000 ppm; est. 0, 637.3 (males); 0, 773.6 (females); Diet; 78 weeks, followed by 26 weeks recovery  (Moore 1998a)  DINP-1 68515-48-0	Dose-related increase in incidence of MNCL in both sexes from 6000 ppm (males: 22/65, 23/55, 21/55, 32/65, 30/65; females: 17/65, 16/49, 9/50, 30/65, 29/65 at 0, 29 – 36, 88 – 109, 359 – 442, 733/885 mg/kg bw/day, respectively).  Significant increase in hepatocellular carcinoma in males at the highest dose tested (1/65, 0/50, 0/50, 1/65, 12/65 at 0, 29, 88, 359, 733 mg/kg bw/day, respectively) but not in females (1/65, 0/49, 0/50, 1/65, 5/65 at 0, 36, 109, 442, 885 mg/kg bw/day, respectively). Significant increase incidence of carcinoma or adenoma in both sexes at the highest dose (males: 5/65, 3/50, 2/50, 7/65, 18/65; females: 1/65, 1/49, 0/50, 2/65, 8/65 at 0, 29 – 36, 88 – 109, 359 – 442, 733 – 885 mg/kg bw/day, respectively).

<b>Strain and species; Doses [mg/kg bw/day]; Route; Duration (Reference)</b>	<b>Result</b>
	<p>LOAEL (non-neoplastic): 358 – 442 mg/kg bw/day (increase in absolute and relative liver and kidney weights, increase in serum ALT and AST, and histopathological findings in both organs) (males/females)</p> <p>Recovery study: Significant increase in MNCL in both sexes and significant increase in renal tubular carcinomas in males (0/65, 4/50 at 0, 637 mg/kg bw/day, respectively).</p>
<p>Fischer 344 rats; 0, 0.03, 0.3, 0.6%; est. 0, 15, 152, 307 (males); 0, 18, 184, 375 (females); Diet; 2 years (Lington et al. 1997)</p> <p>DINP-1 68515-48-0</p>	<p>Increase in incidence of MNCL at the two highest doses tested in both sexes (males: 33/81, 28/80, 48/80, 51/80; females: 22/81, 20/81, 30/80, 43/80 at 0, 15 – 18, 152 – 184, 307 – 375 mg/kg bw/day, respectively).</p> <p>LOAEL (non-neoplastic): 152 – 184 mg/kg bw/day (increase in absolute and relative liver and kidney weights, and increase in histopathological changes in both organs at the two highest doses) (males/females)</p>
<p>Sprague-Dawley rats; 0, 500, 5000, 10 000 ppm; est. 0, 27, 271, 553 (males); 0, 33, 331, 672 (females); Diet; 2 years (Bio/dynamics 1986)</p> <p>DINP-A 71549-78-5</p>	<p>Increase in incidence of hepatocellular carcinoma in females treated with the two highest doses (males: 2/70, 5/69, 6/69, 4/70; females: 0/70, 0/70, 5/70, 7/70 at 0, 27 – 33, 271 – 331, 553 – 672 mg/kg bw/day, respectively).</p> <p>Increased incidence of neoplastic liver nodules at all doses (not significant) (males: 2/70, 5/69, 6/69, 5/70; females: 1/70, 1/70, 5/70, 2/70 at 0, 27 – 33, 271 – 331, 553 – 672 mg/kg bw/day, respectively).</p> <p>Significant increase in testicular interstitial cell hyperplasia in males at the highest dose. Non-significant increase in the incidence of testicular interstitial cell carcinoma (increase was outside the range of historical control) (2/59, 7/60 at 0, 553 mg/kg bw/day, respectively).</p> <p>Slight increase in pancreatic islet cell carcinoma (1/70,</p>

<b>Strain and species; Doses [mg/kg bw/day]; Route; Duration (Reference)</b>	<b>Result</b>
	<p>4/70 at 0, 553 mg/kg bw/day, respectively) and parathyroid gland hyperplasia in males treated with the highest dose.</p> <p>Slight increase in endometrial hyperplasia and adenocarcinoma in high-doses females (hyperplasia: 2/70, 13/69; adenocarcinoma: 0/70, 2/69 at 0, 672 mg/kg bw/day, respectively).</p> <p>LOEL (non-neoplastic): 27 mg/kg bw/day (histologic changes in the liver) (males)</p>
<p>B6C3F1 mice; 0, 500, 1500, 4000, 8000 ppm; est. 0, 90, 276, 742, 1560 (males); 0, 112, 336, 910, 1888 (females); Diet; 2 years</p> <p>Recovery study; 0, 8000 ppm; est. 0, 1377 (males); 0, 1501 (females); Diet; 78 weeks, followed by 26 weeks recovery</p> <p>(Moore 1998b)</p> <p>DINP-1 68515-48-0</p>	<p>Significant increase in incidence of hepatocellular carcinoma at the two highest doses in females and at the highest dose in males (males: 10/70, 8/67, 10/66, 17/65, 20/70; females: 1/70, 2/68, 5/68, 7/67, 19/70 at 0, 90 – 112, 276 – 336, 742 – 910, 1560 – 1888 mg/kg bw/day, respectively). Significant increase in incidence of total liver neoplasms (carcinomas and adenomas) in females from 336 mg/kg bw/day and in males at the two highest dose (males: 16/70, 13/67, 18/66, 28/65, 31/70; females: 3/70, 5/68, 10/68, 11/67, 33/70 at 0, 90 – 112, 276 – 336, 742 – 910, 1560 – 1888 mg/kg bw/day, respectively).</p> <p>LOAEL (non-neoplastic): 276 – 336 mg/kg bw/day (increase in absolute liver weights accompanied with histopathological changes in the liver at the highest dose and decreased body weight gain) (females); (increase incidence of liver masses and decreased absolute kidney weights) (males)</p> <p>Recovery study: Increased incidence of total liver neoplasms in both sexes. Significant increased incidence of carcinoma in females only.</p>

Overall, the lowest oral doses associated with a significant increase in incidence of tumours are 331 – 336 mg/kg-bw per day, based on significant increase in hepatocellular tumours in female rats and mice, respectively (Bio/dynamics 1986; Moore et al. 1998b).

The lowest oral dose associated with chronic non-cancer effects was 27 mg/kg bw/day, based on histologic changes in the liver of male rats exposed to DINP in a two-year carcinogenicity study (Bio/dynamics 1986); however, the incidence of those changes was not dose-related.

DINP CAS number was not provided in the Bio/dynamics study; however, it was reported as DINP-A (71549-78-5) in a risk assessment report of the US Consumer Product Safety Commission (2010) (Babich 1998). DINP-A has an isomeric composition similar to DINP-2 (28553-12-0). In comparison, the LOAEL in the Lington study was 152 – 184 mg/kg bw/day based also on liver effects in both male and female rats exposed to DINP-1. According to US CPSC (2010b), the difference in the toxic potency between the Bio/dynamics and Lington studies may be due to differences in dose selection, differences in toxicity between the two forms of DINP, and/or the use of different rat strains. Since non cancer effects in the Biodynamic study (1986) were not found to be dose-related, the LOAEL of 152 – 184 mg/kg-bw/d (NOAEL of 15 – 18 mg/kg-bw/d) from the Lington et al. (1997) study is considered more relevant.

### Genotoxicity

In the available genotoxicity studies for DINP, negative results were observed in bacterial mutation assays, with and without metabolic activation (EG and G Mason Research Institute 1980; Zeiger 1985; BASF 1986a; BASF 1995; Exxon Biomedical Sciences 1996d; Mckee 2000). Negative results were also observed in *in vitro* mouse lymphoma assays and Chinese hamster ovary (CHO) cell chromosomal aberration assays, with and without metabolic activation (EGandG Mason Research Institute 1981; Litton Bionetics 1985a; BASF 1986b; Hazleton 1986; Exxon Biomedical Sciences 1996e; Mckee 2000). In addition, DINP did not induce unscheduled DNA repair in primary rat hepatocytes (Litton Bionetics 1981a). DINP also tested negative in an *in vivo* cytogenetics test with bone marrow of rats exposed orally for 5 days (Microbiological associates 1981d) and in an *in vivo* mouse micronucleus assay in which CD-1 mice were given up to 2000 mg DINP/kg-bw per day by oral gavage for 2 days (McKee et al. 2000).

In *in vitro* transformation studies of Balb/c 3T3 cells, DINP was shown to be positive (without metabolic activation) in one of seven studies (Microbiological Associates 1981a,b,c; Litton Bionetics 1981b,c; Microbiological Associates 1982; Litton Bionetics 1985b).

#### **9.2.2.6 Other systemic effects: evidence in humans**

Available epidemiological studies examining the potential relationship between any observed systemic effects and exposure to DINP in humans were reviewed (Appendix F; Health Canada 2015b). There was inadequate evidence for an inverse association between MCIOP and insulin-like growth factor I (IGF-I) (Boas et al. 2010). No associations were found between metabolites of DINP and effects on cardiovascular function (albumin/creatinine ratio, risk of stroke) (Trasande et al. 2014; Shiue 2013).

There were no associations with MCOP and allergic symptoms (Hoppin et al. 2013), or oxidative stress (Ferguson et al. 2011, 2012).

More recent studies have found associations between DINP and various endpoints, but these have not yet been assessed using the Downs and Black evaluation approach. Floor dust levels of DINP were reported to be associated with the risk of allergic rhinitis in children, but not for asthma, conjunctivitis, or dermatitis (Bamai et al. 2014). MCOP was shown to be associated with obesity-related parameters (Christensen et al. 2014b; Buser et al. 2014), osteoporosis (Min and Min 2014). MCOP was associated with one of the biomarkers of oxidative stress, but not with inflammation (Ferguson et al. 2014). No significant association was reported between the metabolites of DINP and blood pressure (Shiue 2014a, b; Shiue and Hristova 2014).

## **9.3 Characterization of Risk to Human Health**

### **9.3.1 Characterization of Risk of DINP**

Based principally on the weight of evidence from the available information, critical effects associated with oral exposure to DINP are carcinogenicity and effects in the liver and developmental effects on the male reproductive system.

The OEHHA has recently reviewed the evidence of the potential carcinogenicity of DINP and it has been concluded that DINP has been clearly shown, through scientifically valid testing according to generally accepted principles, to cause cancer and should be listed under Proposition 65 as a carcinogen (OEHHA 2013a). Accordingly, DINP has been listed at the end of 2013 (OEHHA 2013b). DINP has not been classified for its potential carcinogenicity by other international agencies. DINP has been tested in a number of oral carcinogenicity studies in rats and mice. Statistically significant increases in several tumour types were observed in animals exposed to high doses of this phthalate, from 331 and 336 mg/kg bw/day, in female rats and mice, respectively. It has been assumed that the increased incidence of liver neoplasms observed in both rats and mice is related to peroxisome proliferation. However, the mode of action of liver carcinogenicity in rodents with peroxisome proliferators has not been fully elucidated and the carcinogenic potential of DINP in human liver remains unclear (Health Canada 2015d).

Mononuclear cell leukemia of the spleen was also reported in Fischer rats. However, this type of lesion is likely specific to aging rats of this strain and is unlikely to be relevant to humans (Health Canada 2015d). Renal tubular cell carcinomas were also reported in one chronic study in rats. It has been suggested that the mechanism responsible for these tumours was related to accumulation of  $\alpha_2$ -globulin, a protein specific to the male rat (Health Canada 2015d). While this type of neoplastic lesion has not been observed in female rats, increased kidney weights accompanied by histopathological changes were noted in female rats exposed for 2 years (Moore 1998a) and treatment-related nephropathy was noted in female mice in another chronic study conducted by the same author (Moore 1998b). Those kidney effects cannot be explained by an  $\alpha_2$ -globulin mode of action. Overall, findings in the kidneys of rodents

could be considered of little or unclear relevance to humans. In addition, other tumour types were noted in DINP-treated animals: renal transitional cell carcinoma, pancreatic islet cell carcinoma, testicular interstitial (Leydig) cell carcinoma, and uterine adenocarcinoma. Non-statistically significant increases in incidences of those tumour types were noted but since these types of tumours are considered to be rare and/or uncommon in rats, those findings have uncertain biological significance. As a result, the increase of those tumour types could be considered of little or unclear relevance to humans. The lowest oral doses associated with a significant increase in incidence of tumours are 331 – 336 mg/kg-bw/day, based on significant increase in hepatocellular tumours in female rats and mice, respectively (Bio/dynamics 1986; Moore et al. 1998b).

Consideration of the available information on genotoxicity indicates that DINP is not likely to be genotoxic.

With respect to non-cancer effects, several repeat-dose studies have shown that the liver and kidney are the primary target organs in rodents and dogs following repeated oral exposure to this phthalate. The main effects observed were increases in liver and kidney weights, increased peroxisomal enzyme levels and histological changes in both organs. No liver effect was observed in monkeys exposed orally for up to 13 weeks, which may indicate that monkeys and maybe humans are less sensitive than rodents and dogs to liver effects, which is consistent with the hypothesis that species differences in the activation of PPAR $\alpha$  or its signaling network by peroxisome proliferation may exist. Likewise, no kidney effect was reported in monkeys. However, it is difficult to conclude on the non-relevance of those liver and kidney effects in humans since the monkey studies are limited (only two studies available, no long-term administration, small numbers of monkeys tested, etc.).

For non-cancer effects, the lowest oral LOAEL identified was 152 – 184 mg/kg bw/day (NOAEL of 15 – 18 mg/kg bw/d) based on liver effects in both male and female rats in a chronic carcinogenicity study (Lington et al. 1997).

DINP is also associated with developmental effects on the male reproductive system following exposure *in utero* in rats, but at higher doses compared to those inducing effects on liver and kidneys. These effects were reported to include decreased testicular and serum testosterone levels, decreased AGD, NR, effects in sperm, and evidence of testicular pathology. Effects on pup weight were observed at lower doses than those observed specifically related to RPS. Specific endpoints of decreased AGD and increased NR are considered adverse and these effects are also considered relevant to humans. For further description of the justification of these aspects in the characterization of risk to human health, please see section 9.3 in the medium chain phthalate ester SOS (Environment Canada and Health Canada 2015b).

The principal source of exposure to DINP, for the general population, is expected to be from food, with dust also being a contributor. Two consumer product scenarios, modelling exposure from contact with plastic articles, were also evaluated. Concentrations of DINP metabolites (MINP, MCiOP, and MHiNP) in urine samples were

converted to daily intake estimates of DINP, using NHANES data as a surrogate for the Canadian general population; these estimates represent an internal-dose from all sources.

Comparison of upper-bounding estimates of exposure to DINP from environmental media and food, for infants and children 6 months to 4 years of age, with the NOAEL of 15 – 18 mg/kg bw/day, [based on liver effects in both male and female rats at the next dose tested (Lington et al. 1997)] results in MOEs ranging from 652 – 8333. These MOEs are considered adequate to address uncertainties in the exposure and health effects database for DINP. See Table 9-21 below.

Comparison of upper-bounding estimates, for infants 0 – 18 months of age mouthing plastic toys and articles containing DINP, with the appropriate critical effect levels results in an MOE of 125 and is considered adequate to address uncertainties in the exposure and health effects database for DINP. See Table 9-21 below. Note, migration rates used to estimate exposure were based on concentrations in toys (12.9 - 77%) that are higher than concentrations observed in recent Health Canada surveys (see Table 9-3).<sup>10</sup>

Comparisons of upper-bounding estimates from dermal exposure to DINP from various plastic manufactured items, for infants 0 – 18 months of age, and adjusting for 4% dermal absorption with the appropriate critical effect levels, results in an MOE of 1744 and is considered adequate to address uncertainties in the exposure and health effect databases for DINP on an individual substance basis. See Table 9-21 below.

Comparison of upper-bounding estimates from 95<sup>th</sup> percentile biomonitoring measurements for males aged 20 plus and children aged 6 – 11 result in MOEs ranging from 577 to 625 and are also considered adequate.

These MOEs are also considered adequate as they address effects on the developing male and female reproductive system that occur at higher doses than those inducing liver and kidney effects. Margins of exposure for pregnant women based on conservative upper bounding estimates and the critical effect level (NOAEL) of decreased pup weights as well as decreased testicular testosterone and testicular pathology in male pups are 2173 and above. Margins of exposure for infants based on conservative upper bounding estimates and the critical effect level (NOEL) of decreased absolute seminal vesicle and LABC weights are 833 and above.

**Table 9-21. Summary of margins of exposure to DINP for subpopulations with highest exposure**

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<sup>10</sup> Currently, Canada (along with the US and EU) have regulations (0.1%) in place limiting the amount of certain phthalates (including DINP) in toys and childcare articles.

<b>Age Group and Exposure Scenario</b>	<b>Central tendency (upper bounding) estimate of exposure (<math>\mu\text{g}/\text{kg}</math> per day)</b>	<b>Margin of exposure (MOE)<sup>d</sup> based on a oral NOAEL of 15 mg/kg bw/day from Lington et al. (1997)</b>
Children (females) 6 – 11 years: biomonitoring, 95th percentile, NHANES <sup>b</sup>	3.8 (26)	3947 (577)
Infants/children 0.5 – 4 years: food and dust, oral	1.8 (19.7)	8333 (761)
Infants (0 – 18 months): mouthing plastic toys and articles, oral	30 <sup>c</sup> (120)	500 (125)
Infants (0 – 18 months): exposure to plastic articles, dermal	1.1 <sup>c</sup> (8.6)	13 636 (1744)
Adults (females) 20 + years: biomonitoring: 95 <sup>th</sup> percentile, NHANES <sup>b</sup>	2.3 (23)	6522 (652)
Adults (males) 20 + <sup>a</sup> years: biomonitoring, 95th percentile, NHANES <sup>b</sup>	2.8 (24)	5357 (625)
Teens 12 – 19 years: food and dust, oral	1.0 (11.4)	15 000 (1315)
Adults (females) 20+ <sup>a</sup> years: exposure to plastic articles, dermal	1.1 <sup>c</sup> (3.4)	13 636 (4412)

<sup>a</sup> MOEs were calculated for non-pregnant individuals (male and female) and pregnant females for this age group.

<sup>b</sup> The highest intakes at the 95<sup>th</sup> percentile (33  $\mu\text{g}/\text{kg}$  bw/d: 12 to 19 males and 27  $\mu\text{g}/\text{kg}/\text{day}$ : 12 – 19 females) were not brought forward to risk characterization because the relative standard error of the data was greater than 30%. For children, aged 6 – 11 years old, 26  $\mu\text{g}/\text{kg}$  bw/d (RSE > 30%) was carried forward to risk characterization in order to be protective of this age group and due to the absence of low variability data, at the upper percentiles, for another comparable age group.

<sup>c</sup> Estimated lower end exposure.

<sup>d</sup> Margin of Exposure: central tendency and (upper bounding)

Comparison of upper-bounding estimates from the highest exposed age group (children aged 6 – 11 years; 95<sup>th</sup> percentile biomonitoring measurements), with the lowest dose associated with a significant increase in incidence of tumours (331 – 336 mg/kg-bw per day, based on significant increase in hepatocellular tumours in female rats and mice, respectively) results in MOEs of 87 105 - 88 421 (central tendency) and 12 731 – 12 923 (upper bounding). These MOEs are considered adequate to address uncertainties in the exposure and health effect databases for DINP. These MOEs are also considered adequate as they address the significant increase in incidence of other types of tumours associated with exposure to DINP (MNCL and renal tubular cell carcinomas) that occur at higher doses than those inducing hepatocellular tumours in rats and mice and the non-statistically significant increases in incidences of tumour types considered to be

rare and/or uncommon which have unclear relevance to humans (e.g., uterine adenocarcinoma, observed in female rats from 672 mg/kg bw/d). Based on the information available, it can be concluded that DINP has effects on the developing male reproductive system based on the evidence of male reproductive developmental effects of RPS at high doses, indicating that DINP may be a weak antiandrogen. Although the above MOEs are considered adequate on an individual basis, this does not address potential risk of concurrent exposure to DINP and other phthalates exhibiting a similar mode of action.

## Considerations

For DINP, the driver of indirect exposure is from food with dust also being a contributor. Due to the notification of this substance, in manufactured items that may come in contact with skin, an exposure scenario was conducted to evaluate dermal exposure from dermal contact (adults and infants). Finally, as DINP may also be present in children's toys and articles, potential oral exposure from mouthing these products was also evaluated.

With respect to the use of adhesive, sealants, and coatings which contain DINP, exposure would not be considered to be of concern for human health based on the following:

Dermal absorption of DINP in rats is low (4%), and evidence shows that human skin is less permeable than rat skin to phthalate diesters. Also, retention in skin is 3 to 6 fold higher in rat compared to human (Mint and Hotchkiss 1993; Mint et al. 1994). Distribution in tissues of rats is generally low showing no accumulation, and excretion is rapid, within hours to days.

Exposure from use of these products would be of very short duration (acute) via the dermal route.

Phthalates in general, are not considered acute toxicants, with LD<sub>50</sub> levels from dermal exposure being at minimum 2 – 5 fold higher than oral values (Draize et al. 1948; Eastman Kodak 1978; David et al. 2001; Monsanto Company 1970, cited in US EPA 2006, 2010).

Acute dermal toxicokinetic information indicates that reproductive organs are not a target organ, and that presence and residence time in other tissues (adipose and muscle) is extremely low after 7 days (0.02 to 0.3% of applied-dose; Elsis et al. 1989).

This is consistent with the assessments of other jurisdictions who have focussed their assessment on repeated exposures (ECHA 2013a; US CPSC CHAP 2014).

## 9.4 Uncertainties in Evaluation of Risk to Human Health

The majority of the studies in the health effects database for DINP related to reproductive-developmental toxicity are limited to one species (rat) and mainly in males. There is some uncertainty associated not only with the potential biological significance of effects, but also in sensitivity of effects after exposure to this substance group in both female and male humans, but current information does not allow for conclusions to state otherwise.

There are also no, or limited, information on repeated-dose effects via inhalation and dermal routes of exposure, respectively. There are limited to no studies by any route of administration on neurodevelopmental toxicity of DINP.

There is uncertainty associated with the mode of induction of tumours. Postulated modes of action have been identified for some tumour-types, but they have not been fully elucidated.

Although a rigorous evaluation approach was conducted with the available human epidemiological data, uncertainty still exists in the relevance of these studies implicating the potential hazard of DINP to humans. Thoroughly conducted epidemiologic studies showing robust and consistent associations between an exposure factor and an outcome may provide strong implication for causal inference. However, observational studies in diverse populations pose challenges in both the measure of exposure and the measure of the outcome, and inherently have biases and confounding factors (Lucas and McMichael 2005). The majority of epidemiological studies examined were cross-sectional in which a temporal sequence whereby exposure precedes the outcome cannot be established. In addition, several outcomes associated with phthalate exposure in human epidemiological studies have long latencies (such as cancer, diabetes, obesity, cardiovascular disease) and multifactorial etiologies (geographical location, socioeconomic status, diet, lifestyle factors, genetic propensity, nonchemical stressors) and are chronic in nature, whereas phthalates have short biological half-lives and their measurement therefore reflects a snapshot of recent exposure. Moreover, biomonitoring data shows that exposure to certain phthalates is ubiquitous and therefore cannot be dichotomized as present or absent but is instead a continuous variable, often with a limited range.

While it has been argued that even in the absence of consistent methods, a robust association should yield consistent findings (La Kind et al., 2012), poor reproducibility continues to feature prominently in epidemiological studies involving phthalates. Adding to the lack of clarity is the fact that humans are simultaneously exposed to multiple phthalates from multiple sources via multiple routes, as well as other environmental agents that may share coinciding effect domains, including bisphenol A, certain metals and organochlorine compounds, such as PCBs, dioxins and various persistent organic pesticides. In its final report in 2014, the US Chronic Hazard Advisory Panel (CHAP) on Phthalates concluded that although there is a growing body of studies reporting associations between phthalate exposure and human health, and many of the reported

health effects are consistent with testicular dysgenesis syndrome in humans, there are acknowledged limitations of these studies similar to those described above. These were therefore not used in risk characterization (US CPSC CHAP 2014). Another recent review also found that epidemiological evidence for associations with reproductive and developmental effects from phthalates is minimal to weak in most cases (Kay et al. 2014).

There are uncertainties associated with estimating intakes of DINP from environmental media due to minimal monitoring data available in air, drinking water and soil. Confidence is moderate to high that derived intake estimates from household dust are representative of the potential exposure of the general Canadian population, since the exposure estimates are based on a Canadian house dust monitoring study.

There is uncertainty in estimating dermal exposure from contact with products used by consumers (e.g., plastic articles), based on limited substance specific information with regards to the presence and migration over time of phthalates from these products. Therefore it is unclear as to how much phthalate is available for transfer to skin from contact. Additional uncertainty is associated with parameters used (e.g., dermal absorption, migration rate) in estimating exposure from products used by consumers; however, there is confidence that the assumptions used were conservative. Finally, there is uncertainty related to exposure of DINP from presence in automotive applications and medical devices, as there is insufficient information to quantify exposure from these sources.

Confidence is moderate to high that derived dietary exposure estimates for DINP are representative of the general population of Canada, as recent Canadian dietary monitoring data were available; however, uncertainty exists regarding methodology issues of measuring mixtures in various food matrices.

There are a number of assumptions that have been made to derive intake estimates from biomonitoring data which represent a source of uncertainty; i.e., assumptions that spot urine samples are representative of steady state daily concentrations, and assumptions around the use of creatinine corrected concentrations. However, there is confidence that the assumptions used in deriving estimates of intakes are appropriate and conservative.

Additionally, there is uncertainty related to the use of NHANES derived DINP intakes as a surrogate for the Canadian population and around the quantification of mixtures of metabolites in urine samples (observed multiple peaks). However, confidence in the biomonitoring database for DINP is moderate to high as it represents a substantially large number of data points collected recently in North American individuals spanning over a wide age spectrum, and including Canadian subpopulations such as pregnant women and children.

Due to the lack of or limited health effects data for all relevant routes and durations of exposure, route-to-route extrapolation was required and/or use of effect levels from

studies with longer durations of exposure than the exposure scenarios was applied. In the case of inconsistencies in duration scenarios, provided that the daily exposure is being compared with health effect levels from animal studies of longer duration, confidence is high that the derived MOEs are conservative.

Uncertainty is recognized in the potential oral bioavailability of medium chain phthalates, in particular the estimated systemic exposure at which effects were observed in animal studies after administration. Information exists that absorption of these phthalates are highly variable (30 – 95%) and are influenced by rates of metabolism and excretion of an organism and by different routes at any given time of measurement. These limitations do not allow for accurate adjustments in characterization of risk for each phthalate. However, estimated MOEs are considered adequate to account for this uncertainty.

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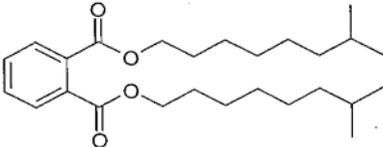
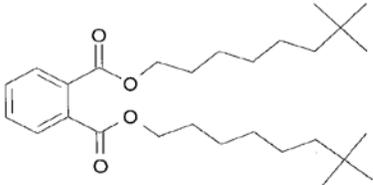
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## Appendices

### Appendix A. Information on Analogues used for DINP

**Table A-1. Structures and property data for DINP and analogue substance used to inform the assessment of DINP**

CAS RN Common name	Representative chemical structure <sup>a</sup>	Representative molecular formula / molecular weight (g/mol) / chemical properties <sup>a</sup>	Similarity index (%) <sup>b</sup>
Target substance: 28553-12-0 68515-48-0 Diisononyl phthalate (DINP)		$C_{26}H_{42}O_4$ MW: 418.62 Low water solubility ( $6.1 \times 10^{-4}$ mg/L) Log $K_{ow}$ : 8.8 Log $K_{oc}$ : 5.5 – 5.7 $D_{max}$ , $D_{eff}$ : 28 – 30, 19 – 20 nm	n/a
Analogue substance: 26761-40-0 68515-49-1 Diisodecyl phthalate (DIDP)		$C_{28}H_{46}O_4$ MW : 446.68 Low water solubility ( $1.7 \times 10^{-4}$ mg/L) Log $K_{ow}$ : > 8 Log $K_{oc}$ : 5.5 – 6.5 $D_{max}$ , $D_{eff}$ : 27 – 30, 19 – 20 nm	with DINP: 85 – 94

Abbreviations:  $D_{eff}$ , effective molecular cross-sectional diameter;  $D_{max}$ , maximum molecular diameter; MW, molecular weight.

Sources: Chemical structure and property data for DINP are from Table 2-1 and Appendix B of this report; data for DIDP are from Environment Canada and Health Canada 2015c. All  $D_{max}$  and  $D_{eff}$  values are from CPOPs 2012.

<sup>a</sup> DIDP and DINP are isomeric mixtures. As such, the chemical structures, formulae and molecular weights provided in Table A-1 are considered to be representative for the substances.

<sup>b</sup> Source: OECD QSAR Toolbox 2012.

## Appendix B. Physical and Chemical Properties for DINP

Table B-1. Physical and chemical properties for DINP

CAS RN Acronym	Physical form	Melting point (°C)	Boiling point (°C)	Density (kg/m <sup>3</sup> )	Vapour pressure (Pa)
28553-12-0	Liquid <sup>a</sup>	-34 – -54 <sup>†</sup> (Exp) <sup>a</sup>  84.91 (Mod) <sup>b</sup>	331 – 341 <sup>†</sup> (Exp) <sup>c</sup>  440.16 (Mod) <sup>b</sup>	967 – 983 (Exp) <sup>a</sup>	7.2 × 10 <sup>-5</sup> (Exp, 25°C) <sup>d</sup>  6.7 × 10 <sup>-5†</sup> (Exp, 25°C) <sup>e,f</sup>  6.8 × 10 <sup>-6</sup> (Cal, 25°C) <sup>g</sup>  2.87 × 10 <sup>-3</sup> (Mod, 25°C) <sup>b</sup>
68515-48-0	Liquid <sup>a</sup>	-48 <sup>†</sup> (Exp) <sup>a</sup>  115.29 (Mod) <sup>b</sup>	> 400 <sup>†</sup> (Exp) <sup>c</sup>  454.14 (Mod) <sup>b</sup>	974 (Exp) <sup>a</sup>	6.0 × 10 <sup>-5</sup> (Exp, 20°C) <sup>c</sup>  6.7 × 10 <sup>-5†</sup> (Exp, 25°C) <sup>e,f</sup>  6.8 × 10 <sup>-6</sup> (Cal, 25°C) <sup>g</sup>  1.11 × 10 <sup>-3</sup> (Mod, 25°C) <sup>b</sup>

Abbreviations: Cal, calculated value; Exp, experimental value; Mod, modelled value.

<sup>†</sup>Indicates selected value for modelling.

<sup>a</sup> European Commission 2000. <sup>b</sup> MPBPVWIN 2010. <sup>c</sup> ECHA 2014. <sup>d</sup> Howard et al. 1985. <sup>e</sup> Staples et al. 1997. <sup>f</sup> Mackay et al. 2006. <sup>g</sup> Cousins and Mackay 2000.

Table B-2. Physical and chemical properties for DINP (continued)

CAS RN	Water solubility (mg/L)	Henry's law constant (Pa·m <sup>3</sup> /mol)	Log K <sub>ow</sub> (unitless)	Log K <sub>oc</sub> (unitless)	Log K <sub>oa</sub> (unitless)
28553-12-0	6.1 × 10 <sup>-4†</sup> (Exp, 22°C) <sup>a</sup>				
	0.2 (Exp, 25°C) <sup>b</sup>				
	< 0.001 (Exp, 25°C) <sup>c,d</sup>	9.26 (Cal, 25°C) <sup>e</sup>	8.8 <sup>†</sup> – 9.7 (Exp) <sup>l</sup>		
	3.08 × 10 <sup>-4</sup> (Cal, 25°C) <sup>e</sup>	2.11 (Mod, Bond estimate, 25°C) <sup>j</sup>	8.6 (Cal) <sup>e</sup>	5.52 (Mod, MCI estimate) <sup>n</sup>	11.03 (Cal) <sup>e</sup>
	3.57 × 10 <sup>-4</sup> (Mod, 25°C) <sup>f</sup>	2.06 (Mod, Group estimate, 25°C) <sup>j</sup>	9.37 (Mod) <sup>m</sup>	5.66 (Mod, Log K <sub>ow</sub> estimate) <sup>n</sup>	13.02 (Mod) <sup>o</sup>
	1.15 × 10 <sup>-4</sup> (Mod, 25°C) <sup>g</sup>	45.7 (Mod, VP/WS estimate, 25°C) <sup>j,k</sup>	8.70 (Mod) <sup>h</sup>		
	1.6 × 10 <sup>-2</sup> (Mod, 25°C) <sup>h</sup>		8.41 (Mod) <sup>i</sup>		
	0.100 (Mod, 25°C) <sup>i</sup>				

CAS RN	Water solubility (mg/L)	Henry's law constant (Pa·m <sup>3</sup> /mol)	Log K <sub>ow</sub> (unitless)	Log K <sub>oc</sub> (unitless)	Log K <sub>oa</sub> (unitless)
68515-48-0 DINP	6.0 × 10 <sup>-4†</sup> (Exp, 20°C) <sup>l</sup>				
	3.08 × 10 <sup>-4</sup> (Cal, 25°C) <sup>e</sup>	9.26 (Cal, 25°C) <sup>e</sup>	8.8 <sup>†</sup> (Exp) <sup>l</sup>		
	3.57 × 10 <sup>-4</sup> (Mod, 25°C) <sup>f</sup>	2.11 (Mod, Bond estimate, 25°C) <sup>j</sup>	8.6 (Cal) <sup>e</sup>	5.67 (Mod, MCI estimate) <sup>n</sup>	11.03 (Cal) <sup>e</sup>
	4.11 × 10 <sup>-5</sup> (Mod, 25°C) <sup>g</sup>	1.43 (Mod, Group estimate, 25°C) <sup>j</sup>	9.52 (Mod) <sup>m</sup>	5.66 (Mod, Log K <sub>ow</sub> estimate) <sup>n</sup>	11.87 (Mod) <sup>o</sup>
	3.5 × 10 <sup>-5</sup> (Mod, 25°C) <sup>h</sup>	46.5 (Mod, VP/WS estimate, 25°C) <sup>j,k</sup>	10.23 (Mod) <sup>h</sup>		
	0.028 (Mod, 25°C) <sup>i</sup>		8.74 (Mod) <sup>i</sup>		

Abbreviations: Cal, calculated value; Exp, experimental value; log K<sub>oc</sub>, organic carbon-water partition coefficient; log K<sub>ow</sub>, octanol-water partition coefficient; log K<sub>oa</sub>, organic carbon-air partition coefficient; Mod, modelled value.

<sup>†</sup>Indicates selected value for modelling.

<sup>a</sup> Letinski et al. 2002. <sup>b</sup> Howard et al. 1985. <sup>c</sup> Staples et al. 1997. <sup>d</sup> Mackay et al. 2006. <sup>e</sup> Cousins and Mackay 2000. <sup>f</sup> WSKOWWIN 2010. <sup>g</sup> WATERNT 2010. <sup>h</sup> ACD/Percepta c1997–2012. <sup>i</sup> VCCLab 2005. <sup>j</sup> HENRYWIN 2011. <sup>k</sup> VP/WS estimate derived using empirical values for vapour pressure and water solubility. <sup>l</sup> ECHA 2014. <sup>m</sup> KOWWIN 2010. <sup>n</sup> KOCWIN 2010. <sup>o</sup> KOAWIN 2010.

## Appendix C. Dust and Food Intakes of DINP for the General Population

**Table C. Central tendency and (upper-bounding) estimates of daily intake of DINP ( $\mu\text{g}/\text{kg}/\text{day}$ )**

Route of exposure	0 – 0.5 year <sup>a</sup> ; Breast milk fed <sup>b</sup>	0 – 0.5 year <sup>a</sup> ; Formula fed <sup>c</sup>	0 – 0.5 year <sup>a</sup> ; Not formula fed	0.5 – 4 years <sup>d</sup>	5 – 11 years <sup>e</sup>	12 – 19 years <sup>f</sup>	20 – 59 years <sup>g</sup>	60+ years <sup>h</sup>
Food and beverage <sup>s</sup>		N/A (9.2)	N/A (9.2)	1.4 (17.8)	1.3 (14.0)	1.0 (11.4)	0.69 (6.9)	0.52 (8.6)
Dust <sup>j</sup>	0.57 – (2.7)	0.57 – (2.7)	0.57 – (2.7)	0.40 (1.9)	0.19 (0.88)	0.0068 (0.032)	0.0065 (0.030)	0.0062 (0.029)
Total Oral intake	0.57 (2.7)	0.57 (11.9)	0.57 (11.9)	1.8 (19.7)	1.5 (14.9)	1.0 (11.4)	0.70 (6.9)	0.53 (8.6)

<sup>a</sup> Assumed to weigh 7.5 kg, to breathe 2.1 m<sup>3</sup> of air per day, to drink 0.2 L/day (not formula fed) and to ingest 30 mg of soil per day. Consumption of food groups reported in Health Canada (1998). Median and 90th dietary intake estimates (food) for the < 6 months age group, as presented in Table 9-2, were used to represent dietary intake for this age group (applicable to the non-formula fed group). F notates that coefficients of variation associated with intake estimates were not sufficiently low to allow for reporting the intake value.

<sup>b</sup> Infants 0 – 6 months assumed to ingest 0.742 litre breast milk/day (USEPA, 2011). No data were identified on the levels of DINP in breast milk. The P4 study, also reported non detection of the monoester MINP.

<sup>c</sup> Formula-fed infants are assumed to have an intake rate of 0.75 kg of formula per day. DINP is present in 5 of 32 infant formula samples (range ND – 0.590 ppm, Personal communication Food Directorate to Existing Substances Risk Assessment Bureau, April 2014)

<sup>d</sup> Assumed to weigh 15.5 kg, to breathe 9.3 m<sup>3</sup> of air per day, to drink 0.7 L of water per day and to ingest 100 mg of soil per day. Consumption of food groups reported in Health Canada (1998). Median and 90th dietary intake estimates (food) for the 1 to 3 years age group, as presented in Table 9-2, were used to represent dietary intake for this age group.

<sup>e</sup> Assumed to weigh 31.0 kg, to breathe 14.5 m<sup>3</sup> of air per day, to drink 1.1 L of water per day and to ingest 65 mg of soil per day. Consumption of food groups reported in Health Canada (1998). Median and 90th dietary intake estimates (food) for the 4 to 8 years age group, as presented in Table 9-2, were used to represent dietary intake for this age group.

<sup>f</sup> Assumed to weigh 59.4 kg, to breathe 15.8 m<sup>3</sup> of air per day, to drink 1.2 L of water per day and to ingest 30 mg of soil per day. Consumption of food groups reported in Health Canada (1998). Highest median and 90th dietary intake estimates (food) for the 9 – 13 years age group, as presented in Table 9-2, were used to represent dietary intake for this age group

<sup>g</sup> Assumed to weigh 70.9 kg, to breathe 16.2 m<sup>3</sup> of air per day, to drink 1.5 L of water per day and to ingest 30 mg of soil per day. Consumption of food groups reported in Health Canada (1998). Highest median and 90th dietary intake estimates (food) for the 19 to 30 years age group, as presented in Table 9-2, were used to represent dietary intake for this age group.

<sup>h</sup> Assumed to weigh 72.0 kg, to breathe 14.3 m<sup>3</sup> of air per day, to drink 1.6 L of water per day and to ingest 30 mg of soil per day. Consumption of food groups reported in Health Canada (1998). Highest median and 90th dietary intake estimates (food) for the > 71 years age group, as presented in Table 9-2, were used to represent dietary intake for this age group.

<sup>i</sup> Probabilistic intakes (median and 90<sup>th</sup>) were incorporated into dietary intake table for comparison purposes. Intakes and methodology are outlined in Appendix D (see also Table 9-2). Note gender and age groups do not match fully; therefore the highest intake from within an age group was inputted into the table: e.g., Female intakes (>71 years) were inputted into the 60+ (unisex) column because this age group had the highest intake of all the groups in the 51 – 71 year range. N/A notates significant variation therefore estimates not presented

<sup>j</sup>The ingestion of indoor dust is considered a significant source of indoor exposure to Phthalates, including DINP, and the amount of indoor dust ingested each day is based on Wilson et al. (2013). The median (112 ug/g) and 95<sup>th</sup> (527 ug/g) percentile concentrations of DINP identified in indoor dust were used for exposure characterization (Kubwabo et al. 2013).

## **Appendix D. Derivation of dietary intakes**

### **Occurrence data – DINP**

Phthalate occurrence data for DINP were available from foods sampled as part of the 2013 – 2014 Food Safety Action Plan (FSAP) survey conducted by the Canadian Food Inspection Agency (CFIA); this dataset was determined to be the most recent and comprehensive Canadian survey of the occurrence of these phthalates in foods. Duplicate foods were included in earlier CFIA FSAP surveys (i.e., 2011 – 2012 and 2012 – 2013) therefore only data from the most recent (i.e., 2013 – 2014) FSAP survey were employed in the exposure assessment. Occurrence data for DINP in foods not analysed as part of the CFIA surveys, were obtained from an American total diet study (Schechter et al. 2013) and any remaining data gaps were filled using data from a British total diet study (Bradley et al. 2013). Note that these data were only used to fill data gaps. Duplicate occurrence data from these studies for a given food or phthalate were not included if such data were already available from the CFIA's 2013 – 2014 FSAP survey.

Occurrence data for these five phthalates in food that were reported as less than the analytical LOD were assigned values of ½LOD. However, a value of 0 (zero) was assigned to all samples within a broad food category when no phthalates were detected above the LOD in any sample in that category.

### **Food consumption data and matching to occurrence data**

The phthalate concentrations in individual foods were matched to consumption figures for these foods from the Canadian Community Health Survey (CCHS) Cycle 2.2 on Nutrition, (Statistics Canada, 2004), to generate distributions of phthalates exposure for various age-sex groups. The CCHS included 24-hour dietary recall information for over 35 000 respondents of all ages across Canada.

If a food line item belonged to a recipe that was matched to a set of the assayed foods, then the associated phthalate levels matched to the recipe were assigned to the ingredient. Otherwise, if the food line item itself matched to a set of the assayed foods then the phthalate levels matched to the food line item were assigned. For DINP, 1003 foods and 153 recipes were matched with the list of assayed foods.

### **Body weight information**

For the purpose of determining per kilogram body weight exposure estimates, infant body weights were set to the mean body weights as derived from the body weight data from United States Department of Agriculture Continuing Survey of Food Intakes by Individuals (CSFII; 1994-96, 1998). For all age groups, body weights reported in the CCHS, whether measured or self-reported, were used and where missing were imputed using the median for the corresponding age-sex group and quintile of energy intake.

### Probabilistic exposure assessment

For each food consumed by a respondent in the CCHS survey, phthalate concentrations were randomly selected from the matching list of assayed values. For each individual respondent, exposure estimates from each food were summed, generating a distribution of exposure for all respondents. This was repeated 500 times (500 iterations) to model the variability of the distribution of exposures due to the variability of the phthalates levels. For each age-sex group, the median and 90th percentile exposures were derived from the empirical distribution generated by the 500 iterations.

## Appendix E. Derivation of daily intakes for DINP based on biomonitoring

### P4 and MIREC CD+:

#### Equation 1:

$$\text{Daily intake} \left( \frac{\mu\text{g}}{\text{kg bw. day}} \right) = \frac{C_{\text{SUM}} \left( \frac{\text{moles}}{\text{g Cr}} \right) \times \text{CER} \left( \frac{\text{g}}{\text{day}} \right) \times \text{MW}_{\text{parent}} \left( \frac{\text{g}}{\text{mole}} \right)}{\text{FUE}_{\text{Sum}} \times \text{BW (Kg)}}$$

Where,

$C_{\text{SUM}} \left( \frac{\text{moles}}{\text{g Cr}} \right)$  = sum of molar concentrations of the metabolites

$\text{CER} \left( \frac{\text{g}}{\text{day}} \right)$  = Creatinine excretion rate using Mage equation

$\text{MW}_{\text{parent}} \left( \frac{\text{g}}{\text{mole}} \right)$  = Molecular weight, DINP: 418 g/mol

$\text{FUE}_{\text{Sum}}$  = Sum of fractional urinary excretion values of the metabolites MHINP and MOINP = 0.18

$\text{BW (Kg)}$  = Body weight of the participant

**Step 1:** Converting the urinary metabolite concentration from  $\mu\text{g/g Cr}$  to moles/g Cr

**Equation 2:**

$$C_{\text{metabolite}} \left( \frac{\text{moles}}{\text{g Cr}} \right) = \frac{C_{\text{metabolite}} (\mu\text{g/g Cr})}{MW_{\text{metabolite}}}$$

DINP metabolites: MHINP and MOINP

For MHINP,

$$C_{\text{MHINP}} \left( \frac{\text{moles}}{\text{g Cr}} \right) = \frac{C_{\text{MHINP}} (\mu\text{g/g Cr})}{308 \text{ g/mol}}$$

For MOINP,

$$C_{\text{MOINP}} \left( \frac{\text{moles}}{\text{g Cr}} \right) = \frac{C_{\text{MOINP}} (\mu\text{g/g Cr})}{306 \text{ g/mol}}$$

**Step 2:** Sum the metabolite concentration (moles/g Cr) from Step 1

$$C_{\text{SUM}} \left( \frac{\text{moles}}{\text{g Cr}} \right) = \Sigma C_{\text{MHINP}} + C_{\text{MOINP}}$$

**Step 3:** Compute CER for individual participants using Mage equation

**Step 4:** Calculate intake using Equation 1

## NHANES

Statistical analysis: The data were analyzed with SAS 9.2 (SAS Institute Inc., USA) and SUDAAN 10.0.1 software (RTI International, USA). Variance estimates were produced using the Taylor Series Linearization approach as recommended by the NHANES analytical guidelines. All analyses were weighted using the NHANES survey weights (environmental subsample) in order to be representative of the U.S. population. Phthalates concentrations that were below LOD were assigned a value of LOD/2.

Estimation of creatinine excretion rate (CER): For each study participant creatinine excretion rate was calculated using the Mage equations (Huber et al. 2010). The adiposity adjustment (discussed in the supplemental information (Huber et al. 2010)) was applied for all participants and the body surface area adjustment was applied for children under the age of 18. Median BMIs by age for the adiposity adjustment were computed using the entire NHANES sample. The 2009-2010 and 2011-2012 NHANES phthalates datasets had 58 and 49 children who exceeded the height limits in the Mage equations (186 cm for males and 172 cm for females). The Mage equations were

applied directly to the observed heights in order to extrapolate creatinine excretion rates for these participants. The predicted excretion rates for these individuals appeared to be reasonable despite the extrapolation.

Daily intake estimation: The daily intake of each phthalate was estimated for each participant using the following equations and procedure (David et al. 2000; Koch et al. 2007):

### Equation 1:

$$\text{Daily intake} (\mu\text{g}/\text{kg bw. day}) = \frac{C_{\text{SUM}} \left( \frac{\text{moles}}{\text{g Cr}} \right) \times \text{CER} \left( \frac{\text{g}}{\text{day}} \right) \times \text{MW}_{\text{parent}} \left( \frac{\text{g}}{\text{mole}} \right)}{\text{FUE}_{\text{Sum}} \times \text{BW (Kg)}}$$

Where,

$C_{\text{SUM}} \left( \frac{\text{moles}}{\text{g Cr}} \right)$  = sum of molar concentrations of the metabolites

$\text{CER} \left( \frac{\text{g}}{\text{day}} \right)$  = Creatinine excretion rate using Mage equation

$\text{MW}_{\text{parent}} \left( \frac{\text{g}}{\text{mole}} \right)$  = Molecular weight, DINP: 418 g/mol

$\text{FUE}_{\text{Sum}}$  = Sum of fractional urinary excretion values of the metabolites MINP and MCIOP = 0.13

$\text{BW (Kg)}$  = Body weight of the participant

. **Step 1:** Converting the urinary metabolite concentration from  $\mu\text{g/g Cr}$  to moles/g Cr

### Equation 2:

$$C_{\text{metabolite}} \left( \frac{\text{moles}}{\text{g Cr}} \right) = \frac{C_{\text{metabolite}} (\mu\text{g}/\text{g Cr})}{\text{MW}_{\text{metabolite}}}$$

DINP metabolites: MINP and MCIOP

For MINP,

$$C_{\text{MINP}} \left( \frac{\text{moles}}{\text{g Cr}} \right) = \frac{C_{\text{MINP}} (\mu\text{g}/\text{g Cr})}{292 \text{ g/mol}}$$

For MCIOP,

$$C_{\text{MCIOP}} \left( \frac{\text{moles}}{\text{g}} \right) = \frac{C_{\text{MCIOP}} (\mu\text{g}/\text{g Cr})}{322 \text{ g/mol}}$$

**Step 2:** Sum the metabolite concentration (moles/g Cr) from Step 1

$$C_{\text{SUM}} \left( \frac{\text{moles}}{\text{g Cr}} \right) = \Sigma C_{\text{MINP}} + C_{\text{MCIOP}}$$

**Step 3:** Compute CER for individual participants using Mage equation

**Step 4:** Calculate intake using Equation 1

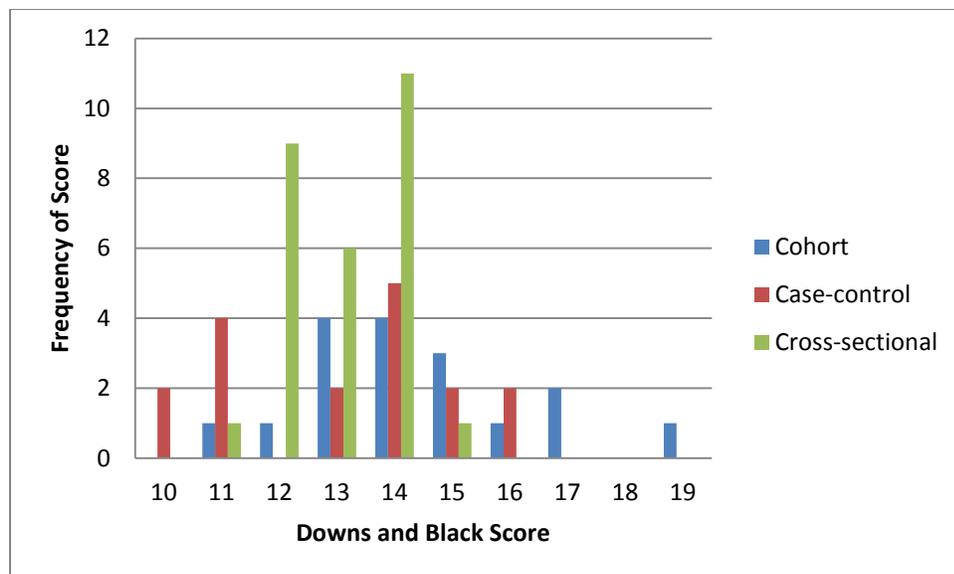
For each selected phthalate diester, the daily intake for each study participant was computed using equation 1. Arithmetic and geometric means, and selected percentiles along with their 95% confidence intervals of daily intake were produced for the U.S. population by age group and sex. Descriptive statistics were computed using SUDAAN proc DESCRIPT.

## **Appendix F. Description and Application of the Downs and Black Scoring System and Guidance for Level of Evidence of An Association**

### **Evaluation of study quality**

A number of systematic approaches for assessing the quality of epidemiologic studies were identified and evaluated. The Downs and Black method was selected based on (1) its applicability to the phthalate database; (2) applicability to multiple study designs; (3) established evidence of its validity and reliability; (4) simplicity; (5) small number of components; and (6) epidemiologic focus. Downs and Black consists of a checklist of 27 questions broken down into the following five dimensions 1) reporting; 2) external validity; 3) internal validity study bias; 4) internal validity confounding and selection bias; and 5) study power. Overall study quality is based on a numeric scale summed over the five categories. The range of the scale allows for more variability in rating study quality. The 27 questions are applicable to observational study designs including case-control, cohort, cross-sectional, and randomized controlled trials.

Studies retained for further assessment were scored for quality using the Downs and Black tool. As previously mentioned, the Downs and Black allows for a range of scores from 27 questions and each epidemiological study design has a maximum score (the maximum score for cohort studies is 21, case-control studies 18, and cross-sectional studies 17). Studies were divided into quartiles based on the scoring distribution for each study design and the distribution of scores for cohort, case-control and cross-sectional studies appears in Figure F-1. The average scores for cross-sectional and case-control studies were 13.1, whereas cohort studies had higher scores than both other study designs with an average score of 14.4.



**Figure F-1. Distribution of Downs and Black scores by study design.**

### Guidance for level of evidence of an association

The potential for an association between phthalate exposure and each health outcome was assessed based on strength and consistency as well as the quality of the epidemiology studies as determined by the Downs and Black scores. Descriptions of the levels of evidence of association are as follows:

1. **Sufficient evidence of an association:** Evidence is sufficient to conclude that there is an association. That is, an association between exposure to a phthalate or its metabolite and a health outcome has been observed in which chance, bias and known confounders could be ruled out with reasonable confidence. Determination of a causal association requires a full consideration of the underlying biology/toxicology and is beyond the scope of this document.
2. **Limited evidence of an association:** Evidence is suggestive of an association between exposure to a phthalate or its metabolite and a health outcome; however, chance, bias or confounding could not be ruled out with reasonable confidence.
3. **Inadequate evidence of an association:** The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of an association.
4. **Evidence suggesting no association:** The available studies are mutually consistent in not showing an association between the phthalate of interest and the health outcome measured.