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Memorandum

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TO : Mary Ann Danello, Ph.D., Associate Executive Director for Health Sciences

THROUGH: Lori E. Saltzman, M.S., Director, Division of Health Sciences

FROM : Michael A. Babich, Ph.D., Chemist, Division of Health Sciences
Cheryl A. Osterhout, Ph.D., Pharmacologist, Division of Health Sciences

SUBJECT : Toxicity Review of Diisononyl Phthalate (DINP)[1]

The attached report provides the U.S. Consumer Product Safety Commission's (CPSC's) Health Sciences' staff assessment of the potential toxicity associated with diisononyl phthalate (DINP).

CPSC staff assesses a product's potential health effects to consumers under the Federal Hazardous Substances Act (FHSA). The FHSA is risk-based. To be considered a "hazardous substance" under the FHSA, a consumer product must satisfy a two-part definition.[2] First, it must be toxic under the FHSA, or present one of the other hazards enumerated in the statute. Second, it must have the potential to cause "substantial illness or injury during or as a result of reasonably foreseeable handling or use." Therefore, exposure and risk must be considered in addition to toxicity when assessing potential hazards under the FHSA.[3]

The FHSA addresses both acute and chronic hazards. While the FHSA does not require manufacturers to perform any specific battery of toxicological tests to assess the potential risk of chronic health hazards, the manufacturer is required to label a product appropriately according to the requirements of the FHSA.

The first step in the risk assessment process is hazard identification, that is, a review of the available toxicity data for the chemical under consideration and a determination of whether the chemical is considered "toxic" under the FHSA. Chronic toxicity data (including carcinogenicity, neurotoxicity, and reproductive and developmental toxicity) are assessed by the CPSC staff using guidelines issued by the Commission. If it is concluded that a substance is toxic under the FHSA due to chronic toxicity, then a quantitative assessment of exposure and

[1] These comments are those of the CPSC staff, have not been reviewed or approved by, and may not necessarily reflect the views of, the Commission.

[2] 15 USC 1262 (f)(1)(A).

[3] Labeling requirements for art materials presenting chronic hazards; guidelines for determining chronic toxicity of products subject to the FHSA; supplementary definition of "toxic" under the Federal Hazardous Substances Act; final rules. Federal Register 57: 46626-46674. October 9, 1992.

risk is performed to evaluate whether the chemical may be considered a "hazardous substance" under the FHSA.

Purpose and Scope of the Report

The primary purpose of the attached report is to complete the first two steps in the risk assessment process, that is, the hazard identification and dose response assessment. In addition, the report briefly summarizes published information relating to exposure, as well as published exposure and risk assessments. The sections on exposure and risk assessment are included to provide additional context for the reader, and as background for future risk assessments. However, quantitative exposure and risk assessments are beyond the scope of this report. Cumulative risks from exposure to multiple phthalates are also not included in this report, although they are briefly discussed. The information in this report will be provided to the Chronic Hazard Advisory Panel on Phthalates. This panel will convene in 2010 to assess the potential health effects of cumulative exposure to phthalates from all sources, including children's articles and cosmetics.

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Summary

DINP is a complex substance comprised of multiple isomers. In addition, DINP is currently available in two forms (DINP-1 and DINP-2) manufactured by different processes. The two forms are considered commercially interchangeable, that is, they can be used interchangeably in products. Differences in toxicity between the two forms appear to be minor, although there are few direct comparisons. DINP is used in a variety of products, including wire and cable insulation, household furnishings, building materials, and footwear. DINP use in toys in the U.S. declined following voluntary removal from teethers and pacifiers in 1999, and declined further following the Consumer Product Safety Improvement Act of 2008, which prohibits the use of DINP in certain child care articles and toys that can be placed in a child's mouth.

A considerable amount of toxicity data on DINP is available. DINP has been tested in two-year bioassays in rats and mice. DINP has also been tested in a two-generation study of reproductive toxicity, standard prenatal developmental toxicity screens, and perinatal developmental studies. Genotoxicity has been well-studied. Numerous mechanistic studies relating to carcinogenesis are also available. Neurotoxicity has not been tested. Except for developmental studies, there are no data on the relative susceptibility of immature animals to DINP.

DINP exposure causes hepatocellular tumors in rats and mice. The tumors are believed to result from peroxisome proliferation, which does not occur significantly in humans. Therefore, the hepatocellular tumors are generally regarded as not relevant to humans. DINP also induced mononuclear cell leukemia in Fischer rats, but not in mice. Mononuclear cell leukemia has a high background rate and is a common finding in Fischer rats. Therefore, mononuclear cell leukemia is considered to be of uncertain relevance to humans. DINP induced a low incidence of renal tubular cell carcinoma in male rats only. These were shown to arise by a mechanism that occurs only in male rats.

Principal non-cancer endpoints in animal studies include the liver and kidney. The liver is the most sensitive target site. The 2001 Chronic Hazard Advisory Panel (CHAP) on DINP derived an acceptable daily intake (ADI) of 120 μg/kg-d, based on the incidence of spongiosis hepatis (cystic degeneration) in rats. The CPSC staff derived a preliminary ADI of 0.88 mg/kg-d for kidney toxicity.

Prenatal DINP exposure is associated with various malformations in offspring, while perinatal exposure leads to reproductive malformations in male pups. DINP is relatively less potent than several other phthalates with respect to developmental effects. The CPSC staff preliminarily derived an ADI of 1.2 mg/kg-d for the developmental effects of DINP. Recently, it has been demonstrated that DINP and other phthalates may act in a dose-additive fashion to induce male reproductive effects following perinatal exposure.

The potential health effects of DINP have been assessed by several agencies. The 2001 CHAP on DINP concluded that the risk to children from mouthing teethers and toys

containing DINP was “minimal to non-existent.” The CPSC staff concluded that exposure to DINP from mouthing teethers and soft plastic toys did not present a hazard to children. The U.S. Center for the Evaluation of Risks to Human Reproduction (CERHR) concluded in 2003 that there was “minimal risk” of developmental or reproductive effects from current exposure levels. However, none of these assessments considered the effects of cumulative exposure to multiple phthalates.

An assessment of the potential risks from cumulative exposure to phthalates is beyond the scope of this report. The information presented here will contribute to a cumulative risk assessment of phthalates by a Chronic Hazard Advisory Panel on Phthalates to convene in 2010.

List of Abbreviations

ACC	American Chemistry Council
ADI	Acceptable daily intake
AGD	Anogenital distance
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATBC	Acetyltributyl citrate
BBP	Butylbenzyl phthalate
BMD	Benchmark dose
BMDL	Lower confidence limit of the benchmark dose
CAS	Chemical Abstracts Service
CDC	Centers for Disease Control and Prevention, U.S.
CERHR	Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program, U.S.
CHAP	Chronic Hazard Advisory Panel
CI	Confidence interval
CMA	Chemical Manufacturers Association (currently American Chemistry Council)
CO_2-MINP	Mono(carboxy-isooctyl) phthalate
CPSC	Consumer Product Safety Commission, U.S.
CPSIA	Consumer Product Safety Improvement Act (2008)
CSTEE	Scientific Committee on Toxicity, Ecotoxicity, and the Environment, European Commission
o-DAP	Dialkyl *ortho*-phosphate
DBP	Dibutyl phthalate
DEHA	Di(2-ethylhexyl) adipate
DEHP	Di(2-ethylhexyl) phthalate
DIBP	Diisobutyl phthalate
DIDP	Diisodecyl phthalate
DINP	Diisononyl phthalate
DINP-1	Diisononyl phthalate, type 1 (68515-48-0)
DINP-2	Diisononyl phthalate, type 2 (28553-12-0)
DINP-A	Diisononyl phthalate, type A (71549-78-5)
DME	Danish Ministry of the Environment
DNOP	Di-*n*-octyl phthalate
DNP	Di-*n*-nonyl phthalate
ECB	European Chemicals Bureau
EPA	Environmental Protection Agency, U.S.
EPL	Experimental Pathology Laboratories
ER	Estrogen receptor
F344	Fischer 344
FHSA	Federal Hazardous Substances Act
FSH	Follicle stimulating hormone
GD	Gestational day
GJIC	Gap junction intercellular communication

GRN	Granulin precursor
HRIPT	Human repeated insult patch test
HSDB	Hazardous Substances Data Bank
IL	Interleuken
JRC	Joint Research Centre, European Commission
LH	Luteinizing hormone
LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
MAFF	Ministry of Agriculture, Fisheries, and Food, United Kingdom
MEHP	Mono(2-ethylhexyl) phthalate
MINP	Mono(isononyl) phthalate
MNCL	Mononuclear cell leukemia
NERI	Danish National Environmental Institute
NICNAS	National Industrial Chemicals Notification and Assessment Scheme, Australia
NLM	National Library of Medicine, U.S.
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NTP	National Toxicology Program, U.S.
OH-MINP	Mono(hydroxyl-isononyl) phthalate
Oxo-MINP	Mono(oxo-isononyl) phthalate
PA	*Ortho*-Phthalic acid
PND	Postnatal day
PPAR	Peroxisome proliferator-activated receptor
PPRE	Peroxisome proliferator response element
PVC	Polyvinyl chloride
PWG	Pathology Working Group
RIVM	Rijksinstituut voor Volksgesondheid en Milieu (National Institute of Public Health and Environment), the Netherlands
RT-PCR	Reverse transcriptase- polymerase chain reaction
SD	Sprague-Dawley
SDN	Sexually dimorphic nucleus
SHBP	Sex-hormone binding protein
TMA	Toy Manufacturers Association (currently the Toy Industry Association)
TNO	Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek (Netherlands Organisation for Applied Scientific Research)

1. Introduction

Dialkyl-*ortho*-phthalates (*o*-DAP's) have been used as plasticizers in many household products made from polyvinyl chloride (PVC), including children's products such as soft plastic teethers, rattles, and toys. Because plasticizers are not chemically bound to PVC, they may be released when children place PVC products in their mouths. Dermal exposure from these products is also possible, but probably to a lesser extent (CPSC 1983; CPSC 2001, 2002; ECB 2003). Significant inhalation exposure is not likely, due to the low vapor pressure of DINP. Until about 1985, di(2-ethylhexyl) phthalate (DEHP) was the predominant *o*-DAP in PVC children's products such as teethers, rattles, and soft toys. However, DEHP was found to be carcinogenic in laboratory animals (NTP 1982). Thus, the U.S. Consumer Product Safety Commission (CPSC) staff performed a cancer risk assessment of exposure to DEHP in children's products (CPSC 1983), initiated a rulemaking procedure to limit the use of DEHP in children's products, and convened a Chronic Hazard Advisory Panel (CHAP) to review the available information on the possible health effects of DEHP (CPSC 1985). The rulemaking was later withdrawn when manufacturers voluntarily agreed to remove DEHP from pacifiers, teethers, and rattles (TMA 1986). The voluntary withdrawal of DEHP from pacifiers, teethers, and rattles was later incorporated into a voluntary standard for toy safety, ASTM F963. DEHP was replaced with another phthalate, diisononyl phthalate (DINP).

In 1998, the CPSC staff began investigating the potential chronic hazards from DINP in children's products (CPSC 1998a). Subsequently, the National Environmental Trust and 11 other organizations petitioned the Commission to: (i) ban the use of PVC in toys and other products intended for use by children five years of age and under and (ii) issue "a national advisory on the health risks that have been associated with soft plastic vinyl (PVC) toys to inform parents and consumers about the risks associated with PVC toys currently in stores and homes." The petitioners cited concerns about the adverse effects of phthalates, lead, and cadmium additives in PVC. The petition was docketed in December 1998 (CPSC 1998b). CPSC took the following steps to assess the potential chronic hazards from DINP in children's products:

- Convened a CHAP to assess the potential chronic hazards associated with DINP in children's products and, in particular, to consider the human relevance of tumors induced in animals. DINP and certain other *o*-DAP's induce liver tumors in rodents that are associated with the activation of the peroxisome proliferator activated receptor alpha (PPARα).
- Conducted an observational study of children's mouthing behavior.
- Participated in the development a laboratory method for DINP migration that accurately predicts the amount of DINP released when children mouth products.
- Tested children's products to determine the prevalence of DINP and other plasticizers, plasticizer concentration, and migration rate.

At the request of CPSC, manufacturers voluntarily removed DINP from children's products "intended to be mouthed," including teethers and rattles. Thus, in 1999 manufacturers began manufacturing teethers and rattles with plastics such as polypropylene that do not require plasticizers. Manufacturers were still permitted to use DINP in soft plastic toys, but the

prevalence of DINP use declined. In Canada, manufacturers and distributors also voluntarily removed phthalates from children's products intended to be mouthed. In 1999, the European Commission issued a temporary ban of the use of six phthalates in children's products intended to be mouthed, namely, DINP, DEHP, di-n-octyl phthalate (DNOP), dibutyl phthalate (DBP), benzylbutyl phthalate (BBP), and diisodecyl phthalate (DIDP). The temporary ban was made permanent in 2005 (EC 2005).

The CHAP convened in May 2000 and published a final report in June 2001 (CPSC 2001). The CHAP concluded, "For the majority of children, the exposure to DINP from DINP containing toys would be expected to pose a minimal to non-existent risk of injury." The CHAP further concluded, "The PPARα-mediated mechanism of hepatocarcinogenesis is pronounced in rodents, but believed not readily induced in humans, especially at the doses resulting from current use of consumer products. The human risk was therefore seen as negligible or non-existent."

The CPSC staff completed an observational study of children's mouthing behavior (Greene 2002a; Kiss 2002), participated in efforts to develop a laboratory method for measuring DINP migration from children's toys (Rijk and Ehlert 1999; Rijk et al. 1999; Simoneau et al. 2001), and conducted additional migration measurements using the new laboratory method (Chen 2002). Finally, the staff completed a risk assessment of the potential chronic hazards associated with DINP in children's products (Babich 2002; Babich et al. 2004; CPSC 2002) and concluded that oral exposure to DINP from mouthing soft plastic toys is not likely to present a health hazard to children. The petition to ban the PVC in children's products was denied in 2003 (CPSC 2003).

Consideration of DINP continued following the enactment of the Consumer Product Safety Improvement Act of 2008 (CPSIA)[*] on August 14, 2008. Section 108 of the CPSIA permanently prohibits the sale of any "children's toy or child care article" containing concentrations of more than 0.1 percent of DEHP, DBP, or BBP (Table 1-1). Section 108 prohibits on an interim basis the sale of "any children's toy that can be placed in a child's mouth" or "child care article" containing concentrations of more than 0.1 percent of DINP, DIDP, or DNOP. In addition, section 108 of the CPSIA directs CPSC to convene a CHAP "to study the effects on children's health of all phthalates and phthalate alternatives as used in children's toys and child care articles." The CHAP will recommend to the Commission whether any phthalates (including DINP) or phthalate alternatives other than those permanently banned should be declared banned hazardous substances.

The CPSC staff is performing the following tasks to support the work of the CHAP on Phthalates:

- Toxicity reviews of the six phthalates banned by the CPSIA.
- Toxicity reviews of five phthalate substitutes.
- A review of published exposure studies on phthalates.
- Laboratory studies of plasticizers in children's articles.

[*] Public Law 110-314.

The European Commission and U.S. Congress have regulated the six phthalates specified above. However, there are numerous phthalates and phthalate mixtures in commerce. Some phthalates, including dipropyl phthalate, diisobutyl phthalate, and pentyl phthalate may also contribute to the cumulative health risks to be assessed by the CHAP on Phthalates.

Table 1-1. Dialkyl *ortho*-phthalates (*o*-DAP's) banned by the Consumer Product Safety Improvement Act of 2008 (CPSIA)

Phthalate	CAS number [a]
Permanent ban	
Dibutyl phthalate (DBP)	84-74-2
Benzyl butyl phthalate (BBP)	85-68-7
Di(2-ethyhexyl phthalate) DEHP	117-81-7
Interim ban	
Di-n-octyl phthalate (DNOP)	117-84-0
Diisononyl phthalate (DINP)	28553-12-0, 68515-48-0
Diisodecyl phthalate (DIDP)	26761-40-0, 68515-49-1

[a] CAS, Chemical Abstracts Service

Purpose and Scope of the Report

The primary purpose of the attached report is to complete the first two steps in the risk assessment process, that is, the hazard identification and dose response assessment, for diisononyl phthalate (DINP). In addition, the report briefly summarizes published information relating to exposure, as well as published exposure and risk assessments. The sections on exposure and risk assessment are included to provide additional context for the reader, and as background for future risk assessments. However, quantitative exposure and risk assessments are beyond the scope of this report. Cumulative risks from exposure to multiple phthalates are also not included in this report, although they are briefly discussed. The information in this report will be provided to the CPSC Chronic Hazard Advisory Panel on Phthalates. This panel will convene in 2010 to assess the potential health effects of cumulative exposure to phthalates from all sources, including children's articles and cosmetics, as required by the CPSIA.

The toxicity of DINP has been reviewed previously by several authors and agencies (Babich 2002; Babich et al. 2004; CERHR 2003; CPSC 2001; ECB 2003; Gill et al. 2001; HSDB 2009; Madison et al. 2000; NICNAS 2008). This report updates the previous CPSC staff review of DINP (Babich 2002).

2. Chemistry and Use

Chemistry

DINP (68515-48-0; 28553-12-0) is a mixture of C9-rich, di-C8 to C10, branched chain dialkyl esters of *ortho*-phthalic acid (Hellwig et al. 1997; NLM 2009; ECB 2003). Different processes are used to produce the isononyl alcohols used as feedstock in manufacturing DINP. This results in DINP's with different isomeric compositions and multiple CAS numbers. Two commercial processes are currently in use.

DINP-1 (68515-48-0) contains alcohol groups made from octane, by the "polygas" process (ECB 2003). At least 95 percent of these alcohol groups comprise roughly equal amounts of 3,4-, 3,5-, 3,6-, 4,5-, 4,6-, and 5,6-dimethyl heptan-1-ol (Hellwig et al. 1997) (Table 2-1). DINP-1 is also known by the tradename Jayflex®. DINP-2 (28553-12-0) contains alcohol groups made from *n*-butene, which results mainly in methyl octanols and dimethyl heptanols. DINP-2 is also known by the tradenames Palatinol N® and Palatinol DN® (NLM 2009a). DINP-3 (also 28553-12-0) contains alcohol groups made from *n*-butene and *i*-butene, resulting in 60 percent methylethyl hexanols. DINP's generally contain 70% or more nonyl alcohol moieties, with the remainder being octyl or decyl (Madison et al. 2000). According to the American Chemistry Council, the composition of each type of DINP is stable (CERHR 2003). However, data on the composition of DINP-1 and DINP-2 suggest that there may be some variability (Table 2-2.). Although their isomeric composition differs, the different types of DINP are considered commercially interchangeable. DINP-3 is no longer produced.

Table 2-1. Comparison of different forms of diisononyl phthalate (DINP) [a]

Type	CAS no.	Starting material	Composition of alcohol groups	Production
DINP-1	68515-48-0	Octene	≥95% 3,4-, 3,5-, 3,6-, 4,5-, 4,6-, and 5,6-dimethyl heptan-1-ol	>10,000 lbs./year
DINP-2	28553-12-0	*n*-butene	Mainly methyl octanols and dimethyl heptanols	>10,000 lbs./year
DINP-A [b]	71549-78-5	*n*-butene	Mainly methyl octanols and dimethyl heptanols	Never produced commercially
DINP-3	28553-12-0	*n*-butene + isobutene	60% methylethyl hexanols	Not currently produced
NA [c]	14103-61-8	NA	3,5,5-trimethyl hexanol	<10,000 lbs./year

[a] Sources: Hellwig et al. 1997; Madison et al. 2000; NLM 2009a.
[b] This product is reported to be similar in composition to DINP-2 (Harmon 2000).
[c] NA, not applicable.

Some manufacturers add small amounts of diisodecyl phthalate (DIDP) to their DINP. Manufacturers also add small amounts of bisphenol A as a stabilizer at the request of the customer.

Another form of DINP, Santicizer 900® (71549-78-5), was never produced on a commercial scale (Menza 1985). However, this product was apparently made from *n*-butene and has an isomeric composition similar to the DINP-2 that is currently produced (Harmon 2000). This product has been referred to as DINP-A (Smith et al. 2000).

Bis(3,5,5-trimethylhexyl) phthalate (14103-61-8) is a branched-chain dinonyl phthalate that comprises a single isomer. This compound has an annual production of less than 10,000 pounds per year (Madison et al. 2000) and, therefore, is not a commercially significant plasticizer. It is marketed as a laboratory reagent (www.sigma-aldrich.com/).

Table 2-2. Percent composition of diisononyl phthalate (DINP) [a]

	DINP-1	DINP-2	DINP-3
Methylethyl hexanols	5 - 10	5 - 10	65 - 70
Dimethyl heptanols	45 - 55	40 - 45	20 - 25
Methyl octanols	5 - 20	35 - 40	--
n-Nonanol	0 - 1	0 - 10	--
Isodecanol	15 - 25	--	--

[a] ECB 2003.

Physico-Chemical Properties

DINP is an extremely hydrophobic compound with low vapor pressure and low water solubility (reviewed in Staples et al. 1997) (Table 2-3). Due to its extreme hydrophobic nature, the octanol-water partition coefficient (log K_{ow}) and water solubility are not amenable to direct measurement. Thus, a range of estimates for these properties has been reported. The values in the table are as recommended in the CERHR report (CERHR 2003).

Table 2-3. Physico-chemical properties of DINP [a]

Molecular formula	$C_{26}H_{42}O_4$
Molecular weight	418.6 g/mol
Melting point	-48 °C
Boiling point	370 °C
Vapor pressure	5×10^{-7} mm Hg
Specific gravity	0.97
Water solubility	<0.001 mg/L [b]
Log K_{ow}	~9 [b]

[a] Sources: CERHR 2003; Staples et al. 1997.
[b] A range of estimates was reported in the literature. Values shown are from CERHR 2003.

Uses of DINP

Over 90% of DINP is used as a plasticizer for PVC (ECP 2003; ExxonMobil 2009). DINP is used as a plasticizer in a variety of products manufactured from PVC, including vinyl flooring, wire and cable insulation, stationery, coated fabrics, gloves, toys, tubing, garden hoses, artificial leather, footwear, automobile undercoating, and roofing (CERHR 2003; ECB 2003; ExxonMobil 2009). The use of DINP in toys represents less than 1% of total DINP consumption. Most of the DINP in toys imported into the U.S. is manufactured by Asian companies (ExxonMobil 2009). DINP has limited use in food packaging in the U.S. and is not used in medical devices (CERHR 2003).

Non-PVC uses of DINP account for less than 10% of DINP production (ECB 2003; ExxonMobil 2009). Non-PVC uses include rubbers, inks, paints, lacquers, adhesives, and sealants (ECB 2003).

Domestic consumption of DINP was estimated to be 178,000 metric tons (392 million pounds) in 1998. DINP represents approximately 10 to 15 percent of total dialkyl phthalate plasticizer production (Madison et al. 2000). DINP production in the U.S. currently exceeds that of DEHP (ExxonMobil 2009). DINP production is expected to increase at the expense of DEHP. Worldwide, more DEHP is produced than any other plasticizer (ExxonMobil 2009).[*]

[*] The CPSC staff is in the process of compiling updated information on phthalate production.

3. Acute Toxicity, Skin and Eye Irritation

Acute Toxicity

DINP exhibits a low degree of acute toxicity (reviewed in ECB 2003; HSDB 2009; NICNAS 2008). Oral LD_{50} values of DINP-1 and DINP-2 in rats ranged from >9,800 mg/kg to >50,000 mg/kg. Clinical signs in treated animals included labored breathing, spastic gait, tremors, and apathy.

The inhalation LC_{50} of DINP-1 was reported to be >0.067 mg/L in rats, mice, and guinea pigs. The LC_{50} of DINP-2 was >0.07 mg/L in Sprague-Dawley (SD) rats. In these studies, DINP was presumably present as an aerosol (ECB 2003). The inhalation LC_{50} was reported to be >4.4 mg/L of vapor in SD rats. All treated animals exhibited lacrimation and clear nasal discharge. One male had blood around the eyes, brown discharge from the eyes, and an opacity on one eye. No other symptoms were reported. According to the authors, there were no compound-related gross or microscopic lesions in the lungs, liver, or kidney.

The dermal LD_{50} of DINP-1 in rabbits was reported to be >3,160 mg/g (ECB 2003; HSDB 2009; NICNAS 2008).

There were no deaths or symptoms in mice administered 9,800 mg/kg DINP-2 by the intraperitoneal route (ECB 2003; HSDB 2009; NICNAS 2008).

No clinical reports of human exposures were identified.

DINP does not meet the CPSC regulatory definition of acute toxicity.[*] The oral LD_{50} values exceed 5,000 mg/kg in rats. The inhalation studies were not at sufficiently high exposures to estimate an LC_{50}. Furthermore, exposures to high concentrations of DINP vapor or aerosol is not likely to occur.

Skin and Eye Irritation

DINP (types 1 and 2) were mild skin and eye irritants in rabbits (reviewed ECB 2003; HSDB 2009; NICNAS 2008).

DINP-1 was not irritating to human skin when tested in 14 volunteers (ECB 2003; HSDB 2009; NICNAS 2008).

A case report described a case of "sqwish ball dermatitis" involving a 10-year old girl (Brodell and Torrence 1992). Tiny erythematous papules and papulovesicles appeared on the child's hands the morning after contact with the liquid contents of the ball, which according to the manufacturer contained DINP and other unspecified ingredients. The manufacturer reported that five other consumers complained of dermatitis after contacting the liquid. The authors

[*] 16 CFR 1500.3 (c)(2)(ii).

concluded that the dermatitis was due to irritation, rather than atopic dermatitis. The irritation cannot be specifically attributed to DINP.

Overall, DINP is considered to have low potential for skin and eye irritation. In addition, in most products DINP is present in a solid matrix such as PVC, which greatly reduces the potential exposure.

Sensitization

Two Buehler tests (occluded application test to measure delayed contact hypersensitivity) were performed on female guinea pigs with DINP-1 (reviewed in ECB 2003; HSDB 2009; NICNAS 2008). The first study showed evidence of sensitization on day 37 in 3/20 animals, following the second challenge (Exxon Biomedical Sciences 1992). It is noteworthy that 4/10 control animals displayed a score of 1 on the same day. In the second test, there was no evidence of sensitization (Huntingdon Research Centre 1994).

A human repeated insult patch test (HRIPT) was performed on 104 subjects with several phthalates, including DINP. There was no evidence of dermal irritation after repeated applications with undiluted DINP (Medeiros et al. 1999).

Overall, it is unlikely that DINP is a skin sensitizer in animals or humans.

4. Toxicokinetics

Oral Toxicokinetics

As with di(2-ethylhexyl) phthalate (Albro and Thomas 1973) and other *o*-DAP's, DINP is initially de-esterified by the intestinal mucosa (Frederiksen et al. 2007; Koch and Angerer 2007; Smith et al. 2000; Silva et al. 2006a,b). Studies of DINP metabolism are complicated by the fact that DINP is a mixture of isomers.

Rats

Hazleton Laboratories. Oral absorption of ^{14}C-DINP was studied in male albino rats (Hazleton 1972; reviewed in CERHR 2003 and CPSC 2001). Most of the administered dose (85%) was eliminated in the feces within 72 hours. The remainder was eliminated in urine (12%) or remained in the tissues (3%).

Midwest Research Institute. In studies performed at the Midwest Research Institute, male and female Fischer 344 rats were given either a single oral (gavage) dose at 50 or 500 mg/kg or five daily doses at 50, 150, or 500 mg/kg of ^{14}C-DINP (El-hawari et al. 1983; El-hawari et al. 1985; Lington et al., 1985; reviewed in CERHR 2003 and CPSC 2001). Elimination of radioactivity in urine and feces was followed for up to 72 hours. Blood and tissue levels of radioactivity were determined in animals sacrificed at times up to 72 hours.

In rats given a single dose, radioactivity levels in blood and tissue were generally greatest at one hour following administration. Levels were greatest in the liver, followed by blood and kidney, and very low in fat. At least 49 percent of the applied dose was absorbed at 50 mg/kg; less was absorbed at 500 mg/kg (CERHR 2000a), suggesting that absorption was saturated at the high dose. Administered radioactivity was largely recovered in urine and feces within 72 hours. Roughly equal amounts of radioactivity were recovered in urine and feces at the low dose, while at the high dose more radioactivity was recovered in feces. Most of the ^{14}C-DINP collected in urine was in the form of phthalic acid (PA) or unspecified side-chain oxidation products of the monoester. Elimination of PA decreased at the high dose. Feces included up to 41 percent DINP, as well as PA and side-chain oxidation products.

In animals given five daily doses, blood and tissue levels were greatest at one hour following the last dose. DINP levels were highest in the liver, followed by kidney, blood, and skin. Administered radioactivity was largely recovered in urine and feces within 72 hours. Excretion of radioactivity was higher in urine than in feces at all three doses. Most of the ^{14}C-DINP collected in urine was in the form of PA or side chain oxidation products of the monoester. Elimination of PA decreased at the high dose.

Smith et al. 2000. DINP and its metabolites monoisononyl phthalate (MINP) and PA were studied in Fischer 344 rats (0, 0.1, or 1.2% in feed) and B6C3F1 mice (0.05 or 0.6% in feed) for four weeks (Smith et al. 2000). DINP, MINP, and PA were measured in liver and serum at two and four weeks. DINP levels in the livers of rats and mice were greater at the high dose at two weeks, but not at four weeks; DINP was not detected in serum. The levels of MINP in liver and

serum increased over time, and were greater than DINP and PA levels. In rats, PA increased with time in liver and serum. In mice, PA increased with time in liver and serum at the low dose, but not at the high dose. PA concentrations were not dose-dependent in the liver or serum of mice or rats.

Oxidative Metabolites

Three major oxidative metabolites of DINP have been identified in urine from rats (Silva et al. 2006a) and humans (Koch and Angerer 2007; Koch et al. 2007; Silva et al. 2006b; reviewed in Frederiksen et al. 2007; Wittasek and Angerer 2008). MINP can be oxidized at the ω-carbon position of the side chain to form mono(carboxyisooctyl) phthalate or carboxy-MINP (CO_2-MINP). MINP can also be oxidized at the ω-1 position to form hydroxy-MINP (OH-MINP), which can be further oxidized to the ketone (oxo-MINP) (Figure 4-1). Each oxidative metabolite comprises a mixture of isomers. The oxidative metabolites are then subject to β-glucuronidation.

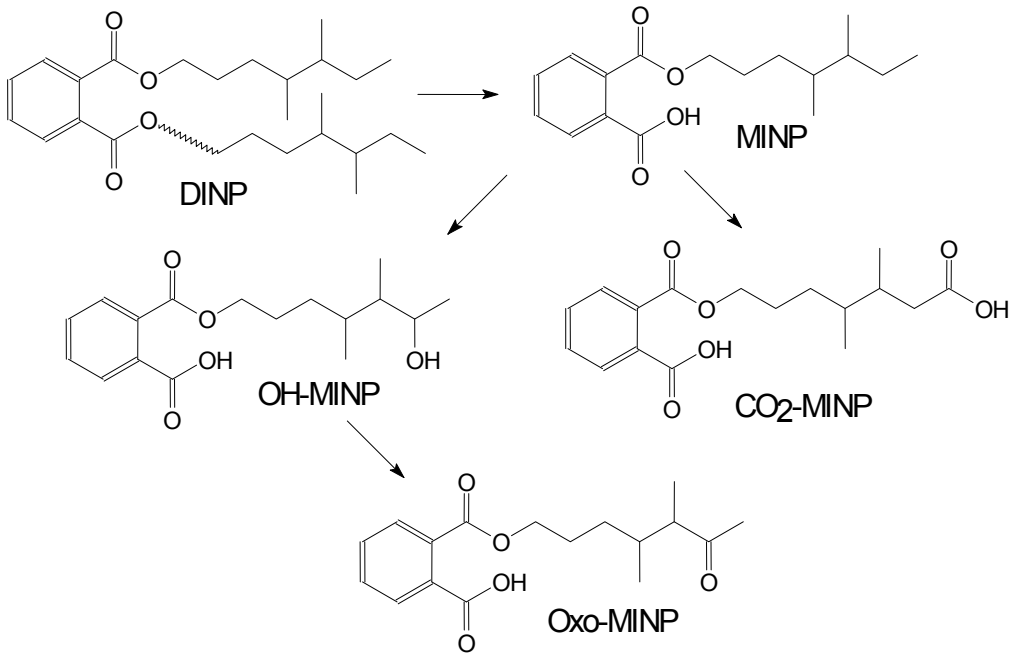

Figure 4-1. Proposed metabolic pathway for DINP in rats and humans (adapted from Koch and Angerer 2007). DINP is a mixture of branched-chain isomers. The metabolism of a single isomer, di(4,5-dimethyl heptyl) phthalate, is shown. Oxidized metabolites are subject to β-glucuronidation.

Over 80% of DINP metabolites isolated from rat urine were in the form of the carboxylated metabolite of MINP (Silva et al. 2006a). The hydroxyl- and oxo- metabolites were also present at substantial levels (Table 4-1). MINP was a very minor metabolite, accounting for only 0.038% of total metabolites. According to the authors, the identities and relative abundance of the metabolites from DINP-1 and DINP-2 were similar.

Table 4-1. Levels of major DINP metabolites identified in rat urine [a]

Metabolite	µg/mL	Percent
MINP	0.059	0.038
CO_2-MINP	126.7	82.0
OH-MINP	11.8	7.6
Oxo-MINP	4.9	3.27
Other [b]	10.3	6.7
PA	0.7	0.46
Total metabolites	154.6	100

[a] Silva et al. 2006a. Adult female SD rats were given 300 mg/kg DINP-1 or DINP-2 by gavage. Metabolites were treated with bacterial β-glucuronidase prior to analysis.

[b] Metabolites of C8 and C10 isomers.

Humans

Koch and Angerer (2007) administered 1.27 mg/kg of ring-labeled [3,4,5,6-D]-DINP to a single volunteer. The D4-DINP was administered in spiked butter spread on bread during breakfast. Urine was collected for 48 hours. A total of 43.6% of the applied dose was recovered, 20.2 % as OH-MINP, 10.7% as CO_2-MINP, 10.6% as oxo-MINP, and 2.2% as MINP. Elimination was biphasic. The oxidative metabolites had half-lives of approximately 5 hours in the first phase, 8 to 24 hours following exposure. In the second phase, beginning at 24 hours, the half life was about 12 hours for OH-MINP and oxo-MINP and about 18 hours for CO_2-MINP. A significant portion of high molecular weight *o*-DAP's is probably excreted in feces, although this was not tested by Koch and Angerer.

In a German study of 25 non-occupationally exposed individuals, CO_2-MINP was present at twice the level of OH-MINP (Koch et al. 2007) (Table 4-2). In a U.S. study of 129 people, OH-MINP present at higher levels than CO_2-DINP (Silva et al. 2006b). MINP was not detected in any of the U.S. samples. The reason for the difference between the U.S. and German populations is unknown. It might reflect exposure to different DINP types, since DINP-2 is manufactured in Europe and DINP-1 in the U.S. However, metabolism of the two types was similar in rats (Silva et al. 2006a). The difference could also be due to inter-individual differences in metabolism.

Fromme et al. (2007) measured *o*-DAP metabolites in urine over an 8-day period (n=27 women and 23 men). They found significant intra-individual, daily variations in DINP metabolites. However, there were no significant effects of gender or age.

The finding that oxidative metabolites of DINP are present at much higher levels than MINP has important implications for biomonitoring studies. In earlier biomonitoring studies (Blount et al. 2000; Brock et al. 2002), MINP was non-detectable in most individuals, which led to the

conclusion that human exposure to DINP was low (David 2001; Kohn et al. 2000) (see Exposure section). However, studies based on MINP may underestimate human exposure. OH-MINP and CO_2-MINP are more sensitive and should lead to more accurate estimates of exposure. The daily variation in metabolite levels should also be considered in designing and analyzing biomonitoring data.

In short, DINP is rapidly absorbed and eliminated. DINP was de-esterified to the monoester, which was further metabolized either by hydrolysis to phthalic acid or by side-chain oxidation of the ester group. The oxidative metabolites of higher molecular weight *o*-DAP's such as DINP are more-sensitive biomarkers than the monoesters when studying urinary metabolites.

Table 4-2. Median levels (ng/mL) of major DINP metabolites in human urine [a]

Metabolite	Koch et al. 2007 n=25	Silva et al. 2006b n=129
CO_2-MINP	5.0	8.4
OH-MINP	2.5	13.2
Oxo-MINP	1.3	1.2
MINP	Not tested	Not detected

[a] Subjects were from the general population of Germany (Koch et al.) or the U.S. (Silva et al.). Metabolites were treated with bacterial β-glucuronidase prior to analysis.

Percutaneous Absorption

Pecutaneous absorption of ^{14}C-DINP was studied in male Fischer 344 rats (Stoltz and El-hawari 1983; Stoltz et al. 1985). A volume of either 0.1 mL or 0.2 mL of neat DINP was applied to a 12 cm^2 area of skin and occluded. DINP remained on the skin during the 7-day study. Some animals were pre-conditioned by applying non-labeled DINP to the skin for three days prior to applying ^{14}C-DINP. After seven days, roughly 2 to 4 percent of the applied dose (as ^{14}C) was recovered in urine, feces, gastrointestinal tract, blood, or tissue, in descending order. More than half of the absorbed dose was recovered in urine. In naïve animals, either 3.1 or 3.7 percent of the applied dose was absorbed, at the low and high dose, respectively. Absorption was 2.0 percent in pre-conditioned animals.

Other Phthalates

Related *o*-DAP's have also been studied. *o*-DAP's were applied to the skin of male Fischer 344 rats in ethanol at a dose of 5 to 8 mg/kg (Elsisi et al. 1989). Approximately 5 percent of the applied dose of DEHP was excreted in five days. Only 0.5 percent of the applied dose of diisodecyl phthalate was excreted in seven days.

Percutaneous absorption of DEHP was also studied in humans *in vivo* and in the isolated perfused porcine skin flap (IPPSF) system (Wester et al. 1998). In humans, 1.8 percent of the applied dose was absorbed, compared to 2.4 percent in the IPPFS system.

Deisinger et al. (1998) studied the percutaneous absorption of ^{14}C-DEHP from PVC film containing 40 percent DEHP. A 15 cm^2 piece of PVC containing about 400 mg DEHP was applied to the skin and occluded, then removed after 24 hours. Excretion was monitored for seven days. From 0.064 to 0.126 percent of the applied DEHP was transferred to the skin in 24 hours. Approximately 0.01 percent of the applied dose (261.5 to 505.6 μg) was absorbed in seven days.

Percutaneous absorption of DEHP was also studied in two *in vitro* studies. Barber et al. (1992) reported a permeability constant of 4.3×10^{-7} cm/h for full-thickness rat skin. The permeability constant was 1.05×10^{-7} cm/h with human stratum corneum (Barber et al. 1992). Scott et al. (1987) reported permeability constants of 2.28×10^{-5} cm/h and 0.57×10^{-5} cm/h using rat and human epidermis, respectively.

5. Systemic Health Effects

The non-neoplastic systemic effects of DINP have been reviewed previously (Babich 2002; CERHR 2003; CPSC 2001). DINP-1 (68515-48-0) was tested in a two-year study in Fischer 344 rats at doses of 0, 0.03, 0.3, and 0.6 percent in feed (Lington et al. 1997). DINP-1 from a different supplier was tested in Fischer 344 rats at doses of 0, 0.05, 0.15, 0.6, and 1.2 percent in feed (Moore 1998a) and in B6C3F1 mice at doses of 0, 0.05, 0.15, 0.4, and 0.8 percent (Moore 1998b). The studies by Moore are also referred to as the Covance studies (CPSC 1998a; CPSC 2001). DINP-A (71549-78-5), which is believed to be similar to DINP-2, was tested in Sprague-Dawley rats at doses of 0, 0.05, 0.5, and 1.0 percent in feed (Bio/dynamics 1986). In addition, rodent studies from 1 to 13 weeks in duration have been reported (reviewed in CERHR 2003 and CPSC 2001). Two studies in primates of 2 weeks and 13 weeks in duration have also been reported (Hall et al. 1999; Pugh et al. 2000).

Liver

Hepatomegaly

Hepatomegaly is an early observable effect of DINP exposure in rodents. Increases in absolute and relative liver weights have been reported in studies ranging from 1 week to 2 years in duration (Table 5-1). In Fischer rats exposed to DINP-1 for 2 years, the no observed effect level (NOEL) was 0.15 percent in feed (88 mg/kg-d) (Moore 1998a), while the lowest observed effect level (LOEL) was 0.3 percent (307 mg/kg-d) (Lington et al. 1997). Hepatomegaly was somewhat reversible. In animals fed 1.2 percent DINP for 79 weeks followed by a 26 week recovery period, liver weights returned to near control levels (Moore 1998a). In mice exposed to DINP-1 for two years, the NOEL was 0.15 percent (276 mg/kg-d) in males (Moore 1998b). Liver weight increases were also reversible in recovery group animals. In females, the increase in absolute and relative liver weights was not statistically significant. In Sprague-Dawley rats exposed to DINP-A for two years, liver weights were significantly increased in males at the mid and high doses and females at the high dose. Thus, the NOEL was 0.05 percent (33 mg/kg-d) and the LOEL was 0.5 percent (331 mg/kg-d) (Bio/dynamics 1986).

Hepatomegaly was reported following one week of exposure in SV129 wild type mice, but not in PPAR-α null mice (Valles et al. 2000). The PPAR-α protein (alpha isoform of the peroxisome proliferator activated receptor) is believed to mediate many of the effects of DINP and other PPARα agonists[*] (Lee et al. 1995; Peters et al. 1997a; Ward et al. 1998). Hepatomegaly was not observed in cynomolgus monkeys exposed to 500 mg/kg-d DINP by gavage for 14 days (Pugh et al. 2000). Non-statistically significant increases in liver weight were observed in marmosets exposed to 100, 500, or 2,500 mg/kg-d DINP by gavage for 13 weeks, the greatest increase occurring at the low dose (Hall et al. 1999). The primate studies are limited by the small number of animals (4) per dose/sex/group.

[*] Also referred to as "peroxisome proliferators." The term "PPARα agonist" is now preferred.

Histopathology and Clinical Chemistry

Rats

Lington study. Lington et al. (1997) found several non-neoplastic lesions in Fischer 344 rats exposed to DINP for two years, including focal necrosis, spongiosis hepatis, hepatopathy associated with leukemia, and a slight centrilobular to midzonal hepatocellular enlargement. The authors attributed these lesions to the presence of mononuclear cell leukemia (MNCL). In high dose males, the incidences of focal necrosis ($p=0.0018$), spongiosis hepatis ($p=7.3x10^{-10}$), hepatopathy associated with leukemia, and hepatocellular enlargement were significantly elevated (Table 5-2). In mid dose males, spongiosis hepatis and hepatopathy associated with leukemia were significantly elevated, while focal necrosis was non-significantly elevated ($p=0.13$). In high dose females, hepatopathy and hepatocellular enlargement were significantly elevated, while focal necrosis was non-significantly elevated. In mid-dose females, focal necrosis ($p=0.15$) and hepatopathy ($p=0.093$) were non-significantly elevated over controls. The hepatocellular enlargement (hypertrophy) is probably related to the occurrence of hepatomegaly (see above) and peroxisome proliferation (below). In male rats, the severity of spongiosis was minimal to moderate and exhibited a modest dose response (Brown 2000). The average number of foci per animal was more strongly dose dependent, ranging from 1.45 in the controls to 4.26 at the high dose (Brown 2000).

Some statistically significant increases in serum enzymes associated with liver function were reported in the 6, 12, and 18-month interim sacrifices. At 24 months, alkaline phosphatase was significantly increased in mid and high dose males. The no observed effect level (NOEL) for liver effects in this study was 0.03 percent DINP (15 mg/kg-d).

Covance study. In the Covance rat study in Fischer 344 rats, diffuse hepatocellular enlargement, centrilobular to midzonal hepatocellular enlargement, and increased cytoplasmic eosinophilia were observed in rats sacrificed as early as week 1 (Moore 1998a, p. 41). In males exposed to 1.2 percent DINP, the incidences of several lesions were significantly elevated, including: individual cell degeneration/ necrosis ($p=0.0029$), spongiosis hepatis ($p=0.0051$), hepatocellular enlargement ($p=3.1x10^{-14}$), and increased cytoplasmic eosinophilia ($p=4.4x10^{-17}$) (Moore 1998a, Table 10E; see Table 5-3). Spongiosis hepatis was also significantly elevated in males at 0.6 percent DINP ($p=0.0068$). In females exposed to 1.2 percent DINP, focal necrosis, hepatocellular enlargement, and increased cytoplasmic eosinophilia were significantly elevated. Focal necrosis was observed in a few animals at 0.05, 0.15, and 0.6 percent DINP, but did not reach statistical significance. The incidences of the various liver histopathological lesions generally declined in the recovery group, in which animals were exposed to 1.2 percent for 78 weeks, then allowed to recover for 26 weeks. However, the incidence of spongiosis hepatis remained significantly elevated in males in the recovery group ($p=0.037$). At terminal sacrifice, spongiosis hepatis was generally of low severity (grades 1 and 2), with only one animal in the high dose at grade 5 (Moore 1998a, Table 10C). The average grade among animals with spongiosis hepatis tended to increase with increasing dose, although the dose response was not smooth (CPSC 2001).

Table 5-1. Increased liver weight following DINP exposure [a]

Species/strain	Duration	Doses	Effect	NOEL [b]	Reference
Rat, F344	2, 4 weeks	0, 0.1, 1.2 % in feed	Yes [c]	0.1 %	Smith et al. 2000
Rat, F344	3 weeks	0, 0.06, 0.12, 0.25 % in feed (M: 0, 639, 1192, 2195 mg/kg-d F: 0, 607, 1198, 2289 mg/kg-d)	Yes	ND [d]	BIBRA 1985 Barber et al. 1987
Rat, F344	4 weeks	0, 0.2, 0.67, 2.0 % in feed	Yes	0.2 %	Shellenberger et al. 1983
Rat, F344	13 weeks	0, 0.1, 0.3, 0.6, 1.0, 2.0 % in feed (0, 50, 150, 320, 530, 1260 mg/kg-d)	Yes	0.1 %	Bird et al. 1986
Rat, F344	13 weeks	0, 0.25, 0.5, 1.0, 2.0 % in feed (M: 0, 146, 292, 584, 1168 mg/kg-d F: 0, 182, 364, 728, 1456 mg/kg-d)	Yes	0.25 %	Myers 1991
Rat, F344	2 years	0, 0.03, 0.3, 0.6 % in feed (M: 0, 15, 152, 307 mg/kg-d F: 0, 18, 184, 375 mg/kg-d)	Yes	0.03 %	Lington et al. 1997
Rat, F344	2 years	0, 0.05, 0.15, 0.6, 1.2 % in feed (M: 0, 29, 88, 359, 733 mg/kg-d F: 0, 36, 109, 442, 885 mg/kg-d)	Yes	0.15 %	Moore 1998a
Rat, SD	2 years	0, 0.05, 0.5, 1.0 % in feed (M: 0. 27, 271, 553 mg/kg-d F: 0, 33, 331, 672 mg/kg-d)	Yes	0.05 %	Bio/dynamics 1986
Mouse, B6C3F1	1 week	0, 0.8 % in feed	Yes	ND	Valles et al. 2000
Mouse, SV129 wt	1 week	0, 0.8 % in feed	Yes	ND	Valles et al. 2000
Mouse, SV129 (-/-) [e]	1 week	0, 0.8 % in feed	No	0.8 %	Valles et al. 2000
Mouse, B6C3F1	2, 4 weeks	0, 0.05, 0.6 % in feed	Yes	0.05 %	Smith et al. 2000

Table 5-1. Increased liver weight following DINP exposure (continued)

Species/strain	Duration	Doses	Effect	NOEL [b]	Reference
Mouse, B6C3F1	13 weeks	0, 0.15, 0.4, 1.0, 2.0 % in feed (M: 0, 340, 904, 2365, 5472 mg/kg-d F: 0, 389, 1041, 2834, 6070 mg/kg-d)	Yes	0.4 %	Bankston 1992 Moore 2000
Mouse, B6C3F1	2 years	0, 0.05, 0.15, 0.4, 0.8 % in feed (M: 0, 90, 276, 742, 1560 mg/kg-d) F: 0, 112, 336, 910, 1888 mg/kg-d)	Yes	0.15 % [f]	Moore 1998b
Cynomolgus monkey	14 days	0, 500 mg/kg-d by gavage	No	500 mg/kg-d	Pugh et al. 2000
Marmoset	13 weeks	0, 100, 500, 2500 mg/kg-d by gavage	ND [g]	2,500 mg/kg-d	Hall et al. 1999

[a] Adapted from CPSC 2001 with changes. Where there were differences between sexes, the NOEL is for the more sensitive sex.
[b] NOEL, no observed effect level; ND, not determined.
[c] Positive at 4 weeks only.
[d] Data presented in graphical form. Statistical significance not reported.
[e] PPAR-α null.
[f] Males only.
[g] Non-statistically significant increases in liver weight were reported, with the greatest increase at 100 mg/kg-d.

Beginning at week 52, increased levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were found in males and females exposed to 0.6 or 1.2 percent DINP (Moore 1998a, p. 45). In males, these enzymes remained elevated in the recovery group. This suggests that these increases in liver-specific enzymes were not reversible, at least in the high dose group males.

The no observed effect level (NOEL) for liver enzyme effects in this study was 0.15 percent DINP (88 mg/kg-d). The LOEL was 0.6% (359 mg/kg-d).

Bio/dynamics study. The Bio/dynamics study (1986) was a two-year bioassay of DINP-A in Sprague-Dawley rats, at 0, 0.05, 0.5, and 1% in feed. In males, the incidence of focal necrosis was significantly elevated at the low ($p=0.0042$) and high ($p=0.0001$) doses, while the mid dose was non-significantly elevated ($p=0.086$) (Table 5-4). Spongiosis hepatis was significantly elevated at the mid and high dose. In females, spongiosis hepatis was elevated at the high dose, with borderline statistical significance ($p=0.051$). A no observed adverse effect level (NOAEL) was not established in this study. The lowest observed adverse effect level (LOAEL) in males was 0.05 percent DINP in feed or 27 mg/kg-d.

Serum glutamic oxaloacetic transaminase values were elevated in the mid and high dose males at 6, 12, and 18 months and in the high dose males at 24 months. Serum glutamic pyruvic transaminase values were elevated in the mid and high dose males at 6 and 12 months, and in the high dose males at 24 months. Serum alkaline phosphatase was elevated in the mid and high dose males at 6 and 12 months only. The LOEL for liver effects in this study was 0.05 percent DINP (27 mg/kg-d), the lowest dose tested.

Subchronic studies. Hepatocellular enlargement was reported in a 13-week study in male and female Fischer 344 rats given 2.0 percent DINP in feed (Myers 1991).

Spongiosis hepatis. Spongiosis hepatis, also described as cystic or microcystic degeneration, is a focal degeneration of the perisinusoidal (Ito) cells of the liver (reviewed in Karbe and Kerlin 2002). It is classified as a degenerative lesion (CPSC 2001; Karbe and Kerlin 2002). Spongiosis hepatis is characterized by the appearance of cystic spaces or vacuoles between hepatocytes that are not lined by endothelium and contain granular or flocculent eosinophilic material or fluid (Boorman 1997; EPL 1999; Hardisty 2000; Karbe and Kerlin 2002). The vacuoles may also be filled with erythrocytes. Spongiosis hepatis is typically associated with strong liver carcinogens, such as nitrosamines, and has been observed in fish (i.e., medaka) (e.g., Bannasch et al. 1981; Boorman et al. 1997; Brown-Peterson et al. 1999) and rats (Braunbeck et al. 1992; Zerban and Bannasch 1983). Spongiosis hepatis/cystic degeneration has not been reported in human liver (Karbe and Kerlin 2002). However, cystic degeneration has been reported in human pancreas, frequently in association with squamous carcinoma or adenocarcinoma (Colarian et al. 2000), and in human kidney (Woodward 1990).

Spongiosis hepatis was observed in a chronic study with DEHP in Fischer 344 rats (David et al. 2000). Spongiosis hepatis has been reported in Fischer 344 rats in several NTP* studies, including: tetrafluoroethylene, methyleugenol, malonaldehyde (sodium salt), benzyl acetate, anthraquinone, 3,3'-dimethylbenzidine dihydrochloride, C.I. pigment red 3, pentachlorophenol, chlorendic acid, and 3,3'-dimethoxybenzidine dihydrochloride (Maronpot 2000). Among the compounds tested by NTP, tetrafluoroethylene, methyleugenol, 3,3'-dimethylbenzidine dihydrochloride, chlorendic acid, and 3,3'-dimethoxybenzidine dihydrochloride were hepatocarcinogens in rats (i.e., clear evidence of carcinogenicity). To our knowledge, none of these compounds has been reported to be a PPARα agonist.

Table 5-2. Incidence of selected liver lesions in Fischer rats exposed to DINP for two years—all deaths [a] (Lington et al. 1997) [b]

Lesion	Percent DINP in Feed			
	0	**0.03**	**0.3**	**0.6**
Males				
Number examined	81	80	80	80
Focal necrosis	10	9 (0.51)	16 (0.13)	26 (0.0018)
Spongiosis hepatis	24	24 (0.55)	51 ($1.2x10^{-5}$)	62 ($7.3x10^{-10}$)
Hepatopathy associated with leukemia	22	17 (0.25)	34 (0.030)	33 (0.043)
Centrilobular to midzonal hepatocellular enlargement	1	1 (0.75)	1 (0.75)	9 (0.0084)
Females				
Number examined	81	81	80	80
Focal necrosis	13	11 (0.41)	19 (0.15)	21 (0.082)
Spongiosis hepatis	4	1 (0.18)	3 (0.51)	4 (0.63)
Hepatopathy associated with leukemia	16	18 (0.42)	24 (0.093)	33 (0.0025)
Centrilobular to midzonal hepatocellular enlargement	1	0 (0.50)	0 (0.50)	11 (0.0024)

[a] All deaths includes terminal sacrifice and spontaneous deaths.

[b] Numbers in parentheses are Fisher's exact p-values for pair-wise comparisons with controls.

* Spongiosis hepatis is described as "cystic degeneration" in NTP reports.

Table 5-3. Incidence of selected liver lesions in Fischer rats exposed to DINP for two years—all deaths (Moore 1998a, Table 10E) [a]

Lesion	Percent DINP in Feed					
	0	**0.05**	**0.15**	**0.6**	**1.2**	**1.2** [b]
Males						
Number examined	80	50	50	65	80	50
Individual cell degeneration/necrosis	0	0	0	1 (0.45)	5 (0.0029)	0
Focal necrosis	3	1	0	0	3 (0.69)	4 (0.27)
Spongiosis hepatis	5	5	2	14 (0.0068)	21 (0.0051)	9 (0.037)
Diffuse hepatocellular enlargement	0	0	0	0	37 ($3.1x10^{-14}$)	0
Increased cytoplasmic eosinophilia	0	0	0	0	43 ($4.4x10^{-17}$)	0
Females						
Number examined	80	50	50	65	80	50
Individual cell degeneration/necrosis	0	0	0	0	1 (0.50)	0
Focal necrosis	1	3 (0.17)	4 (0.078)	4 (0.13)	7 (0.034)	3 (0.17)
Spongiosis hepatis	0	0	0	1 (0.45)	2 (0.25)	0
Diffuse hepatocellular enlargement	0	0	0	0	52 ($6.6x10^{-22}$)	0
Increased cytoplasmic eosinophilia	0	0	0	0	45 ($4.3x10^{-18}$)	0

[a] Numbers in parentheses are Fisher's exact p-values for pair-wise comparisons with controls.
[b] Treated for 78 weeks, followed by a 26-week recovery period.

Table 5-4. Incidence of selected liver lesions in Sprague-Dawley rats exposed to DINP-A for two years—all deaths (Bio/dynamics 1986, Volume II, page 11) [a]

Lesion	Percent DINP in Feed			
	0	**0.05**	**0.5**	**1.0**
Males				
Number examined	70	69	69	70
Focal necrosis	5	17 (0.0042)	11 (0.086)	23 (0.0001)
Spongiosis hepatis	16	11 (0.89)	30 (0.0079)	32 (0.0036)
Females				
Number examined	70	70	70	70
Focal necrosis	10	15 (0.19)	7	10 (0.60)
Spongiosis hepatis	4	3	6 (0.38)	11 (0.051)

[a] Numbers in parentheses are Fisher's exact p-values for pair-wise comparisons with controls.

Spongiosis hepatis was observed in the two lifetime feed studies in Fischer 344 rats (Lington et al. 1997; Moore 1998a). However, the study by Lington et al. (1997) exhibited a more pronounced dose response for this effect (Figure 5-1). The NOAEL was 15 mg/kg-d in the Lington study and 88 mg/kg-d in the Moore study. Both studies used Fischer 344 rats, and both used the same type of DINP (DINP-1), although the DINP was from two different suppliers. The Chemical Manufacturers Association (currently the American Chemistry Council) convened a pathology working group (PWG) to review histological slides from both studies (CMA 2000). The PWG attributed the disparity between the two studies to differences in methodology (EPL 1999). According to the PWG, Lington et al. routinely prepared sections from each liver lobe plus gross lesions, resulting in 4 to 5 sections per liver. The number of liver sections routinely examined was not specified in the methods sections of either study. In contrast, Moore routinely reviewed only one section from each liver plus gross lesions. Because the spongiosis hepatis was generally a microscopic lesion, Lington et al. had a higher probability of finding a lesion if one existed. The difference in methodology makes it difficult to compare the two studies.

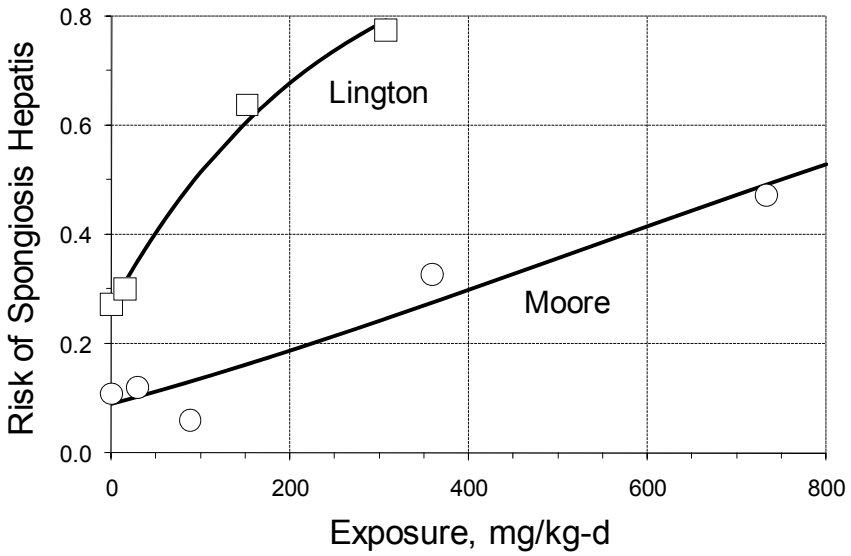

Figure 5-1. Risk of spongiosis hepatis in male F344 rats fed diisononyl phthalate (DINP) for two years: squares, Lington et al. 1997; circles, Moore 1998a (Covance); lines, polynomial (multistage) model for either data set (EPA 2000). Incidence data at terminal sacrifice are as revised by the pathology working group (EPL 1999).

Although there is no way to ascertain what results would have been obtained if Moore had reviewed four slides from each liver, the effect of reviewing additional slides can be modeled (Babich and Greene 2000). Assuming that Moore looked at exactly one slide from each liver examined, then the risk of spongiosis hepatis in the Moore data set represents the probability $p_M(D)$ of finding a lesion on one slide, at a given dose level D. Then, the risk of finding a lesion on four slides $p_4(D)$ is:

$$p_4(D) = 1 - [1 - p_M(D)]^4 \qquad (5\text{-}1)$$

Thus, the data from the Moore (1998) study were scaled to make them roughly comparable to the Lington et al. (1997) study (Table 5-5). The only dose level common to both studies is zero dose. The zero dose incidence in the Moore study (6 of 55) was scaled to 20 of 55. This is not significantly different from the zero dose incidence in the Lington study (22 of 81) by a two-tailed Fisher's exact test (P=0.34). This observation tends to support the validity of the scaling process.

With the Moore data scaled to 4 slides, the two dose responses appear to be comparable (Figure 5-2). A marginal fit to the pooled data is obtained with a polynomial model (P=0.0075) (models are described below). A considerably better fit was obtained when the observation at 88 mg/kg-d, an apparent outlier, was ignored (P=0.64).

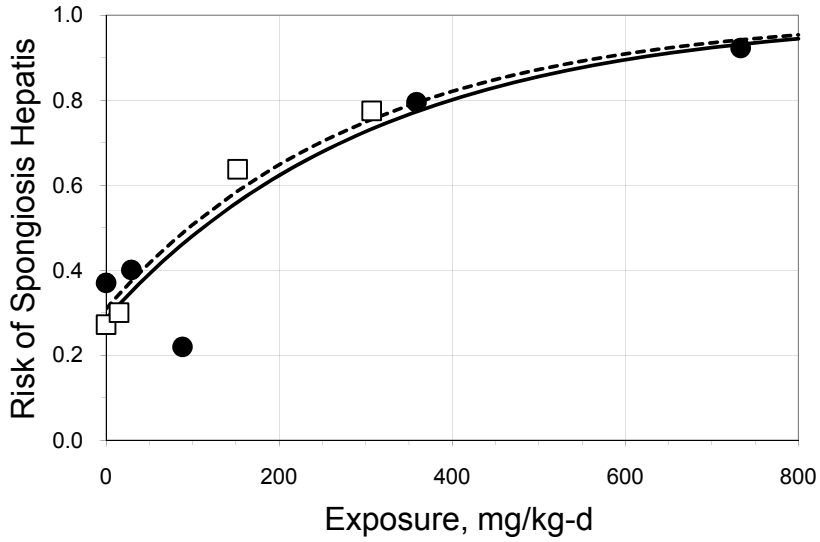

Figure 5-2. Risk of spongiosis hepatis--scaled data (see text): squares, Lington et al. 1997; filled circles, Moore 1998a scaled to 4 slides with equation (5-1); solid line, polynomial (multistage) model for the pooled data (EPA 2000); broken line, polynomial model without the observation at 88 mg/kg-d.

The Lington data can also be scaled to one slide per liver:

$$p(D) = 1 - [1 - p_L(D)]^{1/4} \qquad (5\text{-}2)$$

where: p_L is the probability of finding a lesion by viewing 4 slides.

This also makes the two dose responses comparable (not shown) (see Babich and Greene 2000). It should be noted that the number of slides viewed affects the incidence of spongiosis hepatitis in both the control and treated animals. Thus, viewing more than 4 slides could result in a higher background incidence than was observed in the Lington et al. study.

The sponsor of the Lington study also commissioned a reevaluation of slides from this study (Brown 2000). Slides from male rats diagnosed with spongiosis hepatis were evaluated to determine the number of slides with spongiosis hepatis, the number of foci per slide, and the severity of the effect. The purpose of the reevaluation was to further investigate the effect of the number of slides per liver on incidence. The dose responses for each liver lobe are plotted in Figure 5-3. These dose responses are roughly consistent with the dose response for the Lington study scaled to one slide. The observed incidence of spongiosis hepatis was slightly greater in the left and right lobes than in the median and caudate lobes.

The preceding analyses are consistent with the conclusion of the pathology working group that the difference in dose response between the two studies can be attributed to differences in methodology, that is, the number of slides from each liver that were

examined microscopically. Furthermore, the observed incidence at the NOAEL in the Moore study is apparently an outlier (Figure 5-1). These findings support the use of the more sensitive study, that is, Lington et al., to establish a NOAEL (15 mg/kg-d) for liver effects.

Lington et al. concluded that spongiosis hepatis and other non-neoplastic liver lesions were associated with mononuclear cell leukemia (MNCL) (Lington et al. 1997). However, the pathology working group found that about half of the animals with spongiosis hepatis did not have MNCL (EPL 1999, see p. 16 and Tables 11-12; see also Brown 2000). Therefore, they concluded that, although spongiosis hepatis was somewhat associated with MNCL, it was not a consequence of MNCL. Furthermore, spongiosis hepatis and hepatic necrosis were also observed in the Bio/dynamics study with DINP-A, in which hematological neoplasms were not reported (Bio/dynamics 1986). Therefore, it seems reasonable to conclude that spongiosis hepatis may be induced by chronic exposure to DINP independently of MNCL (Babich 2002; CPSC 2001; EPL 1999; Lee 1998).

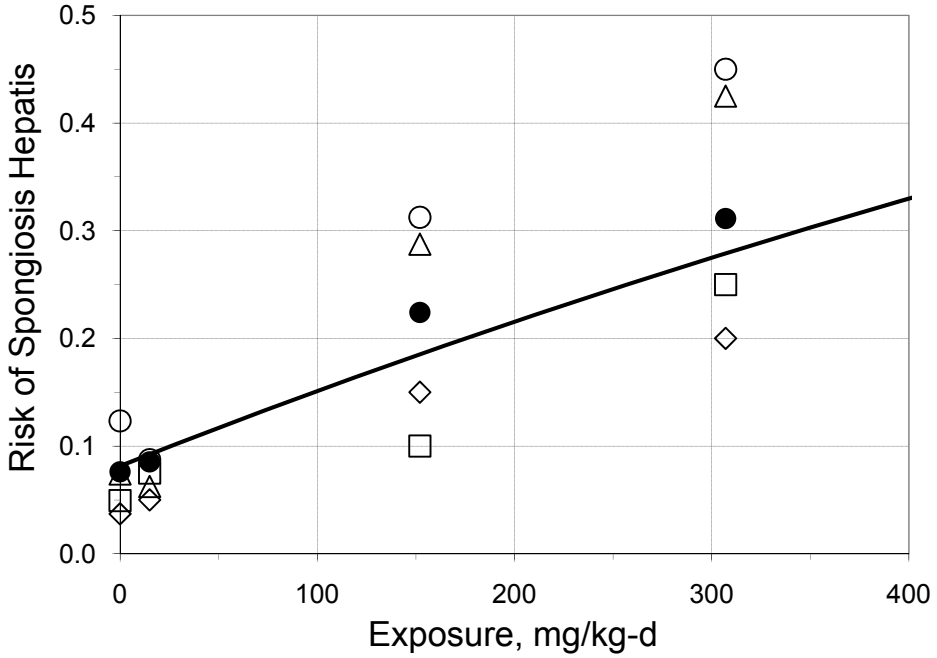

Figure 5-3. Risk of spongiosis hepatis by liver lobe (see text): filled circles, Lington et al. 1997 scaled to 1 slide with equation (5-2); open circles, Lington et al. left lobe; squares, Lington et al. median lobe; triangles, Lington et al. right lobe; diamonds, Lington et al. caudate lobe; line, polynomial (multistage) model for pooled data, one slide for liver (see text) (EPA 2000). Lobe-specific incidence data from Brown (2000).

One may also consider whether spongiosis hepatis was associated with liver tumors or peroxisome proliferation. Spongiosis hepatis was sometimes associated with liver tumors, but frequently was not (Moore 1998a). Spongiosis hepatis was found primarily in male rats, whereas peroxisome proliferation is induced in both sexes of mice and rats

(see below). Therefore, the CHAP concluded that spongiosis hepatis likely occurred independently of liver tumors and peroxisome proliferation (CPSC 2001).

As discussed above, spongiosis hepatis is a focal, degenerative liver lesion found in aging male rats. The incidence (Lington et al. 1997; Moore 1998a) and number of lesions per animal (Brown 2000) increased in a dose-dependent manner following exposure to DINP. Spongiosis hepatis is considered to occur independently of the occurrence of liver neoplasms (Moore 1998a), MNCL (CPSC 2001; EPL 1999; Lee 1998), and peroxisome proliferation (CPSC 2001). The more sensitive study (Lington et al. 1997) may be used to establish a NOAEL for liver effects (Babich and Greene 2000; CPSC 1998; CPSC 2001; CSTEE 2001; Lee 1998).

Table 5-5. Incidence of spongiosis hepatis in male rats at terminal sacrifice—pooled data when 4 slides are viewed (Babich and Greene 2000).

Dose mg/kg-d	Study	N	Observed [a]		4 Slides per liver [b]	
			X	P	X'	P'
0	Lington	81	22	0.272	22 [c]	0.272
0	Moore	55	6	0.109	20 [c]	0.364
15	Lington	80	24	0.300	24	0.300
29	Moore	50	6	0.120	20	0.400
88	Moore	50	3	0.060	10	0.200
152	Lington	80	51	0.638	51	0.638
307	Lington	80	62	0.775	62	0.775
359	Moore	55	18	0.327	43	0.782
733	Moore	55	26	0.473	50	0.909

Dose, dose in feed, milligrams per kilogram per day; Study, Lington et al. (1997) or Moore (1998a) (Covance); N, number of animals at risk; X, observed number of animals with spongiosis hepatis at terminal sacrifice; P, observed fraction of animals with spongiosis hepatis, that is, P = X / N; X', number of animals with spongiosis hepatis assuming 4 slides per liver; P'; fraction of animals with spongiosis hepatis assuming 4 slides per liver.

[a] Incidence data are as reevaluated by the Pathology Working Group (EPL 1999).
[b] Data from Moore (1998a) were adjusted according to equation (5-1) (see text).
[c] The two incidences at zero dose are not significantly different by a two-tailed Fisher's exact test (P=0.34).

Mice

In the Covance study in B6C3F1 mice, focal necrosis, diffuse hepatocellular enlargement, and cytoplasmic eosinophilia were significantly elevated in both males and females at 0.8 percent DINP (Moore 1998b, Table 11D). The incidences of the focal necrosis declined to background levels in the recovery group, in which animals were exposed to 0.8 percent DINP for 78 weeks, then allowed to recover for 26 weeks. The incidences of

hepatocellular enlargement and cytoplasmic eosinophilia also declined dramatically, although not to background levels.

In a 13 week study in B6C3F1 mice, diffuse hepatocelluar enlargement and individual cell necrosis were reported in male and female mice at feed levels of 1.0 and 2.0 percent, respectively (Bankston 1992; Moore 2000). The NOEL for liver histopathological effects in mice was 0.4 percent (Moore 1998b).

Primates

No histopathological, clinical chemistry or hematological changes were reported to occur in the two primate studies. However, these studies are limited by the small numbers of animals (4) per dose/sex group and somewhat by their short duration. Cynomolgus monkeys were treated with 500 mg/kg-d for 14 days (Pugh et al. 2000), while marmosets were treated with up to 2500 mg/kg-d for 13 weeks (Hall et al. 1999). For comparison, histopathological effects were reported to occur in rats treated for 13 weeks at doses of at least 584 mg/kg-d (Myers 1991).

Kidney

Effects of DINP exposure on the kidney are summarized in Table 5-6 (reviewed in CERHR 2003; CPSC 2001). Increased kidney weights were reported in rats following as little as 3 weeks exposure (BIBRA 1985). In a 13 week study, increased kidney weights and blood urea nitrogen levels were found in both sexes, with microscopic lesions occurring only in males, at a dietary level of 0.25 percent DINP or greater (Myers 1991). In the 2-year Lington study, increased kidney weights were reported in both sexes beginning at 6 months at 0.3 and 0.6 percent DINP (Lington et al. 1997). In the Covance study in rats, increased kidney weights and blood urea nitrogen were found in males and females at 0.6 and 1.2 percent DINP (Moore 1998a). Kidney weights returned to control levels in the recovery group animals, but blood urea nitrogen remained elevated in recovery group males. Increases in mineralization of renal papillae and pigmentation of tubular cells were found in males at 0.6 and 1.2 percent DINP, which were not reversed in the recovery group. Urine volume was increased and urine electrolytes decreased in males at 1.2 percent DINP and in the recovery group.

In the 2-year Covance study in mice, kidney weights were significantly decreased in males given 0.15 percent or more DINP (Moore 1998b). The decrease in kidney weight was somewhat reversible in the recovery group. Increased incidence and severity of chronic progressive nephropathy was reported in females at 0.8 percent DINP. Increased urine volume and decreased electrolytes were found in males at 0.4 and 0.8 percent, and in females at 0.8 percent, which the authors attributed to an alteration in the concentrating ability of the renal tubule epithelium. In the 13-week pre-chronic study, kidney weights decreased in males, but increased in females (Moore 2000). Tubular nephrosis was found in males at 2.0 percent DINP.

Hematology

In the Lington study, erythrocyte count, hemoglobin, and hematocrit were reduced in animals fed 0.3 or 0.6 percent DINP, but were only statistically significant in males at 0.6 percent DINP (Lington et al. 1997). In the Covance study in rats, erythrocyte count, hemoglobin, and/or hematocrit were significantly reduced at certain doses and time points in rats of both sexes at 0.15, 0.6, or 1.2 percent DINP and in recovery group animals (Moore 1998a). However, these were only consistently reduced at doses of 0.6 percent or greater. At 104 weeks, hemoglobin (1.2 %) and hematocrit (0.6%) were significantly reduced in males, while erythrocytes, hemoglobin, and hematocrit were significantly reduced in females at 0.6 and 1.2 percent DINP. These effects were apparently reversed in the recovery group animals. The NOAEL for hematological effects was 0.3 percent DINP (Lington et al. 1997).

Endocrine Effects

Humans

A Danish-Finish cohort study examined the levels of phthalate monoester metabolites in breast milk of mothers of 3-month old male infants with (n=62) or without (n=68) cryptorchidism (Main et al. 2006). The authors found no correlations between lactational phthalate monoester exposure and the presence of cryptorchidism. However, some effects on reproductive hormone levels were observed. Boys with and without cryptorchidism (n=96) were analyzed together. MINP, the primary metabolite of DINP, was significantly positively correlated with levels of luteinizing hormone (LH) ($p=0.019$). A 10-fold increase in MINP level was associated with a doubling of serum LH. Increasing LH is an indirect indicator of antiandrogenic effects. Lower LH:free testosterone leads to increased LH production by the pituitary, which in turn stimulates the Leydig cells to increase in size and number to increase testosterone production. MINP was also associated with non-significant increases in sex-hormone binding globulin (SHBG) ($p=0.076$), an indicator of Leydig cell function, LH:free testosterone ($p=0.099$), and total testosterone ($p=0.078$). Increases in LH, SHGB, and LH:testosterone are indirect indicators of antiandrogenic effects. Roughly similar results were obtained when only boys without cryptorchidism (n=46) were analyzed. The positive correlation of MINP with LH levels was not statistically significant ($p=0.078$). However, MINP was significantly positively correlated with LH:testosterone and LH:free testosterone.

Table 5-6. Kidney effects following DINP exposure [a]

pecies/ Strain	Duration	Doses	Effect	NOEL [b]	Reference
Rat, F344	3 weeks	0, 0.06, 0.12, 0.25 % in feed (M: 0, 639, 1192, 2195 mg/kg-d F: 0, 607, 1198, 2289 mg/kg-d)	Increased relative kidney weight	ND [c]	BIBRA 1985
Rat, F344	13 weeks	0, 0.1, 0.3, 0.6, 1.0, 2.0 % in feed (0, 50, 150, 320, 530, 1260 mg/kg-d)	Increased kidney weight (both sexes); nephrotic and regenerative changes (males)	0.1 %	Bird et al. 1986
Rat, F344	13 weeks	0, 0.25, 0.5, 1.0, 2.0 % in feed (M: 0, 146, 292, 584, 1168 mg/kg-d F: 0, 182, 364, 728, 1456 mg/kg-d)	Increased kidney weight, BUN, (both sexes); microscopic lesions (males)	0.25 %	Myers 1991
Rat, F344	2 years	0, 0.03, 0.3, 0.6 % in feed (M: 0, 15, 152, 307 mg/kg-d F: 0, 18, 184, 375 mg/kg-d)	Increased relative kidney weights (both sexes); increased urine volume (high dose males); increased tubular cell pigmentation associated with MNCL	0.03 %	Lington et al. 1997
Rat, F344	2 years	0, 0.05, 0.15, 0.6, 1.2 % in feed (M: 0, 29, 88, 359, 733 mg/kg-d F: 0, 36, 109, 442, 885 mg/kg-d)	Increased kidney weight and BUN (both sexes); mineralization of renal papillae, increased pigmentation of tubular cells, increased urine volume, decreased electrolytes (males)	0.15 %	Moore 1998a
Rat, SD	2 years	0, 0.05, 0.5, 1.0 % in feed (M: 0. 27, 271, 553 mg/kg-d F: 0, 33, 331, 672 mg/kg-d)	Increased kidney weight (high dose males; mid and high dose females); increased mineralization at high dose (significant in males)	0.05 %	Bio/dynamics 1986

Table 5-6. Kidney Effects following DINP exposure (continued)

Mouse, B6C3F1	13 weeks	0, 0.15, 0.4, 1.0, 2.0 % in feed (M: 0, 340, 904, 2365, 5472 mg/kg-d F: 0, 389, 1041, 2834, 6070 mg/kg-d)	Increased kidney weights (females, 2.0%); decreased kidney weights (males, ≥0.4%); tubular nephrosis (males, 2.0%).	0.15 %	Bankston 1992 Moore 2000
Mouse, B6C3F1	2 years	0, 0.05, 0.15, 0.4, 0.8 % in feed (M: 0, 90, 276, 742, 1560 mg/kg-d) F: 0, 112, 336, 910, 1888 mg/kg-d)	Decreased kidney weights (males, ≥0.15%); increased urine volume (males, 0.04%, both sexes, 0.8%); increased progressive nephropathy (females, 0.8%)	0.05 %	Moore 1998b

[a] Adapted from CPSC 2001 with changes.
[b] NOEL, no observed effect level.
[c] ND, not determined.

In this study, lactational exposure to ortho-phthalate monoesters was correlated with hormone levels in 3-month old male infants, including increased LH and LH:testosterone. This suggests that exposure to these compounds may have antiandrogenic effects in male infants. As with other human studies (e.g., Swan et al. 2005), this study is complicated by exposures to multiple phthalates, including some (monomethyl phthalate and monoethyl phthalate) that do not cause similar effects in animals. Thus, it is difficult to determine which of the monoesters contribute to the effects. In animal studies, DINP/MINP is less potent than other "active" phthalates (see Developmental Effects). Furthermore, this study does not report levels of oxidative metabolites. The data presented in the publication are not amenable to dose response assessment.

Animals

Reduced testicular weights, in the absence of histopathological effects, were reported in B6C3F1 mice (Bankston 1992) and in Fischer 344 rats (Myers 1991) given ≥1.0 percent DINP in feed for 13 weeks. Small numbers of immature or abnormal sperm were also present at 1.0 percent DINP in mice (Bankston, 1992).

Reduced testicular weights without histopathological effects were also reported in chronic studies in B6C3F1 mice at ≥0.4 percent DINP-1 (Moore 1998b), but not in rats at doses up to 1.2 percent (Lington et al. 1997; Moore 1998a). Relative adrenal gland weights were slightly increased at 0.6 percent DINP at terminal sacrifice in the Lington study in Fischer 344 rats (Lington et al. 1997). Spleen weights were increased in female rats at ≥0.6 percent at terminal sacrifice in the Covance study (1998a).

In a Hershberger assay, castrated prepubertal SD Crl:CD rats (six per group) were given zero, 20, 100, and 500 mg/kg/day DINP by gavage in combination with 0.4 mg/kg/day testosterone for 10 days (Lee and Koo 2007). There were no changes in total body weight, kidney, liver or adrenal weight. Changes were not seen in the weights of ventral prostate, Cowper's gland or the glans penis. There was a decrease in seminal vesicle weight in all treatment levels of DINP and a decrease in the levator ani/bulbocavernosus at 500 mg/kg/day DINP. There was also an increase in luteinizing hormone and a decrease in testosterone in rats treated with 500 mg/kg/day DINP compared to the testosterone treated controls. These results suggest that acute exposure to DINP causes antiandrogenic activity in rats. The LOEL in this study was 20 mg/kg-d; a NOEL was not established.

DINP did not increase uterine wet weight or vaginal epithelial cell cornification in immature or mature ovariectomized rats, two classical *in vivo* estrogen mediated responses (Zacharewski et al. 1998).

In Vitro Studies

The *in vitro* studies discussed below are limited by the fact that the cells were exposed to DINP, rather than MINP. The *o*-DAP's are rapidly metabolized to the monoesters *in vivo*. The monoesters are believed to be the active species, and are more likely to yield a

positive response *in vitro*. The lack of an effect of DINP i*n vitro* is meaningless, since MINP is the active form.

However, DINP-1 did not affect the expression of the androgen receptor as evaluated through an *in vitro* transcriptional reporter gene assay in transiently transfected CHO-K1 cells (Krüger et al. 2008).

DINP was inactive in an *in vitro* assay that measured binding of phthalates to estrogen receptors (Zacharewski et al. 1998) or in an *in vitro* assay of estrogen-induced gene expression (Harris et al. 1997).

Further *in vitro* studies were performed with DINP to study potential endocrine disrupting effects. Ovarian granulosa cells were treated with low and high concentrations of DINP (10^{-8}M and 10^{-4}M) (Mlynarcikova et al. 2007). Porcine primary ovarian granulosa *in vitro* cell culture model contains the majority of follicular cells and represents the main source of female sex hormones. Under appropriate media and nutritional conditions, the steroidogenic function of these cells can be studied. DINP did not affect basal estradiol levels. Normally, granulosa cells treated with follicle stimulating hormone (FSH) respond by producing hormones; this response was studied in combination with DINP treatment. FSH-stimulated estradiol was significantly inhibited back to, or below, control levels by all concentrations of DINP ($p<0.001$). These results suggest that DINP may have the potential to inhibit the FSH-stimulated release of estradiol.

Metabolically active cultured rat thyroid cells treated with DINP showed a statistically significant increase (2.3-fold) in iodide uptake (Wenzel et al. 2005). This increase was significantly blocked by a specific sodium/iodide symporter (NIS) inhibitor ($KClO_4$). These results suggest that DINP increases uptake of iodide by thyroid cells via the sodium/iodide symporter, and has the potential to modulate thyroid hormone biosynthesis.

Summary of Endocrine Effects

A Hersberger assay suggests that DINP has anti-androgenic effects in the developing male rat (Lee and Koo 2007) (Table 5-7). Rat and mice studies show decreased testicular weight with no histopathological changes, following subchronic or chronic exposures. I*n vitro* and *in vivo* data suggest that DINP does not possess estrogenic activity. *In vitro* studies suggest that DINP may modulate thyroid hormone biosynthesis. There is inadequate evidence that lactational exposure to MINP affects hormone levels in male infants (Main et al. 2006).

Perinatal DINP exposure leads to developmental malformation in rats through an anti-androgenic mechanism that does not involve direct interaction of DINP and the androgen receptor (Gray et al. 2000; Parks et al. 2000). This is discussed in detail below (Developmental Effects).

Table 5-7. Endocrine effects of DINP and MINP exposure in males [a]

Species/ strain	Study design	Effect	NOEL [b]	LOEL [b]	Reference
Human	Lactational exposure to MINP	Increased LH and LH:testosterone ratio	ND	ND	Main et al. 2006
Rat, F344	13 weeks in feed	Reduced testicular weight	292	584	Myers 1991
Rat, F344	2 years in feed	Reduced testicular weight	733	ND	Moore 1998a
Mouse, B6C3F1	13 weeks in feed	Reduced testicular weight; abnormal sperm	904	2365	Bankston 1992; Moore 2000
Mouse, B6C3F1	2 years in feed	Reduced testicular weight	276	742	Moore 1998b
Rat, SD	Hershberger assay	Decreased seminal vesicle weight	ND	20	Lee and Koo 2007
		Decreased levator ani/ bulbocavernous weight; increased LH; decreased testosterone	100	500	

[a] LH, luteinizing hormone; LOEL, lowest observed effect level; MINP, monoisononyl phthalate; ND, not determined; NOEL, no observed effect level.
[b] Doses in mg/kg-d.

Immunologic Effects

Human Studies

A human repeated insult patch test (HRIPT) was performed on 104 subjects with several phthalates, including DINP. There was no evidence of dermal irritation after repeated applications with undiluted DINP (Medeiros et al. 1999).

A nested case-control study was performed in Sweden analyzing the possible association of the presence of phthalates in house dust and children displaying asthma, rhinitis, and/or eczema (Bornehag et al. 2004). DINP was present in dust collected from children's rooms of 346 homes, with a median (mean) concentration of 0.041 (0.639) mg/g dust. In comparing the association of children with doctor-diagnosed asthma, rhinitis, or eczema, the dust collected from the bedrooms of cases had a median (mean) concentration of 0.000 (0.671) mg/g dust, while the control rooms had 0.047 (0.589) mg/g dust. While BBP and DEHP were found to have associations with these disease states in children, a similar association was not observed with DINP. The large difference between the median and mean exposures in this study indicates that there were a large number of non-detects, which makes interpretation of the data difficult, at best.

A case study involving a 10-year old girl described the onset of tiny erythematous papules and papulovesicles on the child's hands the morning after contact with the contents of a "sqwish" ball. When the child opened the ball, the material inside came into contact with her hands and it was difficult to wash off. After unsuccessful washing with soap and water, a soft cleanser was used to scrub off the sticky residue. The

manufacturer of the toy (C.A.P. Toys Inc., Cleveland Ohio) reported that there were 20 consumer complaints after the toys were opened. In five of the cases, consumers observed dermatitis. The manufacturer reported that DINP was one of the ingredients of the inside of the toy, although the exact ingredients were trade secret. The authors believe the clinical appearance of the eruption to be the result of an irritant dermatitis and not allergic contact dermatitis, resulting from contact with DINP from the sqwish ball or scrubbing with the soft cleanser. Patch testing was not performed (Brodell and Torrence 1992).

Animal Studies

Two Buehler tests (occluded application test to measure delayed contact hypersensitivity) were performed on female guinea pigs with DINP-1 (reviewed in ECB 2003; HSDB 2009; NICNAS 2008). The first study showed evidence of sensitization on day 37 in 3/20 animals, following the second challenge (Exxon Biomedical Sciences 1992). It is noteworthy that 4/10 control animals displayed a score of 1 on the same day. In the second test, there was no evidence of sensitization (Huntingdon Research Centre 1994).

Butala et al. 2004. DINP-1 and other phthalates—50 µL/flank, neat, with a semi-occlusive dressing—were applied topically to female B6C3F1 mice (15/group) 5 days per week for two weeks (Butala et al. 2004). On study day 14, five animals per group were sacrificed to measure liver weight. On day 21, the remaining animals (10/group) were challenged by applying 25 µL phthalate to each ear. There were significant increases in the liver weights of DINP-treated mice, compared to control animals, indicating DINP was absorbed systemically. There was a large increase in total serum IgE in animals treated with trimellitic anhydride (TMA), a known respiratory sensitizer. However, DINP application did not lead to increased serum IgE levels, relative to vehicle controls. Cytokine levels are used as markers of respiratory sensitizing potential. TMA elicited significant increases in the levels of IL-4 and IL-13 protein as well as a significant induction of IL-4 and IL-13 mRNA. DINP and the other phthalates treatment did not lead to significant changes in either the IL-4 and IL-13 proteins or mRNA levels. The results of this study suggest that DINP does not elicit the expected responses of a respiratory allergen.

Larsen et al. 2002. Allergic asthma and allergic rhinitis (type I allergy) are characterized by an increase in IgE production in humans and in mice by production of IgE and/or IgG1 antibodies. This IgG1 response has been used as an assay for the potential of developing a Type I allergy in humans. Female BALB/cJ mice (9-11/group) were sensitized to ovalbumin alone or in combination with 50 µL DINP-1 at concentrations of 2, 20, 200 or 2000 µg/mL, by subcutaneous injection (Larsen et al. 2002). Several other phthalates were also tested. The total amount injected was 0.1, 1, 10, or 100 µg DINP per animal. Animals received booster injections of ovalbumin on days 10 and 15 following the primary injection. Blood was collected 4 days following each booster, that is, on study days 14 and 19.

There were non-dose dependent, statistically significant increases in IgG1 and IgE levels following the first booster dose of 200 µg/mL DINP, but not at 2000 µg/mL DINP. Following the second booster dose, there were significant increases of IgG1 levels at both 200 and 2000 µg/mL DINP. The results suggest that DINP possess adjuvant activity, leading to elevated IgG1 levels after two booster injections. It is possible that DINP may possess a slight immune-suppressive activity at higher doses, explaining the effect seen at 2000 µg/mL DINP after one booster.

Lee et al. 2004. Lee et al. (2004) studied the ability of DINP or DEHP to induce IL-4 production in activated CD4+ T cells. IL-4 has a key role in the development of respiratory allergies and may contribute to lung inflammation. BALB/c mice (sex unspecified) were immunized with an antigen (keyhole limpet hemocyanine) twice at seven day intervals. Selected animals (6/group) were given 2 or 5 mg/kg DINP by intraperitoneal injection every other day for 14 days, beginning with the first immunization, then blood and lymph nodes were collected. DINP and DEHP treatment led to significant increases in the antigen mediated production of IL-4. This was accompanied with a significant increase in serum levels of total and antigen-specific IgE. Further studies demonstrated that DINP and DEHP stimulation of IL-4 was at the gene level, as evidenced by significant increases in IL-4 mRNA. The IL-4 gene was promoted by NF-AT[*] binding at the P1 and P4 binding sites.[†] CD4+ T cells were the major cell type responsible for IL-4 release (Lee et al. 2004).

Glue et al. 2005. DINP was studied in a basophil histamine release assay that is used as a model for the inflammatory part of allergic disease. DINP treatment of human basophils did not lead to histamine induction and suggests that DINP does not augment the inflammatory response (Glue et al. 2005).

Summary of Immunologic Effects

In summary, DINP did not elicit a dermal allergic reaction in a study of 104 human subjects. A single case study reported a dermal reaction after contact with a DINP containing soft ball ("Sqwish Ball"). However, it is inconclusive as to whether the reaction was to DINP, other contents of the ball, or scrubbing of the girl's hands after contact. *In vivo* studies in guinea pigs suggest that DINP is not a skin sensitizer. *In vitro* studies mouse and human cells support these results, as DINP treatment did not lead to histamine induction.

The DINP content of bedroom dust did not correlate with allergy-induced asthma or rhinitis in children. However, *in vivo* studies in mice show that DINP or other *o*-DAP's may augment an antigen mediated IL-4, IgE, and/or IgG1 reaction. That is, DINP may contribute to the development of allergies and asthma.

[*] NF-AT, nuclear factor for activated T cells; P1…P4, P elements, i.e., binding sites in the IL-4 gene promoter.

[†] The IL-4 gene promoter has 4 binding sites, called P-elements, for NF-AT.

Summary of Systemic Effects

The chronic studies in mice and rats are the most appropriate studies for deriving an ADI for non-cancer systemic health effects (Table 5-8). Liver is the most sensitive organ site for the toxic effects of DINP, with male rats being the most sensitive species and sex. Thus, liver effects in the rat have been considered to be the critical endpoint for assessing the systemic effects of DINP (Babich 1998, 2002; CPSC 1998; CPSC 2001; CSTEE 2001; ECB 2003; Lee 1998; Wilkinson and Lamb 1999). At dietary doses of 0.6 percent DINP or more, adverse effects in the liver include spongiosis hepatis, focal necrosis, and hepatomegaly (Lington et al. 1997; Moore 1998a). These effects are accompanied by increases in serum enzymes indicative of liver damage and hematological changes. Therefore, the CPSC staff concludes that DINP is probably toxic to humans, as defined under the FHSA and implementing regulations, based on sufficient evidence of chronic toxicity in experimental animals (CPSC 1992).

The LOAEL for liver effects was 152 mg/kg-d in the Lington study, at which increased incidence of spongiosis hepatis, a degenerative lesion of the perisinusoidal cells, and increased serum alkaline phosphatase were found. The LOAEL in the Covance study was 359 mg/kg-d, where increased incidence of spongiosis hepatis and increased serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were found. As discussed above, the more sensitive study will be used to determine a NOAEL for systemic effects. Therefore, the NOAEL for systemic effects is 15 mg/kg-d, which is based on increased incidence of spongiosis hepatis and increased serum enzyme levels in male rats (Babich 1998, 2002; CPSC 2001; CSTEE 2001; Lee 1998).

DINP-A (71549-78-5) is a diisononyl phthalate that was never commercialized (see part II). It is believed to be similar to DINP-2. This product was tested in a lifetime feeding study in male and female Sprague-Dawley rats (Bio/dynamics 1986). Hepatic necrosis, as well as spongiosis hepatis, were reported at the LOAEL of 27 mg/kg-d. In comparison, the LOAEL in the Lington study was 152 mg/kg-d. This apparent difference in the toxic potency between the Bio/dynamics study and the Lington study may be due to either differences in toxicity between the two DINP's or to the use of different rat strains. Dose selection may also contribute to the different LOAEL values. Furthermore, the observation of hepatic necrosis at the LOAEL may represent a greater toxicological concern than spongiosis hepatis (CPSC 2001). However, it should be noted that the incidence of necrosis was not dose-dependent. The incidence was significantly different from the control at the low and high doses, but not at the mid dose (see Table 5-4).

It cannot be established with certainty whether DINP-A is, in fact, identical to DINP-2 (compare CSTEE 2001). It may be prudent to presume that DINP-1 and DINP-2 would give similar results if tested in SD rats. However, given the potential regulatory implications, it would be difficult to justify deriving an ADI from a bioassay with a substance that was never in commerce. Therefore, the CPSC staff will use the dose response from the Lington study to derive a non-cancer ADI for DINP (Babich 2002; CPSC 2001; CSTEE 2001). If it is determined in the future that DINP-A is identical to DINP-2, then the use of the Bio/dynamics study may be appropriate.

Table 5-8. Summary of non-cancer systemic effects in 2-year feeding studies [a]

Effect	Most sensitive species/sex	NOEL [b] mg/kg-d	LOEL mg/kg-d	Reference
Liver				
Spongiosis hepatis	Male rat	15 [c]	152	Lington et al. 1997
		88	359	Moore 1998a
Focal necrosis	Male rat	152	307	Lington et al. 1997
Hepatomegaly	Male rat	88	359	Moore 1998a
Liver-specific serum enzymes	Male rat	15	152	Lington et al. 1997
Kidney				
Mineralization of renal papillae	Male rat	88	359	Moore 1998a
Progressive nephropathy	Female mouse	910	1888	Moore 1998b
Kidney weight change	Male rat	152	307	Lington et al. 1997
Hematological Changes	Male rat	152	307	Lington et al. 1997
		88	359	Moore 1998a
Testicular Atrophy	Male mouse	276	742	Moore 1998b

[a] Adapted from CPSC 2001 with changes.
[b] NOEL, no observed effect level; LOEL, lowest observed effect level.
[c] The reason for the difference in NOEL's between the two studies is most likely that Lington et al. examined 4 to 5 slides per liver, while Moore examined only 1 to 2 (see text).

6. Reproductive and Developmental Effects

Reproductive Effects

The reproductive and developmental effects of DINP and six other phthalates were reviewed by the National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction (CERHR 2003; see also Kavlock et al. 2002; McKee et al. 2004). A two generation reproductive study in rats, which includes a one-generation range-finding study, (Waterman et al. 2000) and a three-generation study in medaka fish (Patyna et al. 2006) were available for review.

Reproductive Study in Rats

Reproductive effects were studied in a two-generation feeding study in SD (Crl:CD) rats (Waterman et al. 2000). Groups of 30 males and 30 female rats were fed 0, 0.2, 0.4, or 0.8% DINP-1 in feed for approximately 10 weeks prior to mating. These were designated the first parental (P_1) generation. Males were mated with randomly selected females from the same dose group. Exposure continued through mating and until weaning at postnatal day (PND) 21. A subset of 4 pups per sex per litter was sacrificed at PND 4 and examined. After weaning, 30 offspring per dose-sex were selected for mating; these were designated the second parental (P_2) or first filial (F_1) generation. The remaining offspring were sacrificed and examined. Exposure of P_2 (F_1) rats continued through an 11-week pre-mating period, through mating, and until weaning of the F_2 offspring, when the study was terminated. All animals in the study were examined visually postmortem. Liver, kidneys, individual testes, prostate, seminal vesicles, individual epididymides, individual ovaries, and brains were weighed. The pituitary, testes, epididymides, prostate, seminal vesicles, vagina, uterus, ovaries, mammary glands, and gross lesions from all control and high-dose animals were examined microscopically.

Average body weights of DINP-exposed P_1 animals were not significantly different from the controls during the 10-week pre-mating period or during gestation (females). However, body weights of high-dose P_1 females were significantly reduced by postpartum day 14. On postpartum day 21, high-dose females weighed about 8% less than controls. In P_1 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. In P_1 females, liver weights were increased at the mid- and high doses, and kidney weights were increased in all non-zero doses. The average weight of the left ovary in high-dose females was 17% less than controls. Histological examination of the liver revealed the presence of eosinophilia at all non-zero doses in both sexes, which the authors attributed to peroxisome proliferation. No histological effects were observed in the male reproductive organs.

No significant effects in P_1 mating ability in males or females were observed. However, mean litter sizes were increased by about 20% at all non-zero doses. At birth, the body weights of F_1 (P_2) pups were less than those of the controls, but in most cases the differences were non-significant. At weaning (PND 21), the body weights in all F_1 (P_2)

treatment groups were significantly reduced. The authors suggested that the offspring body weights may be confounded by the increased litter size.

Body weights of high-dose P_2 males were significantly reduced throughout the 11-week pre-mating period. Body weights of high-dose P_2 females were significantly reduced during the first three weeks of the pre-mating period. At the end of the pre-mating period, the body weights of all treatment groups were reduced relative to controls, but the difference was statistically significant only in the high-doses males. At weaning, maternal body weights were significantly reduced at mid- and high- doses. Absolute kidney weights were increased in high-dose males. Absolute liver weights were increased in high-dose females. No other changes in organ weights were observed. Mid- and high-dose males exhibited dilated renal pelves, which the authors attributed to α2u-globulin.

In the P_2 generation, male fertility indices (mating and fertility) were slightly, but non-significantly, reduced at all non-zero doses. Mean litter size was significantly increased at all non-zero doses. The body weights of second-generation (F_2) pups were slightly, but non-significantly reduced at birth. At weaning, the body weights of both male and female pups were significantly reduced at the mid and high doses.

Overall, there were no statistically significant adverse effects on mating, fertility, or reproductive organs. The only effect on reproduction was an increase in litter size in DINP-treated animals. Pup weights were decreased in DINP-treated animals, which are confounded by the increased litter size. Liver eosinophilia was reported in the livers of all DINP-treated parents. P_2 males had dilated renal pelves at the mid and high doses. The CERHR Expert Panel concluded that the reproductive NOAEL was 0.8% DINP in feed, which is equivalent to 665—779 mg/kg-d in males and 696—802 mg/kg-d in females (Table 6-1). A LOAEL was not established for reproductive effects. The CERHR panel also concluded that the LOAEL for developmental effects was 0.2%, based on reduced pup weights, which is equivalent to 143—285 mg/kg-d during gestation and lactation, respectively. A NOAEL was not established for developmental effects. The CERHR panel also estimated a benchmark dose for reduced pup weight. The 95% lower confidence limit (LCL) of the dose at a 5% reduction in pup weight ($BMDL_{05}$) was estimated to be 250 mg/kg-d.

Waterman et al. (2000) conducted a one-generation dose range-finding study prior to the two-generation study. Animals were dosed at 0, 0.5, 1.0, and 1.5% in feed. By the end of the 10 week pre-mating period, the average body weights of males and females were significantly reduced at the mid (1.0%) and high (1.5%) doses. Body weights of the females remained low throughout gestation and lactation. Liver weights were significantly increased in all DINP-treated groups. Kidney weights were significantly increased in all DINP-treated males, but only in the low and mid dose females. Absolute weights of both testes and the right epididymis were significantly increased in high dose males, while ovary weights were reduced in high dose females. Male mating and fertility indices were non-significantly reduced at the high dose. The mean litter size was increased only at the low dose (0.5%). There was a modest, but statistically significant

reduction in pup survival at the high dose. The results of the one-generation study are consistent with a NOAEL for reproductive effects of 1.0%, based on enlarged testes in males and enlarged ovaries in females. The 1.0% dose is equivalent to 622—1157 g/kg-d in males and 734—1169 mg/kg-d in females during pre-mating (Table 6-2). The NOAEL for fetal effects (reduced pup survival) was also 1.0%, which is equivalent to 741—1731 mg/kg-d during gestation and lactation, respectively.

Table 6-1. Estimated doses (mg/kg-d) in the two-generation study with DINP-1 [a]

Percent in feed	0 %	0.2 %	0.4 %	0.8 %
P_1 / F_0				
Males, pre-mating	0	165	331	665
Females, pre-mating	0	182	356	696
Females, gestation	0	146	287	555
Females, lactation	0	254	539	1026
P_2 / F_1				
Males, pre-mating	0	189	379	779
Females, pre-mating	0	197	397	802
Females, gestation	0	143	288	560
Females, lactation	0	285	553	1229

[a] Waterman et al. 2000. Estimated doses are from CERHR 2003.

In its review of the studies by Waterman et al. (2000), the CERHR (2000) concluded that male and female rat reproductive function and structure of reproductive organs are unaffected by exposure to DINP at maternal doses of 555–1,129 mg/kg-d during gestation and lactation, respectively, and adult doses as high as 1,676 mg/kg-d in males and 1,694 mg/kg-d in females.

Table 6-2. Estimated doses (mg/kg-d) in the one-generation study with DINP-1[a]

Percent in feed	0 %	0.5 %	1.0 %	1.5 %
Males, pre-mating	0	301—591	622—1157	966—1676
Females, pre-mating	0	363—624	734—1169	1114—1694
Females, gestation	0	377—404	741—796	1087—1186
Females, lactation	0	490—923	1034—1731	1274—2246

[a] Waterman et al. 2000. Estimated doses are from CERHR 2003.

Reproductive Study in Medaka

Patyna et al. (2006) evaluated the reproductive and developmental effects of DINP and DIDP in a 3-generation study in Japanese medaka fish. Fish were exposed to 0 or 20 ppm DINP-1 in the diet, which consisted of flake food with 5% lipid content. Feed was

prepared using acetone as a vehicle. The authors estimated that a daily dose of 1 mg/kg-d in DINP-exposed fish. For the F_0 generation, two-week old larvae were randomly assigned, 50 per tank, to 5 control tanks, 5 vehicle controls, and 5 DINP-treated tanks, resulting in 250 per dose group. Sex cannot be determined at two weeks, but random assignment ensured that both sexes would be represented at the historical 2:1 male to female ratio. A sufficient number of eggs were allowed to hatch to provide 50 fish per tank for the next (F_1) generation. Remaining eggs were counted to assess fecundity.

There were no significant affects on survival, fertility, or number of eggs. There were no evidence of endocrine-induced effects, such as changes on gonad morphology or weight, sex ratio, testis-ova, intersex conditions, or sex reversal. There was a statistically significant delay in erythrocyte pigmentation in DINP-treated and vehicle control embryos. There was a modest (<2-fold) increase in testosterone hydroxylase activity in DINP- and DIDP- treated fish. The relevance of these minor effects to aquatic species is uncertain. Medaka has been utilized as a model organism for studying reproductive and developmental toxicity and carcinogenesis. DBP induced developmental/reproductive effects in both male and female medaka (Patyna et al. 1999).

Summary of Reproductive Effects

Only one study of the reproductive effects of DINP in mammals has been reported (Waterman et al. 2000). The CERHR (2003) concluded that male and female rat reproductive function and structure of reproductive organs are unaffected by exposure to DINP at maternal doses of 555–1,129 mg/kg-d during gestation and lactation, respectively, and adult doses as high as 1,676 mg/kg-d in males and 1,694 mg/kg-d in females (Table 6-3). No reproductive effects were observed in Japanese medaka fish.

Overall, the CPSC staff concludes that there is inadequate evidence for reproductive toxicity of DINP in animals. No studies on the reproductive effects of DINP in humans are available.

Developmental Effects

The reproductive and developmental effects of DINP and six other phthalates were reviewed by the National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction (CERHR 2003; see also Kavlock et al. 2002; McKee et al. 2004). Several studies involving prenatal and perinatal DINP exposures were available for review. The developmental effects of *o*-DAP's in animals have been well-studied.

Developmental Effects of *ortho*-Dialkyl Phthalates in Animals

Perinatal Exposure. The developmental effects of the *o*-DAP's have been well-studied in animals. A thorough review of the developmental effects of *o*-DAP's in general is beyond the scope of this report. Briefly, perinatal exposure to certain phthalates is associated with the "phthalate syndrome" in rats, which encompasses a range of effects on the development of the male genitourinary system including reduced anogenital

distance (AGD), nipple retention, undescended testes, testicular atrophy, testicular histopathology, underdeveloped gubernacular cords, and hypospadias (reviewed in Foster et al. 2001; Foster 2006; Howdeshell et al. 2008). These effects persist into adulthood, even in the absence of further exposure (Barlow et al. 2004; compare McIntyre et al. 2001).

Table 6-3. Reproductive Studies of DINP in Animals [a]

Study	Doses, species/strain	NOAEL mg/kg-d	LOAEL mg/kg-d	Effects
Waterman et al. 2000	0, 0.2, 0.4, 0.8% in feed (2-generation study)	ND	143-285 (0.2%)	Decreased pup weight
	SD rat	ND	165-189	Histologic effects (hepatic eosinophilia, dilated renal pelves) (parental)
		665-779 (0.8%)	ND	Mating, fertility, testicular histology (males)
		696-802 (0.8%)	ND	Mating, fertility (females)
Waterman et al. 2000	0, 0.5, 1.0, 1.5% in feed (1-generation study)	741—1731 (1.0%)	1087—2246 (1.5%)	Reduced pup survival
	SD rat	622—1157 (1.0%)	966—1676 (1.5%)	Increased testes weights (parental males)
		734—1169 (1.0%)	1114—1694 (1.5%)	Decreased ovarian weights (parental females)
Patyna et al. 2006	0, 20 ppm in feed Japanese medaka fish	1 mg/kg-d (20 ppm)	ND	Survival, fertility

[a] LOAEL, lowest observed adverse effect level; ND, not determined; NOAEL, no observed adverse effect level.

The male developmental effects of *o*-DAP's are mainly due to the inhibition of testosterone synthesis (Mylchreest et al. 1998; Foster et al. 2001; Gray et al. 2000; Parks et al. 2000), along with reduced expression of insulin-like hormone 3 gene (insl3) (Wilson et al. 2004). The specific cellular and molecular targets of *o*-DAP's are unknown (Howdeshell et al. 2008). However, both the Sertoli cells and Leydig cells are affected (reviewed in Corton and Lapinskas 2005). *o*-DAP's alter the expression of numerous genes involved in testosterone and estrogen metabolism (Corton and Lapinskas 2005).

Testicular effects of *o*-DAP's were also reported in guinea pigs (Gray et al. 1982), mice, (Gray et al. 1982; Ward et al. 1998), rabbits (Higuchi et al. 2003), and ferrets (Lake et al.

1976). Hamsters were resistant due to slow metabolism of the phthalate ester to the monoester, which is believed to be the active metabolite, but responded to the monoester (Gray et al. 1982). *o*-DAP's that are known to induce male reproductive effects include straight chain esters with 3 to 6 carbon atoms (Foster et al. 1980), and branched chain esters with 2-alkyl substitution, most importantly, DEHP (Foster et al. 1981; Gray et al. 2000; Ostby et al. 2000) (Table 6-4). DINP is relatively weak in comparison to other active phthalates (Gray et al. 2000). Presumably only some DINP isomers have 2-alkyl substituents. Recently, it has been shown that the effects of simultaneous exposure to multiple *o*-DAP's are additive (Howdeshell et al. 2008). Furthermore, simultaneous exposure to *o*-DAP's and other antiandrogens—vinclozalin, procymidone, linuron, and prochlaz—is also reported to produce cumulative developmental effects (Christiansen et al. 2009; Rider et al. 2008). Cumulative effects may occur even though the modes of action of antiandrogens may differ. Some are androgen receptor antagonists, while others (i.e., the *o*-DAP's) interfere with testosterone synthesis.

The optimal window for induction of male developmental effects is gestational day (GD) 16 through 19, when sexual differentiation occurs (Carruthers and Foster 2005; Gray et al. 1999). Therefore, older developmental screening assays that expose the dams on GD 6 through 15 (e.g., Hellwig et al. 1997; Hellwig and Jackh 1997; Waterman et al. 1999) are likely to miss the optimum window for male development (Foster 2006). Rodents are most sensitive to the antiandrogenic effects of *o*-DAP's *in utero*. However, *o*-DAP exposure also induces testicular effects in adolescent and adult males, with adolescents being more sensitive than adults (Higuchi et al. 2003; Sjöberg et al. 1986).

Prenatal Exposure. In addition to effects on male reproductive development, *o*-DAP's induce various structural abnormalities when exposure takes place earlier in gestation, during organogenesis. The mechanism for induction of these malformations is unknown, but may involve zinc deficiency in the embryo (Peters et al. 1997c).

Role of PPARα. The PPARα receptor mediates many, but not all, of the toxic effects of the *o*-DAP's (reviewed in Peraza et al. 2006). Whether the toxic effects are mediated by PPARα is significant because humans may respond differently than rodents (reviewed in Klaunig et al. 2003). PPARα activation leads to peroxisome proliferation and hepatocarcinoma induction in rodent liver, but not in humans (see Carcinogenicity).

The ability of phthalates to cause malformations during organogenesis appears to be independent of the PPARα receptor (Peters et al. 1997c). Malformations were induced equally in PPARα (+/+) and (-/-) mice when the dams were exposed to DEHP on GD 8 and 9. Ward et al. (1998) exposed 6-week old PPARα (+/+) and (-/-) mice to dietary DEHP for up to 24 weeks. No effects were seen in the livers of PPARα (-/-) mice. The appearance of lesions in the testes and kidneys was delayed, but the lesions were similar to those in the PPARα (+/+) mice. The authors suggested that there may be both PPARα-dependent and PPARα-independent mechanisms for effects seen in the testis and kidney (Ward et al. 1998).

It is not known whether PPARα is required for the induction of the effects observed in males when exposure occurs during sexual development (Corton and Lapinskas 2005). However, DBP is a weak PPARα agonist and a potent developmental toxicant, whereas DINP is relatively good PPARα agonist (compared to other *o*-DAP's), and a weak developmental toxicant. This suggests that the effects associated with the phthalate syndrome are not dependent on PPARα activation.

Human Relevance. Recently, it has been suggested that *o*-DAP's may contribute to the occurrence of the "testicular dysgenesis syndrome" in humans, that is, testicular germ cell cancer, cryptorchidism, hypospadia, and low sperm count (Mahood et al. 2007; Swan et al. 2005). Testicular dysgenesis might be the human equivalent of the phthalate syndrome in rats. Additional research is needed to determine whether this is true.

Table 6-4. Ability of phthalate diesters to induce testicular effects in male rats

Active Compounds	Inactive Compounds
Linear Dialkyl Phthalates	
di-*n*-propyl *o*-phthalate (DPP) [a, b]	dimethyl *o*-phthalate (DMP) [a, c, d]
di-*n*-butyl *o*-phthalate (DBP) [a]	diethyl *o*-phthalate (DET) [a,c, d]
di-*n*-pentyl *o*-phthalate [a]	di-*n*-butyl isophthalate [b]
di-*n*-hexyl *o*-phthalate [a]	di-*n*-butyl terephthalate [b]
	di-*n*-heptyl *o*-phthalate [a]
	di-*n*-octyl *o*-phthalate (DNOP) [a]
Branched Dialkyl Phthalates	
di-*n*-butyl *o*-phthalate (DBP) [b]	di-tert-butyl *o*-phthalate [b]
di-isobutyl *o*-phthalate [b]	di(2-ethylhexyl) terephthalate (DOTP) [c, d]
di-sec-butyl *o*-phthalate [b]	
di(2-ethylhexyl) phthalate (DEHP) [c, d]	
diisononyl phthalate (DINP) [c, d]	
benzyl butyl phthalate (BBP) [c, d]	

[a] Foster et al. 1980. Testicular atrophy in juvenile male SD rats.
[b] Foster et al. 1981. Testicular atrophy in juvenile male SD rats.
[c] Gray et al. 2000. Developmental effects in males following prenatal exposure.
[d] Ostby et al. 2000. Developmental effects in males following prenatal exposure.

Developmental Effects of DINP Exposure in Animals—Prenatal Exposure

Hellwig et al. (1997). Hellwig et al. (1997) studied the developmental effects of three types of DINP (DINP-1, DINP-2, and DINP-3). Wistar rats (10/group) were given doses of 0, 40, 200, and 1,000 mg/kg-d by gavage on gestational day (GD) 6 through 15 (Table 6-5). Relative kidney and liver weights were slightly increased in dams of the highest dose group (5-13%), but in most instances were not statistically significant. Fetal viability and body weight were unaffected in all three studies. The incidences of skeletal

variations (rudimentary cervical ribs, accessory 14th ribs) were elevated, with the number of affected fetuses per litter being significantly higher than controls with DINP-1 and DINP-3. There also were increases in dilated renal pelves (DINP-1 and DINP-3), agenesis of kidneys and ureters (DINP-3), hydroureter (DINP-3), and skeletal (shortened and bent humerus and femur) malformations (DINP-3) in the high-dose group. The authors and the CERHR Expert Panel identified a NOAEL of 200 mg/kg-d and a LOAEL of 1,000 mg/kg-d for maternal and developmental effects in the Hellwig et al. study. This study is limited because the dams were not exposed during the optimum window for antiandrogenic effects (GD 16-19).

Waterman et al. (1999). Waterman et al. (1999) treated Sprague-Dawley rats (25/group) with DINP-1 by gavage at doses of 0, 100, 500, or 1,000 mg/kg-d on GD 6 through 15. Females were sacrificed on GD 21, and pups were delivered surgically. Maternal food intake and body weights were significantly reduced at the high dose during treatment. Only litter effects were reported for fetuses. Mean fetal body weights were significantly increased at 100 and 1,000 mg/kg-d in males and at 100 mg/kg-d in females. There was a statistically significant increase in the percentage of fetuses with dilated pelves at 1,000 mg/kg-d. There was also a significant positive trend in dilated renal pelves. Rudimentary lumbar ribs were also significantly increased at the high dose. No other fetal effects were reported.

The authors concluded that the LOAEL for maternal and developmental toxicity was 1,000 mg/kg-d, with a NOAEL of 500 mg/kg-d. However, the Expert Panel concluded that developmental effects (skeletal variations and rudimentary lumbar ribs) were present at 500 mg/kg-d (CERHR 2003). At the request of the CERHR panel, the study sponsor reanalyzed the data, the results of which supported the Expert Panel's conclusion. Thus, the Expert Panel determined that the NOAEL for the study was 100 mg/kg-d. The Expert Panel also calculated a benchmark dose (BMD_{05}) (dose at which 5% of animals are affected) of 193 mg/kg-d and a lower confidence limit ($BMDL_{05}$) of 162 mg/kg-d, based on the incidence of rudimentary ribs. This study is limited because the dams were not exposed during the optimum window for antiandrogenic effects (GD 16-19).

Hellwig and Jackh 1997 (Isononyl alcohols). Developmental effects of two 2 isononyl alcohols were studied in Wistar rats (Hellwig and Jackh 1997). Isononyl alcohol type 1 is used to manufacture DINP-1 and type 2 is used to manufacture DINP-2. Phthalates are initially metabolized to form the monoester. Thus DINP is metabolized to monoisononyl phthalate and isononyl alcohol Wistar rats (10 per group) were exposed by gavage on GD 6 through 15, at target doses of 0, 1, 5, or 10 mmol/kg. Due to high maternal mortality at 10 mmol/kg, a supplemental experiment was performed at 0 or 7.5 mmol/kg. The doses of 1, 5, 7.5, and 10 mmol/kg correspond to 144, 720, 1080, and 1440 mg/kg-d. As noted by the CERHR, there were some discrepancies in the dose levels reported in the tables and text, especially at the high dose (CERHR 2003, p. II-28).

Isononyl alcohol type 1 demonstrated significant maternal toxicity. Clinical signs of maternal toxicity (apathy and nasal discharge) were evident at 720 and 1080 mg/kg. Maternal body weights were reduced at the 720 and 1080 mg/kg, although only the

higher dose was statistically significant. Maternal mortality was 10/10 at 1440 mg/kg-d in one experiment and 1/10 at 1080 mg/kg-d in the supplemental experiment. The number of resorptions (postimplantation losses) was significantly increased at 1080 mg/kg-d. Fetal weights were reduced at 720 mg/kg-d, but significant only at 1080 mg/kg. The numbers of fetuses and litters with malformations were significantly increased at the 1080 mg/kg-d. Malformations included globular shaped heart, dilated renal pelvis, hydroureter, and one anophthalmia. The number of fetuses with skeletal retardations was significantly increased at 720 and 1080 mg/kg-d. The number of fetuses with skeletal variations was increased at 720 mg/kg-d only.

Table 6-5. Developmental Studies of DINP in Animals—Prenatal Exposure [a]

Study	Doses, species/strain	**NOAEL mg/kg-d (mol/kg-d)**	**LOAEL mg/kg-d (mol/kg-d)**	**Effects**
Hellwig et al. 1997 DINP-1, -2, & -3	0, 40, 200, 1000 mg/kg-d GD 6-15 Wistar rat	200 (4.8) 200	1000 (2.4) 1000	Maternal effects (increased kidney & liver weight) Dilated renal pelves, hydroureter; skeletal malformations & variations
Waterman et al. 1999 DINP-1	0, 100, 500, 1000 mg/kg-d GD 6-15 SD rat	500 (1.2) 100 (0.24)	1000 (2.4) 500 (1.2)	Maternal effects (decreased weight gain) Dilated renal pelves; malformations (cervical ribs); variations (lumbar ribs)
Hellwig & Jackh 1997 Isononyl alcohol types 1 & 2	0, 144, 720, 1080, 1440 mg/kg-d GD 6—15 Wistar rat	720 (5.0)	1080 (7.5)	Malformations (globular shaped heart, dilated renal pelves, hydroureter, anophthalmia (1), absent genitalia (1) skeletal variations/ retardations

[a] GD, gestational day; LOAEL, lowest observed adverse effect level; ND, not determined; NOAEL, no observed adverse effect level; PND, postnatal day.

[b] mol/kg-d, moles per kg-d. The molecular mass of isononyl alcohol is 144.3.

Isononyl alcohol type 2 demonstrated somewhat less maternal toxicity than type 1. Clinical signs of maternal toxicity were evident at the mid and high doses. Maternal body weights were reduced only on GD 10. Maternal mortality at 1080 mg/kg was 3/10 and 0/10 in two experiments. Maternal body weights were reduced at 1080 mg/kg, but were only significant at one time point in one of two experiments. Fetal weights were significantly decreased at the 1080 mg/kg in the first experiment. The number of fetuses

with malformations was significantly increased at 1080 mg/kg in the supplemental experiment. Malformations included the absence of external genitalia in one high-dose pup, dilated renal pelvis, and hydroureter. The numbers of fetuses with variations, skeletal variations, or retardations were significantly increased at 1080 mg/kg in the first experiment.

Clinical signs of maternal toxicity were observed at the mid dose, and maternal mortality was observed at the high dose. Fetal malformations were observed at the high dose, with variations and retardations at the middle dose. These findings are consistent with a NOAEL of 720 mg/kg-d and a LOAEL of 1080 mg/kg-d for developmental effects of isononyl alcohol. This study did not expose fetuses during the optimum window for sexual differentiation. *o*-DAP's are readily metabolized to equimolar amounts of the monoester and alkyl alcohol. The monoester is considered to be the active metabolite with respect to developmental and other toxic effects. This study suggests that the alcohol may also contribute to the occurrence of developmental effects.

Developmental Effects of DINP Exposure in Animals—Perinatal Exposure

Waterman et al. (2000). Adverse effects on weight gain in pups during the perinatal and pre-weaning period of life were evident in the two-generation reproductive study with SD rats described above (Waterman et al. 2000). Dams were fed 0, 0.2, 0.4, or 0.8 percent DINP-1 during mating, gestation, and lactation. Effects on male reproductive tract development, such as those associated with phthalate exposure, were not examined. The study design may have lacked sufficient power to detect such effects (Foster and McIntyre 2002; Foster et al. 2002; McKee 2002). In the F_1 generation, pup weights were significantly reduced in males on postnatal day (PND) 0 at 0.8 percent DINP and in females at 0.4 and 0.8 percent DINP (on PND 7 and 14). By PND 21, pup weights were reduced at doses ≥0.2 percent DINP. In the F_2 generation, male pup weights were reduced at 0.4 percent DINP (PND 7, 14, 21) and in females at 0.2 percent (PND 4, 7, 14, 21). The CERHR expert panel identified a LOAEL for developmental effects of 0.2% DINP in feed (143–285 mg/kg-d during gestation through lactation).

Gray et al. (2000) and Ostby et al. (2000, 2001). Gray et al. (Gray et al. 2000; Ostby et al. 2000, 2001) studied the effects of perinatal exposure to DINP-1 and other phthalates on sexual differentiation in male Sprague-Dawley rats. Dams were given 0 or 750 mg/kg phthalate from GD 14 to PND 3. Male pups exposed to DINP exhibited female-like areolas/nipples (22%, $p<0.01$; controls 0%) on PND 13. Reproductive malformations (7.7%, $p<0.04$; controls 0%) and a lower frequency of areolas/retained nipples (2/52) were observed at necropsy performed at 4 to 7 months old. The frequencies of areolas/nipples (87% on PND 13) and reproductive organ malformations (82%) were greater with DEHP. Malformations associated with DINP exposure included: small or atrophic testes; flaccid, fluid-filled testes, epididymal agenesis with hypospermatogenesis, abdominal (undescended) testes, and fluid-filled scrotal testes devoid of spermatids. In a subsequent study in the same laboratory, Ostby et al. (2001) exposed pregnant SD females to DINP at 0, 1000, or 1500 mg/kg from GD 14 through PND 3. Anogenital distance on PND 2 was decreased in males, but not females, at the

high dose. The incidence of areolas on PND 13 in males was 14% in controls, 55% at 1000 mg/kg, and 75% at 1500 mg/kg-d (Ostby et al. 2001). Taken together, these studies suggest a LOAEL of 750 mg/kg-d for retention of areolas/nipples and reproductive malformations. A NOAEL was not established.

(Hass et al. 2003). Groups of 12 mated female Wistar rats were gavaged with 0, 300, 600, 750, or 900 mg/kg DINP (type unspecified) from GD 7 through PND 17 (Hass et al. 2003). AGD was significantly decreased at birth in male pups at doses ≥600 mg/kg. After adjusting for birth weight, reductions in AGD were significant only at the high dose. Nipple retention examined at PND 13 was significantly increased in male pups at doses ≥600 mg/kg. Significant delays in pup retrieval by mothers were also observed at doses ≥600 mg/kg, suggesting either maternal toxicity or inadequate nutrition at these doses. The NOAEL in this study was 300 mg/kg-d. The authors concluded that DINP induced developmental effects similar to those of DBP and DEHP, but at higher doses.

Borch et al. (2003, 2004). Borch et al. (2003, 2004) studied the effects of DINP alone or DINP in combination with DEHP on fetal testosterone production. Pregnant female Wistar rats (8 per dose group) were exposed by gavage from GD 7 through 21 to 750 mg/kd DINP-2, 300 mg/kg DEHP, or 750 mg/kg DINP plus 300 mg/kg DEHP. Dams were sacrificed GD 21; male pups were examined for hormone levels. The rate of testosterone production and testosterone content in the testes were significantly reduced in all treatment groups at GD21. DINP or DEHP alone reduced testosterone production to about one-third of the control level, while DINP and DEHP together reduced testosterone production to about one-tenth of the control. Plasma testosterone was reduced in all treatment groups, but was significant only with DINP plus DEHP. Plasma luteinizing hormone was increased in all treatment groups, but was significant only with DINP plus DEHP. The results of this study suggest a LOAEL of 750 mg/kg-d for the developmental effects of DINP. They also suggest that the developmental effects of different phthalates are additive.

Masutomi et al. (2003). Masutomi et al. (2003) exposed pregnant SD female rats, 5 to 6 per group, to 0, 400, 4000, or 20,000 ppm DINP-2 in a soybean-free diet from GD 15 to PND 10. These exposures were equivalent to 0, 30.7, 306.7, or 1164.5 mg/kg-d during gestation and 0, 66.2, 656.7, or 2656.7 mg/kg-d during lactation. Offspring were given a standard soy-based diet after weaning. Body weight gain was significantly reduced in high-dose dams during gestation. At PND 2, AGD was non-significantly reduced in DINP-exposed male pups (3.0 mm at the mid and high doses vs. 3.3 mm in controls). The number of pups examined for AGD was not specified.

Five pups per dose/sex group were examined at PND 27 (pre-puberty). The body weights of male pups were reduced in a dose-dependent manner, although only the mid and high doses were statistically significant. Body weights of female pups were significantly reduced at the high dose only. Relative brain weights were significantly increased, while absolute brain weights were significantly reduced in both sexes at the high dose. In male pups, absolute and relative weights of the testes were significantly reduced at the high dose. The absolute testicular weights were reduced by about 20% at

the mid dose (non-significantly) and 50% at the high dose. In female pups, relative weights of the adrenal glands were significantly increased and absolute weights of the uterus and ovaries were significantly decreased at the high dose. The onset of puberty was unaffected in either sex.

Remaining offspring (5 per dose/sex) were sacrificed at PND 77. There were no significant differences in body or organ weights. Degeneration of spermatocytes and Sertoli cells was observed in males at the high dose. The number of corpora lutea per square centimeter was significantly decreased in high-dose females. The size of the sexually dimorphic nucleus of the preoptic area of the brain was unaffected in either sex.

Body weights of male pups were significantly reduced at PND 27 at the mid and high doses. Other effects, including testicular atrophy and histopathology, were significant only at the high dose. However, testicular weights appeared to show a negative trend with dose. Effects on body and organ weights were reversible following cessation of DINP exposure, which was accompanied by a change to a soy-based diet. The results of the study by Masutomi et al. (2003) are consistent with a NOAEL of 400 ppm (30—66 mg/kg-d) and a LOAEL of 4000 ppm (307—657 mg/kg-d), based on reduced pup weight at the mid dose. This study did not detect differences in AGD, which were reported by others (Hass et al. 2003; Ostby et al. 2001). A complete evaluation of malformations and variations was not reported. This study is limited by the small numbers of pups examined per group (n=5).

Masutomi et al. (2004). In subsequent work, Masutomi et al. (2004) studied the expression of pituitary hormones in Wistar rats exposed to DINP perinatally. Dams were exposed to 20,000 ppm DINP-2 in soy-free feed from GD 15 to PND 10. Expression of luteinizing hormone, follicle stimulating hormone, and prolactin was studied in offspring by immunohistochemical analysis of excised pituitary tissue at postnatal weeks 3 (pre-puberty) and 11. DINP exposure had no effect on the percentage of pituitary cells expressing the hormones tested in males or females. Relative pituitary weights and age at onset of puberty were not affected in either sex.

Takagi et al. (2005). Takagi et al. (2005) studied the effect of perinatal DINP exposure on gene expression in the sexually dimorphic nucleus (SDN) of the medial preoptic area (MPOA) of the hypothalamus. Pregnant CD(SD)IGS rats (7 per group) were exposed to 0, 4000 or 20,000 ppm DINP-2 in soy-free feed from GD 15 through PND 10. Food consumption and daily doses were not reported. Pups were sacrificed on PND 10. Gene expression was measured by reverse transcriptase polymerase chain reaction (RT-PCR). In males, DINP exposure had no significant effects on the transcriptional level expression of estrogen receptors α or β, progesterone receptor, or steroidal receptor coactivator-1 (SRC-1). In females, transcription of the progesterone receptor gene was modestly reduced (~2-fold) at the high dose. This was statistically significant only when normalized against glyceraldehydes-3-phosphate dehydrogenase (GADPH), but not when normalized against hypoxanthine-guanine phosphoribosyl transferase (HPRT) or total RNA.

Table 6-6. Developmental Studies of DINP in Animals—Perinatal Exposure [a]

Study	Doses, species/strain	NOAEL mg/kg-d	LOAEL mg/kg-d	Effects
Waterman et al. 2000 DINP-1	0, 0.2, 0.4, 0.8% in feed (2-generation study) SD rat	ND	143-285 (0.2%)	Decreased pup weight
Gray et al. 2000 DINP-1	0, 750 mg/kg-d GD 14 – PND 3 SD rat	ND	750	Areolas/nipples; testicular malformations; epididymal agenesis (males)
Ostby et al. 2001 DINP-1	0, 1000, 1500 mg/kg-d GD 14 – PND 3 SD rat	ND	1000	In males only: areolas; reduced AGD
Hass et al. 2003 [b]	0, 300, 600, 750, 900 mg/kg GD 7—PND 17 Wistar rat	300 300	600 600	Nipple retention (males) [c] Maternal pup retrieval
Borch et al. 2003, 2004 DINP-2	0, 750 mg/kg-d (PND 7—21) Wistar rat	ND	750	Decreased testosterone production & content (males)
Masutomi et al. 2003 DINP-2	0, 400, 4000, or 20,000 ppm in feed GD 15—PND 10 SD rat	30—66 (0.04%) 307—657 (0.4%)	307—657 (0.4%) 1164—2657 (2.0%)	Decreased pup weight Testicular atrophy & histopathology
Masutomi et al. 2004 DINP-2	0, 20,000 ppm in feed (GD 15—PND 10) Wistar rat	20,000 ppm [d]	ND	No effect on pituitary hormone expression. No other endpoints were studied. [c]
Takagi et al. 2005 DINP-2	0, 4000, 20,000 ppm in feed GD 15—PND 10 CD (SD) IGS rat	20,000 ppm [d]	ND	No effect ER gene transcription in the SDN No other endpoints were studied. [c]
Lee et al. 2006 DINP-2	0, 40, 400, 4000, 20,000 ppm in feed GD 15—PND21 Wistar-Imamichi rat	ND	40 ppm [d]	Reduced pup weight Reduced AGD (males) [c] Increased grn gene transcription (females) Increased p130 gene transcription (males) Reduced lordosis quotient (females)

[a] AGD, anogenital distance; ER, estrogen receptor; GD, gestational day; grn, granulin precursor; LOAEL, lowest observed adverse effect level; ND, not determined; NOAEL, no observed adverse effect level; PND, postnatal day; SDN, sexually dimorphic nucleus.

[b] DINP type or source unspecified.

[c] Other malformations, variations, and developmental delays were not studied or not reported.

[d] Food consumption or dose in mg/kg-d were not reported.

Lee et al. (2006). Lee et al. (2006) studied the effects of perinatal DINP exposure on hypothalamic gene expression and sexual behavior. Pregnant Wistar-Imamichi females were fed 0, 40, 400, 4000, or 20,000 ppm DINP-2 in soy-free feed from GD 15 to PND 21 (weaning). Food consumption and doses in mg/kg-d were not reported, but the dietary levels correspond very roughly to 3 mg/kg-d at the low dose through 1,500 mg/kg-d at the high dose. The number of dams was not specified; litter sizes were adjusted to 8 on PND 5. Pup body weights and AGD were measured on PND 1. An unspecified number of pups were sacrificed on PND 7 and the hypothalamic regions of their brains were removed to isolate RNA. Transcription of the granulin precursor (grn) and p130 genes were measured by RT—PCR. These genes are believed to be indicators of sexual differentiation in the rat brain (as cited in Lee et al. 2006). Serum testosterone and estradiol were also measured on PND 7. Mating behavior of males and females was studied at postnatal weeks 20-21.

Between 16 and 47 pups per sex/dose group were examined on PND 1. Body weights in all treatment groups were significantly reduced as compared to controls. AGD was significantly reduced at all doses in males only, with or without normalizing for body weight. However, the magnitude of the reductions in body weight and AGD were small in comparison to other studies (compare Masutomi et al. 2003; Gray et al. 1999, 2000), even at the high dose. Furthermore, the analysis was based on individual pups, not litters, which is the preferred method.

On PND 7, serum estradiol levels in male pups were significantly lower than controls at the low dose (40 ppm) only. There were no other statistically significant differences in estradiol or testosterone in male or females. However, variability in this experiment was fairly high (n=5—7). In another study, serum testosterone was less sensitive to DINP exposure than the testicular testosterone level and production rate (Borch et al. 2004). Transcription of the granulin precursor gene in the hypothalamus was significantly increased in females at all doses. The granulin precursor gene is inducible by both androgens and estrogen (Suzuki et al. 2001; Suzuki and Nishiahara 2002). Granulins are a family of cell growth regulators that are expressed during development and wound healing. Transcription of p130 was significantly increased at all doses in males. The p130 gene is androgen-inducible cell cycle regulator and the normal analog of the retinoblastoma oncogene (Yonehara et al. 2002a, b).

DINP exposure had minimal effects on the sexual behavior of mature males. Mounts, intromissions, and ejaculations were significantly reduced at the low dose (40 ppm) only (Lee et al. 2006). In a previous study, Gray et al. (2000) found no evidence of an effect on sexual behavior in DEHP-treated males that could be attributed to central nervous system changes. Hypothalamic expression of p130 was increased in males at all doses. Serum testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH) levels were unchanged.

In mature female offspring, the lordosis quotient was significantly reduced in a dose-dependent manner at all doses tested (40, 400, and 4000 ppm). Serum estradiol, follicle stimulating hormone, and luteinizing hormone levels were unaffected in females.

Expression of the granulin precursor gene was increased in DINP exposed to DINP during gestation and lactation.

DINP exposure affected hypothalamic gene expression in males, but did not appear to affect behavior. This study reports an effect on female sexual behavior (reduction in lordosis), which may be due to altered gene expression in the hypothalamus. However, there are questions regarding the methodology (analysis of pups rather than litters).

Adamsson et al. 2009. The authors exposed pregnant dams (SD rat) to 0, 250, or 750 mg/kg-d DINP (type unspecified) by gavage on embryonic days 13.5 through 17.5.* Male pups were examined on ED 19.5. Serum corticosterone levels were non-significantly increased at the low dose. No other significant increases were observed in testosterone levels, androgen receptor, or steroidogenic acute regulatory protein. However, there were significant increases in insl3 and GATA-4 mRNA's at the high dose in the testes, but not in the adrenal glands. Insl3 is required for normal development of the gubernacular cords. GATA-4 is a regulatory protein expressed in the gonads. The lack of effect on testosterone or corticosteroid levels is probably due to the delay between dosing and examining the pups.

Developmental Effects of DINP Exposure in Animals—Postnatal Exposure

The antiandrogenic effects of a series of phthalates were studied in the Hershberger assay (Lee and Koo 2007). In this assay, juvenile males are castrated and then treated with testosterone propionate. Testosterone allows the weights of the accessory sex tissues to recover following castration. The assay assesses the ability of test chemicals to interfere with the testosterone-dependent growth, assessed by tissue weight, of accessory sex tissues: seminal vesicles, ventral prostate, levator ani/bulbocavernosus (LABC), Cowper's gland, and glans penis.

Six-week old in male SD (Crl:CD) rats were castrated and allowed to recover for one week before treatment. Six animals per group were exposed by gavage to 0.4 mg/kg-d of testosterone propionate and 0, 20, 100, or 500 mg/kg phthalate by gavage for 10 days. DINP, DEHP, DBP, BBP, DIDP, and di-*n*-heptyl phthalate were tested. Mono(2-ethyhexyl) phthalate, the primary metabolite of DEHP, was tested at 0, 10, 50, or 250 mg/kg-d. Two control groups consisted of untreated animals and animals treated with testosterone propionate only. The type of DINP was not specified.

DINP treatment did not result in any significant differences in absolute body, liver, kidney, or adrenal weights, as compared to testosterone-treated controls. Liver weights were significantly increased at the high dose with DEHP and DIDP. There were no significant differences in body, kidney, or adrenal weights in any other treatment groups. The absolute weights of the seminal vesicles were significantly reduced at all DINP doses, as compared to the testosterone controls. The weight of the LABC was significantly reduced at the high DINP dose only. The ventral prostate weights were not significantly affected by DINP treatment.

* Embryonic day 13.5 is equivalent to gestational day GD 14.

DIDP treatment resulted in decreased weights of the seminal vesicles and ventral prostate at the high dose. DEHP and DBP reduced the weight of the ventral prostate at all doses. DEHP also reduced the weight of the seminal vesicles at the mid and high doses and the LABC at the high dose. MEHP reduced the weights of the seminal vesicles and LABC at the mid and high doses (50 and 250 mg/kg-d, respectively) and the ventral prostate at the high dose only. BBP and di-*n*-heptyl phthalate had no effect. The weights of the Cowper's gland and glans penis were unchanged by any treatment. This assay suggests that DEHP, DBP, MEHP, DIDP, and DINP have antiandrogenic effects in pre-pubertal male rats. The LOAEL for DINP in this assay was 20 mg/kg-d. A NOAEL was not established.

Developmental Effects of DINP in Humans

Main et al. 2006. Main et al. investigated the relationship between the levels of *o*-DAP metabolites in breast milk in 3-month old male infants with (n=62) or without (n=68) cryptorchidism (Main et al. 2006). The study was conducted in Finland and Denmark. In Denmark, controls were selected randomly from the entire birth cohort (case-cohort design); in Finland, controls were matched to cases by maternal parity, gestational age, and date of birth (case-control design). The authors found no correlations between lactational *o*-DAP exposure and the presence of cryptorchidism. However, some effects on reproductive hormone levels were observed. The relationship between monoester exposure and hormone levels was first investigated by multiple regression with log-transformed data. Nationality was the only significant confounder identified. Then, the relationship between monoester exposure (6 monoesters) and hormone levels (7 hormones and 3 ratios) was evaluated by Spearman correlations with nationality as the confounder. Probabilities were estimated by Monte Carlo permutation, and were not adjusted for multiple comparisons. Only correlation coefficients and probabilities were reported. Summary data of hormone levels were not reported.

Boys with (n=50) and without (n=46) cryptorchidism (n=96 total) were analyzed together. MINP, the primary metabolite of DINP, was significantly positively correlated with levels of luteinizing hormone (LH) (p=0.019). A 10-fold increase in MINP level was associated with a doubling of serum LH. Increased LH is an indirect indicator of antiandrogenic effects. MINP was also associated with non-significant increases in sex-hormone binding globulin (SHBG) (p=0.076), an indicator of Leydig cell function, LH:free testosterone (p=0.099), and total testosterone (p=0.078). Increases in LH, SHGB, and LH:testosterone are indirect indicators of antiandrogenic effects. Monomethyl phthalate, monoethyl phthalate, and monobutyl phthalate were positively correlated with the LH:free testosterone ratio. Monobutyl phthalate was negatively correlated with free testosterone. Monoethyl phthalate and monobutyl phthalate were positively correlated with SHBG. MEHP, the principal metabolite of DEHP, was negatively associated with the follicle stimulating hormone (FSH): Inhibin B ratio, an indicator of Sertoli cell function.

Roughly similar results were obtained when only boys without cryptorchidism (n=46) were analyzed. However, MINP and MEHP were significantly positively correlated with LH:testosterone and LH:free testosterone. As in the previous analysis, MBP was significantly associated with SHGB, free testosterone, and LH:free testosterone. In addition, MBP was positively correlated with the LH:testosterone ratio.

MINP was present at higher levels (at least 10-fold greater) in breast milk than any other *o*-DAP measured. Median MINP levels were 101 and 89 µg/L in milk samples from Denmark and Finland, respectively. Calafat et al. (2004) reported a median level of about 16 ng/mL for mono(3,5,5-trimethylhexyl) phthalate (one isomer of MINP). Furthermore, in human milk, diesters were generally present at higher levels than the corresponding monoesters (Högberg et al. 2008). This is in contrast to urinary MINP levels, which are generally lower than the other phthalate monoesters. Urinary MINP was below the detection limit in the majority of the U.S. general population (Blount et al. 2000) and was undetected in a small (n=19) sample of infants (Brock et al. 2002). Main et al. estimated the MINP exposure from breast-feeding to be on the order of 100 µg/kg-d. In contrast, urinary MINP levels are consistent with DINP exposures on the order of 1 µg/kg-d (see Exposure). It is not clear whether the relatively high levels of MINP in breast milk are due to differences in exposure between Europe and the U.S. or to toxicokinetic differences among phthalates.

No statistical adjustments for multiple comparisons were made in this study. Thus, it is likely that some of the observed associations are due to chance, even though they are statistically significant. Levels of the individual phthalate monoesters in breast milk were correlated with one another, except for MINP.

Overall, MBP levels in breast milk were the most strongly associated with perturbations in sex-related hormones. Strong associations were also found for MMP and MEP, which is unexpected because DMP and DEP do not cause antiandrogenic effects in animals. MINP, the principal metabolite of DINP, was significantly associated with either increased luteinizing hormone in one analysis and increased LH:testosterone/free testosterone in the second analysis. Results for MEHP, the principal DEHP metabolite, were inconsistent among the two analyses. The lack of a consistent effect with MEHP is unexpected, because DEHP is a potent antiandrogenic agent in animals. Furthermore, some of the observed associations (MEHP negatively associated with FSH: inhibin B; positive association of MBP with LH:T) are in the opposite sense expected for antiandrogenic effects. However, the positive results with MMP and MEP and weak results with MEHP are consistent with other studies in male infants (Swan et al. 2005).

This study suggests that exposure to mg/kg-d levels of phthalate monoesters in breast milk may have a modest effect on sex-related hormone levels in male infants. This study was designed to investigate differences between boys with or without cryptorchidism, but no such effects were found. Limitations of this study include the study design, small study size, and the potential effects of multiple comparisons and, therefore, reduce the level of confidence in this study.

Swan et al. 2005. No other studies on developmental effects of DINP in humans have been reported. However, studies on other *o*-DAP's may be relevant if the structure-activity relationships in rats apply to humans or if the effects of *o*-DAP's are additive.

Swan et al. reported that reduced AGD was associated with prenatal exposure to DBP, diisobutyl phthalate, diethyl phthalate, and BBP, as measured by urinary metabolite levels (Marsee et al. 2006; Swan et al. 2005). No association was found for DEHP. DINP was not studied. This study suggests that effects similar to those associated with the phthalates syndrome in rats may also occur in humans. However, this study is not considered definitive for several reasons. AGD has been rarely studied in humans. Measurements of AGD were made, on average, at 12 months of age, rather than at birth. Exposures were based on a single measurement during pregnancy and, therefore, may not reflect the average phthalate exposure during pregnancy. The observation of a positive effect with diethyl phthalate is surprising, because it is not active in rats. The lack of an effect with DEHP is equally surprising, because DEHP is one of the most potent *o*-DAP's in animals, and human exposure is widespread. Furthermore, human exposure to *o*-DAP's is generally in the µg/kg-d range, whereas the effects in animals are found at mg/kg-d levels.

Zhang et al. (2009). Zhang et al. (2009) reported an association between low birth weight and DBP levels in maternal blood samples, as well as umbilical cord blood. In the same study, DEHP and MEHP levels were associated with reduced birth length. DINP was not studied. Median DBP levels in the control population were 2.2 mg/L in maternal blood and 1.8 mg/L in cord blood. Median DEHP levels in controls were 0.6 mg/L and 0.5 mg/L in maternal and cord blood, respectively. The median MEHP level in controls were about 0.5 mg/L and MEHP levels were 1.4 and 1.1 mg/L in maternal and cord blood, respectively.

Summary of Developmental Effects

Two authors have studied the effects of prenatal exposure to DINP in rats, in standard developmental assays (Hellwig et al. 1997; Waterman et al. 2000). In these studies, dams were exposed on GD 6—15 (Table 6-5). Developmental effects reported in these studies include dilated renal pelves, hydroureter, skeletal malformation, and skeletal variations & malformations. Maternal effects, such as decreased weight gain and increased liver and kidney weights, were observed at the high dose (1000 mg/kg-d). The lowest NOAEL for fetal effects in these studies is 100 mg/kg-d (Waterman et al. 2000). In these studies, fetuses were not exposed during the critical period for sexual development.

DINP is initially metabolized to isononyl alcohol and MINP; MINP is believed to be the toxicologically active species. However, isononyl alcohol may also contribute to the developmental effects of DINP. The NOAEL for developmental effects (malformations) of the diisononyl alcohols was 720 mg/kg-d and the LOAEL was 1080 mg/kg-d (Helwig and Jackh 1997), which are equivalent to about 5.0 and 7.5 moles/kg-d, respectively (Table 6-5). In a similar assay from the same laboratory (Helwig et al. 1997), the developmental NOAEL for DINP was 200 mg/kg-d (0.5 moles/kg-d), and the LOAEL

was 1,000 mg/kg-d (2.4 moles/kg-d). In another prenatal study, the NOAEL and LOAEL for DINP were 0.24 and 1.2 moles/kg-d, respectively (Waterman et al. 1999). Thus, on an equimolar basis, DINP appears to be several times more potent than isononyl alcohol. Better dose response data are needed for a more precise comparison.

In subsequent developmental studies, dams were exposed, at a minimum, from GD 14 through PND 3 (Table 6-6). Exposures began as early as GD 7 and ended as late as PND 21. In these studies, there was clear evidence of antiandrogenic effects on male offspring, similar to those found with certain other *o*-DAP's. These effects included reduced AGD, reduced testosterone levels, increased retention of areolas/nipples in males, and increased incidences of male reproductive malformations. However, DINP appears to be less potent, in comparison to "active" *o*-DAP's such as DEHP and DBP. Most of these studies exposed the dams at doses of 750 mg/kg-d or higher. Thus, NOAEL's are difficult to establish for most fetal endpoints. In an abstract, Hass et al. (2003) reported a NOAEL of 300 mg/kg-d for nipple retention. The lowest reported NOAEL for testicular malformations and histopathology is 30—66 mg/kg-d (Masutomi et al. 2003). One author reported a NOAEL of 400 ppm in feed for reduced pup weight (Masutomi et al. 2003). Another author reported reduced pup weights at 40 ppm or about 3 mg/kg-d[*] (Lee et al. 2006), although there were concerns about the study's methodology (see above). Thus, it is reasonable to conclude that a NOAEL has not been clearly established for reduced pup weights. In addition, a NOAEL has not been established for decreased rate of testosterone production. Borch et al. (2003, 2004) reported a LOAEL of 750 mg/kg-d for testosterone production. Lee and Koo (2007) reported a LOAEL of 20 mg/kg-d in a Hershberger assay for antiandrogenicity.

A few studies on the prenatal effects of *o*-DAP exposure in humans have been reported (Swan et al. 2005; Zhang et al. 2009). However, DINP was not included in these studies. Human exposure to DINP is generally very low (see Exposure section). Recently, Main et al. compared the levels of *o*-DAP's in breast milk with hormone levels in 3-month old male infants (Main et al. 2006). MINP and MEHP were both significantly associated with increases in the ratios of luteinizing hormone to total testosterone and luteinizing hormone to free testosterone. However, there are several limitations of this study, including the lack of adjustment for multiple comparisons, correlations among *o*-DAP's, and unusually high levels of MINP. The correlation among *o*-DAP levels makes it difficult to assess which *o*-DAP's, if any, are contributing to the observed effects. Furthermore, it is could be that certain *o*-DAP's are acting in an dose-additive manner, as has been shown in the animal studies (Howdeshell et al. 2008).

Overall, the CPSC staff concludes that there is sufficient evidence for developmental effects of DINP in animals, based on the observation of malformations of the kidneys, male reproductive organs, and skeletons in multiple studies in rats. The lowest NOAEL for developmental malformations in animals is 100 mg/kg-d (Waterman et al. 1999). NOAEL's have not been established for reduced pup weight (LOAEL = 143—285 mg/kg-d) (Waterman et al. 2000) or decreased testosterone production (LOAEL = 750 mg/kg-d) (Borch et al. 2003, 2004). Furthermore, the staff concludes that there is

[*] Food intake or daily dose in mg/kg-d were not reported.

inadequate evidence for developmental effects in humans. DINP is considered to be a probable developmental toxicant in humans, based on sufficient evidence in animals.

7. Genotoxicity

PPARα agonists generally exhibit little or no evidence of genotoxicity in standard assays (Galloway et al. 2000). DINP was not mutagenic in *Salmonella* (BASF 1986, 1995; EG&G Mason Research 1980; Exxon, 1996a; McKee et al. 2000; Zeiger et al. 1985) or mouse lymphoma cells (Barber et al. 2000; Cifone, 1986). DINP did not induce unscheduled DNA repair in primary rat hepatocytes (Litton Bionetics 1981a). It failed to induce phenotypic transformation of BALB/C-3T3 mouse cells in one experiment with metabolic activation (Barber et al. 2000; Microbiological Associates, 1981a) and in five experiments without activation (Barber et al. 2000; Litton Bionetics 1981b, c; Litton Bionetics 1985; Microbiological Associates 1981b,c), although one such experiment without metabolic activation gave a small, but statistically significant positive effect (Microbiological Associates, 1981d). In addition, DINP tested negative in various *in vitro* (Exxon 1996b; McKee et al. 2000) and *in vivo* (McKee et al. 2000; Microbiological Associates, 1981e) chromosome damage assays.

8. Carcinogenicity

This section reviews evidence for the carcinogenicity of DINP in rats and mice, and mechanistic data, such as peroxisome proliferation. Carcinogenic mechanisms and their relevance to human risk are also discussed. The primary types of neoplasms induced in rodents by DINP were hepatocelluar adenoma and carcinoma in rats and mice, renal tubular cell tumors in male rats, and mononuclear cell leukemia in Fischer rats. Carcinogenicity data and mechanisms were reviewed in detail by the CHAP (CPSC 2001).

The carcinogenicity of DINP has been tested in four 2-year feeding studies in rats and mice (Table 8-1). DINP-1 was studied in Fischer 344 rats at doses of 0, 0.03, 0.3, and 0.6 percent in feed in a study by Lington et al. (1997). Interim sacrifices were done at 6, 12, and 18 months. DINP-1 from a different supplier was tested in Fischer 344 rats at doses of 0, 0.05, 0.15, 0.6, and 1.2 percent in feed (Moore 1998a) and in B6C3F1 mice at doses of 0, 0.05, 0.15, 0.4, and 0.8 percent (Moore 1998b). The studies by Moore are also referred to as the Covance studies (CPSC 1998; CPSC 2001). Both Covance studies included interim sacrifices at 1, 2, 13, and 79 weeks and recovery groups exposed for 78 weeks at the high dose, followed by a 26-week recovery period. DINP-A (71549-78-5), which is believed to be similar to DINP-2, was tested in Sprague-Dawley CD rats at doses of 0, 0.05, 0.5, and 1.0 percent in feed (Bio/dynamics 1986). An interim sacrifice was performed at one year.

Table 8-1. Lifetime dietary studies of DINP

Study	Test material	Species, strain	Doses	Number per dose/sex group
Lington et al. 1997	DINP-1	F344 rat	0, 0.03, 0.3, 0.6 % in feed (M: 0, 15, 152, 307 mg/kg-d F: 0, 18, 184, 375 mg/kg-d)	~80 per group
Moore 1998a (Covance)	DINP-1	F344 rat	0, 0.05, 0.15, 0.6, 1.2 % in feed [a] (M: 0, 29, 88, 359, 733 mg/kg-d F: 0, 36, 109, 442, 885 mg/kg-d)	65, 50, 50, 65, 65
Bio/dynamics 1986	DINP-A	SD rat	0, 0.05, 0.5, 1.0 % in feed (M: 0. 27, 271, 553 mg/kg-d F: 0, 33, 331, 672 mg/kg-d)	70 per group
Moore 1998b (Covance)	DINP-1	B6C3F1 mouse	0, 0.05, 0.15, 0.4, 0.8 % in feed [a] (M: 0, 90, 276, 742, 1560 mg/kg-d) F: 0, 112, 336, 910, 1888 mg/kg-d)	70 per group

[a] Includes a recovery group in which animals were exposed at the high dose for 78 weeks, followed by a 26 week recovery period.

Liver

Incidence Data

Rats. Lington treated Fischer 344 rats with up to 0.6 percent DINP-1 in feed (Lington et al. 1997). Incidences of hepatocellular carcinoma and neoplastic nodules were reported. There was a small, non-significant increase in the incidence of hepatocellular carcinoma in males at the high dose (0.6%) ($p=0.12$), although the trend test was positive ($p=0.015$) (Table 8-2). Lington et al. also found an increased incidence of a slight centrilobular to midzonal hepatocellular enlargement at the high dose in both sexes, which may be related to peroxisome proliferation (Table 8-2).

In the Covance study, Fischer rats were given dietary doses of up to 1.2 percent DINP (Moore 1998a). The overall incidence of hepatocellular carcinoma ($p<0.001$) was significantly elevated in males at the high dose (1.2%), as was the incidence of carcinoma or adenoma ($p<0.001$) (Table 8-3). In females, the incidence of carcinoma or adenoma was significantly elevated ($p=0.017$), while carcinoma alone was slightly elevated ($p=0.097$). With males and females, the tumor incidences at 79 weeks were at or near background levels. In addition, the incidences of carcinoma in the recovery groups were not significantly different from the controls, which is consistent with the observation that most tumors appeared after 79 weeks. Hepatocelluar proliferation was increased at one week, but not at 2, 13, or 104 weeks, suggesting that DINP induces acute phase, but not chronic, hepatocellular proliferation. Palmitoyl-CoA oxidase activity (an indicator of peroxisome proliferation) and hepatocellular enlargement were elevated at the high dose in males and females.

In the Bio/dynamics study with DINP-A, rats were given dietary doses up to 1.0 percent (Bio/dynamics 1986). The incidence of hepatocellular carcinoma was significantly elevated in females at the mid ($p=0.029$) and high ($p=0.007$) doses (Table 8-4). There was a small, but non-significant increase in males.

Mice. In B6C3F1 mice, benign and malignant liver tumors were elevated in both sexes at the two highest doses (0.4 and 0.8%) (Moore 1998b). In males, the incidence of hepatocellular carcinoma was significantly elevated at 0.8 percent DINP ($p=0.017$), while the incidence of carcinoma or adenoma was significantly elevated at 0.4 ($p=0.008$) and 0.8 percent (Table 8-5). In females, the incidence of carcinoma was significantly elevated at both 0.4 and 0.8 percent, while the combined incidence of carcinoma and adenoma was significantly elevated at doses of 0.15 percent and greater. In males and females, the tumor incidences were generally close to background at 79 weeks. In males, the incidence of carcinoma in the recovery group was not significantly different from the control. In females, however, the incidence of carcinoma in the recovery group remained significantly elevated ($p=3.8\times10^{-5}$ by Fisher's exact test).

Table 8-2. Incidence of hepatocellular neoplasia in a 2-year dietary study of DINP-1 in Fischer 344 rats (Lington et al. 1997) [a, b]

Lesion	Percent DINP in feed			
	0	**0.03**	**0.3**	**0.6**
Males				
Carcinoma [b]	0/81	0/80	0/80	3/80 [c]
Neoplastic nodules or carcinoma	3/81	1/80	1/80	4/80
Females				
Carcinoma	1/81	0/81	0/80	1/80
Neoplastic nodules or carcinoma [d]	1/81	2/81	0/80	2/80

[a] Adapted from CPSC 2001
[b] p=0.015 for Fisher's exact trend test.
[c] p=0.12 for Fisher's exact test for pairwise comparison with control.
[d] "Neoplastic nodules" are regarded as adenomas. Thie distinction is not clear.

Table 8-3. Incidence of hepatocellular neoplasia in a 2-year dietary study of DINP-1 in Fischer 344 rats (Moore 1998a) [a]

Lesion	Percent DINP in feed					
	0	**0.05**	**0.15**	**0.6**	**1.2**	1.2 R [b]
Males						
Carcinoma						
Overall incidence	1/65	0/50	0/50	1/65	12/65	2/50 [f]
At 79 weeks	0/10	NR [e]	NR	0/10	1/10	(p=0.40)
Poly 3 [c, d]	p<0.001	–	–	–	p<0.001	
Carcinoma or adenoma						
Overall incidence	5/65	3/50	2/50	7/65	18/65	–
At 79 weeks	1/10	NR	NR	0/10	1/10	
Poly 3	p<0.001	–	–	–	p<0.001	
Females						
Carcinoma						
Overall incidence	1/65	0/49	0/50	1/65	5/65	2/55 [f]
At 79 weeks	0/10	NR	NR	0/10	0/10	(p=0.44)
Poly 3	p=0.002	–	–	–	p=0.097	
Carcinoma or adenoma						
Overall incidence	1/65	1/49	0/50	2/65	8/65	–
At 79 weeks	0/10	NR	NR	0/10	1/10	
Poly 3	p<0.001	–	–	–	p=0.017	

[a] Adapted from CPSC 2001.
[b] Recovery group. Animals were exposed for 78 weeks, followed by a 26-week recovery period.
[c] Significance value for trend is given in the column for the control group.
[d] Statistical analysis was provided by the National Toxicology Program. Additional statistical tests are found in CPSC 2001, Appendix B, part A.
[e] NR, not reported.
[f] Level of significance for the recovery group computed by Fisher's exact test. (Statistical tests were not run by NTP on the recovery group.)

Table 8-4. Incidence of hepatocellular neoplasia in a 2-year dietary study of DINP-A in Sprague-Dawley rats (Bio/dynamics 1986) [a, b]

Lesion	Percent DINP-A in feed			
	0	**0.05**	**0.5**	**1.0**
Males				
Neoplastic nodules	2/70 (2.9%)	5/69 (7.2%)	6/69 (8.7%)	5/70 (7.1%)
Hepatocellular carcinoma	2/70 (2.9%) p=0.15	2/69 (3.3%) –	6/69 (8.7%) p=0.13	4/70 (5.7%) –
Females				
Neoplastic nodules	1/70 (1.4%)	1/70 (1.4%)	5/70 (7.1%)	2/70 (2.9%)
Hepatocellular carcinoma	0/70 (0) p=0.0004	0/70 (0) –	5/70 (8.3%) p=0.029	7/70 (10%) p=0.007

[a] Adapted from CPSC 2001.
[b] Statistics for pairwise comparison of treated and control incidences by the Fisher exact test are given beneath incidence values for treated animals. Statistics for exact trend tests are given beneath control incidences.

Mechanistic Data

It has been proposed that DINP and other peroxisome proliferators induce liver tumors in rodents by a mechanism or mechanisms directly linked to peroxisome proliferation and related pleiotropic responses. The possible mechanisms by which peroxisome proliferators induce hepatocellular tumors are discussed below (Mode of Action and Human Relevance). This section summarizes data demonstrating that DINP is a peroxisome proliferator and other mechanistic information. Endpoints that may be related to peroxisome proliferation and/or carcinogenic mechanisms include hepatomegaly, peroxisome proliferation, hepatocellular hypertrophy, effects on cell cycle regulation, and gap junction intercellular communication (GJIC). Data relating to these effects are summarized in Table 8-6.

Hepatomegaly and Hepatocellular Hypertrophy. Hepatomegaly is an effect of peroxisome proliferation that is due to increases in both cell number (hyperplasia) and cell size (hypertrophy) (reviewed in IARC 1995). Hepatomegaly was observed in mice following as little as one week of exposure (Valles et al. 2000) and in rats as early as three weeks (Barber et al. 1987). In chronic studies, hepatomegaly was observed following exposure to dietary levels of 0.3 percent in Fischer 344 rats (Lington et al. 1997; Moore 1998a) and 0.4 percent in male B6C3F1 mice (Moore 1998b). Hepatomegaly was observed in SV129 wild type following one week at 0.8% in feed, but not PPARα-null mice (Valles et al. 2000).

Hepatocellular enlargement (hypertrophy) may contribute to the hepatomegaly induced by peroxisome proliferation (IARC 1995). Centrilobular to midzonal or diffuse

hepatocellular enlargement were observed as early as six months and at doses as low as 0.6% in chronic studies (Lington et al. 1997; Moore 1998a,b).

Table 8-5. Incidence of hepatocellular neoplasia in a 2-year dietary study of DINP-1 in B6C3F1 mice (Moore 1998b) [a]

Lesion	Percent DINP in feed					
	0	0.05	0.15	0.4	0.8	0.8 R [b]
Males						
Carcinoma						
Overall incidence	10/70	8/67	10/66	17/65	20/70	12/50 [e]
At 79 weeks	0/15	0/14	1/13	2/14	3/15	(p=0.13)
Poly 3 [c, d]	p<0.001	–	–	p=0.067	p=0.017	
Carcinoma or adenoma						
Overall incidence	16/70	13/67	18/66	28/65	31/70	–
At 79 weeks	1/15	1/14	4/13	3/14	4/15	
Poly 3	p<0.001	–	–	p=0.008	p=0.002	
Females						
Carcinoma						
Overall incidence	1/70	2/68	5/68	7/67	19/70	8/50 [e]
At 79 weeks	0/15	1/15	0/14	0/14	2/15	($3.8x10^{-5}$)
Poly 3	p<0.001	–	p=0.107	p=0.024	p<0.001	
Carcinoma or adenoma						
overall	3/70	5/68	10/68	11/67	33/70	–
Overall incidence	0/15	1/15	1/14	0/14	3/15	
At 79 weeks	p<0.001	–	p=0.043	p=0.014	p<0.001	
Poly 3						

[a] Adapted from CPSC 2001.
[b] Recovery group. Animals were exposed for 78 weeks, followed by a 26-week recovery period.
[c] Significance value for trend is given in the column for the control group.
[d] Statistical analysis was provide by the National Toxicology Program. Additional statistical tests are found in CPSC 2001, Appendix B, part B.
[e] Level of significance for the recovery group computed by Fisher Exact test. (Statistical tests were not run by NTP on the recovery group.)

Peroxisome Proliferation. Peroxisome proliferation is generally assayed by monitoring the induction of peroxisomal beta-oxidation, for example, by measuring palmitoyl-CoA oxidase activity. In some cases, peroxisome number and volume density were ascertained by electron microscopy. Peroxisome proliferation has been observed in rats and mice following as little as one or two weeks of dietary exposure (Moore 1998a; Smith et al. 2000; Valles et al. 2000).

Peroxisome proliferation was evaluated during the course of chronic studies in rats. In the Covance study (Table 8.1), peroxisome proliferation was evaluated in the controls and high dose groups by palmitoyl-CoA oxidase at 1, 2, 13, and 104 weeks, and in the mid-high dose group at 104 weeks (Moore 1998a). Levels of this enzyme were elevated at all time points in both sexes at the high dose (1.2%), and in females at 104 weeks at a dose of 0.6%. The relative increase in enzyme activity at terminal sacrifice was about 4-

fold in males and 2.5-fold in females at 1.2% DINP. Lington et al. reported that there was no treatment-related peroxisome proliferation (by electron microscopy) at doses up to 0.6% DINP (Lington et al. 1997). Thus, in these studies, peroxisome proliferation was not observed at doses below 0.6% and inconsistently at 0.6% DINP. Peroxisome proliferation generally correlated with tumorigenesis. Clear increases in hepatocellular tumors were observed only at 1.2%. However, in the Biodynamics study with DINP-A, a small, but statistically significant increase in carcinomas was found in females at 0.5% (CPSC 2001). Differences between the studies may be due to the use of different rat strains (F344 in Lington and Moore; SD in Biodynamics).

In studies of shorter duration, significant increases in peroxisome proliferation were reported at doses as low as 56 mg/kg-d (roughly equivalent to 0.1%) in rats (Jansen et al. 1992). In a 21-day study, peroxisome proliferation appeared to be a linear function of dose with a range of *o*-DAP's (Barber et al. 1987; Lin 1987). NOEL's were not reported by the authors. However, it was later reported that NOEL in this study was 6000 ppm, or 639 mg/kg-d in males and 607 mg/kg-d in females (Klaunig et al. 2003, Table 6).

Induction of peroxisomal beta-oxidation activity has been demonstrated in rat hepatocytes *in vitro* (Benford et al. 1986; Hasmall et al. 1999). In one study, monoisononyl phthalate (MINP), which is believed to be the proximate peroxisome proliferator, was more potent than DINP (Benford et al. 1986; compare also Mitchell et al. 1985).

In the Covance study in mice (Table 8-3), peroxisome proliferation was evaluated by palmitoyl-CoA oxidase (control and high dose groups only) activity at 78 and 104 weeks (Moore 1998b). Palmitoyl-CoA oxidase activity was elevated at both time points and in both sexes at the high dose (0.8% DINP). In this study, statistically significant increases in hepatocellular tumors were reported in males at 0.4% DINP and in females at 0.15%. At 104 weeks, palmitoyl-CoA oxidase levels were increased by about 8-fold at the high dose, relative to the controls.

In a 4-week study in B6C3F1 mice at the same doses as the Covance study, the lowest dose at which peroxisome proliferation (by palmitoyl CoA oxidase and microscopy) was significantly increased was 0.05 percent DINP in male and 0.15% in females (Bahnemann 2000; Kaufmann et al. 2002). A NOEL was not established (Table 8-7). In another short-term study, peroxisome proliferation was reported to occur in mice at doses as low as 336 mg/kg-d (roughly equivalent to 0.5%) DINP (Wolfe et al. 1992).

Limited data on DINP-induced peroxisome proliferation are available in non-rodent species. Hall et al. (1999) reported that there was no increase in palmitoyl-CoA oxidase activity when marmosets were given 2500 mg/kg-d DINP by gavage for 13 weeks. Benford et al. (1986) reported a roughly 3-fold increase in palmitoyl-CoA oxidase and laurate hydroxylation activity in marmoset hepatocytes treated with up to 0.5 mM MINP. The authors did not report statistical analyses to determine whether the increase was statistically significant. However, the same enzyme activities were induced roughly 7-fold in rat hepatocytes at the same doses.

DINP-induced peroxisome proliferation was also investigated in cynomolgus monkeys (Pugh et al. 2000). Peroxisomal beta-oxidation was increased by 1.5-fold following exposure to 500 mg/kg-d DINP by gavage for 14 days, but the increase was not statistically significant. For comparison, statistically significant increases in peroxisomal beta-oxidation were reported at doses as low as 56 mg/kg-d in rats (Jansen et al. 1992) and 336 mg/kg-d in mice (Wolfe et al. 1992).

Peroxisome Proliferation and Tumor Response. Kaufmann et al. (2002) measured peroxisome proliferation in B6C3F1 mice at the same doses used in the 2-year bioassay by Moore (1998b). A significant tumor response was found only at doses where there was also a significant increase in peroxisome proliferation (Table 8-7). This tends to support the hypothesis that hepatocellular tumors induced by DINP and other peroxisome proliferators are due to peroxisome proliferation.

Although indicators of peroxisome proliferation were not significantly elevated above background at the low dose in this (Kaufmann et al. 2002) and other studies (e.g. Smith et al. 2000), the data are suggestive of a linear dose response with no apparent threshold (Figure 8-1). Although the incidence of adenoma or carcinoma was significantly increased over background at only three of four doses, the tumor dose response is also consistent with a linear relationship in the observable range. There was a non-zero linear term (q_1), when the data were fitted to the multistage model (see Figure 8-2). The tumor risk appears to have a similar dependence on palmitoyl-CoA oxidase activity (Figure 8-3) (Klaunig et al. 2003).

The apparently linear dose response for peroxisome proliferation and tumor incidence in mice could have implications for human risk assessment if the hepatocellular tumors were considered relevant to humans. Although the existence or lack of a threshold cannot be proven, the data are suggestive of a linear, no threshold dose response in mice. This is significant, because several authors have assumed that there is a threshold for peroxisome proliferation and rodent hepatocellular tumors (Budroe et al. 1992; David et al. 1999; Lake 1995). The dose responses of other events associated with peroxisome proliferation, such as cell proliferation and inhibition of apoptosis, may also influence the cancer dose response (Budroe et a. 1992; Wada et al. 1992).

Activation of PPARα. MINP, the primary metabolite of DINP, has been shown to activate both mouse and human PPARα *in vitro* (Bility et al. 2004). PPARα activation was assayed using a plasmid-based trans-activation assay in mouse 3T3-L1 cells. The DNA sequence encoding the ligand-binding domain of either mouse or human PPARα was fused to the sequence for the DNA-binding domain of the yeast transcription factor Gal4. The fused genes were under the control of the SV40 promoter. A luciferase gene under the control of Gal4 was also encoded in the plasmid. Similar constructs with mouse or human PPARβ and PPARγ ligand-binding domains were also prepared.

MINP significantly induced luciferase expression under the control of the mouse PPARα (m PPARα) at concentrations from 3 to 200 μM (Bility et al. 2004, Table 1). The

maximum induction by MINP was 27-fold at 200 μM. Activation of human PPARα (hPPARα) was less pronounced, with 6-fold induction at 200 μM. Several other monoesters demonstrated activity in this assay, including MEHP, monoisoheptyl phthalate, monoisodecyl phthalate, and mono-n-octyl phthalate. Monoethyl phthalate was inactive, while monobutyl phthalate, monobenzyl phthalate, monoisohexyl phthalate, and mono(2-ethylhexyl) adipate elicted weak responses. MINP (100 μM) also induced acyl CoA oxidase gene expression by about 3-fold in rat hepatoma (FaO) cells, but not in human hepatoma cells (HepG2) (Bility et al. 2004).

MINP, MEHP, monoisoheptyl phthalate, monoisodecyl phthalate, and mono-n-octyl phthalate were also able to induce mouse and human PPARγ (Bility et al. 2004). Consistent with this observation, MINP induced adipogenesis (lipid accumulation) in 3T3-L1 cells. Adipogenesis is a PPARγ-dependent process. This significance of PPARγ activation is unknown (Peraza et al. 2006). PPARγ is expressed in adipose tissue. PPARγ agonists such as rosiglitazone are used to increase insulin sensitivity in type II diabetics. PPARγ activation has been reported either to enhance or inhibit liver toxicity, colon cancer, or mammary cancer in rodents.

MINP barely induced mouse PPARβ (3-fold at 200 μM) *in vitro* and did not significantly induce human PPARβ.

Effects on Cell Cycle Regulation. Peroxisome proliferators may also act by stimulating hepatocellular proliferation and/or by inhibiting apoptosis (Cattley et al. 1998; CPSC 2001; IARC 1995). In the Covance rat study, hepatocellular proliferation was increased at the high dose following 1 week of exposure, but not at 2, 13, or 104 weeks (Moore 1998a). This suggests that DINP induces the acute phase of hepatocellular proliferation, but not the chronic phase. Acute phase proliferation is sufficient to contribute to hepatomegaly, but its role in tumorigenesis is uncertain. In a subchronic study, DINP-1 at 1.2 percent in the diet induced hepatocellular proliferation at 2 weeks, but not at 4 weeks (Smith et al. 2000). In the same study, DINP-A was active at both 2 weeks and 4 weeks.

In the Covance mouse study, DINP did not induce hepatocellular proliferation at the high dose at 78 or 104 weeks; earlier time points were not tested (Moore 1998b). In a subchronic study in mice, DINP-1 at 0.6 percent in the diet induced hepatocellular proliferation at 2 weeks, but not at 4 weeks (Smith et al. 2000). In the same study, DINP-A was not active at either 2 weeks or 4 weeks. Kaufmann et al. (2002) exposed B6C3F1 mice to the same doses used in the Covance study. After one week, the labeling index was significantly elevated at all doses in males ($\geq 0.05\%$) and at doses of 0.4% or greater in females. At four weeks, the labeling index remained elevated at all doses in males, but not in females. Apoptosis was increased at 0.8% in males after one week, but unchanged at four weeks. Interestingly, apoptosis was significantly decreased in males at 0.05% at one week. However, the authors regarded this as not biologically significant. Apoptosis was slightly, but non-significantly, elevated in females.

Hasmall et al. (1999) studied the ability of DINP to stimulate DNA synthesis and inhibit apoptosis in primate rat (F344) and human hepatocytes. DINP stimulated DNA synthesis and inhibited apoptosis in rat hepatocytes exposed to 250 to 750 μM DINP for 48 hours. In human hepatocytes from one of three subjects, there was a small, but statistically significant increase in mitotic index at 500 μM DINP, but not at 150 or 750 μM. DINP also failed to suppress apoptosis in human hepatocytes.

Gap Junction Intercellular Communication. The mechanistic role of gap junction intercellular communication (GJIC) in nongenotoxic carcinogenesis is uncertain, but it may involve the regulation of cell proliferation. Numerous nongenotoxic carcinogens have been shown to inhibit GJIC (Trosko et al. 1990; Yamasaki 1990). DINP-1 and DINP-A inhibited GJIC in rats following 2 weeks of exposure and in mice following 4 weeks of exposure (Smith et al. 2000). However, DINP was not active in cynomolgus monkeys following 2 weeks of exposure at 500 mg/kg-d (Pugh et al. 2000).

Mode of Action

It has been proposed that DINP and many other compounds induce liver tumors in rodents by a mode of action (MOA) associated with the process of peroxisome proliferation. While there has been considerable debate regarding the possible modes of action by which peroxisome proliferation may induce liver tumors, a substantial amount of data has now accumulated that is consistent with the role of peroxisome proliferation in hepatocarcinogenesis in rats and mice. At the same time, there are few or no data demonstrating that peroxisome proliferation may be induced substantially in humans or other primates. This section will briefly discuss possible MOA's by which peroxisome proliferators may induce cancer in the rodent liver and the relevance of peroxisome proliferation to human risk assessment. These subjects have been reviewed in detail by others (Ashby et al. 1994; Cattley et al. 1998; Corton et al. 2000; CPSC 2001; IARC 1995, 2000; Klaunig et al. 2003; Peters et al. 2005; Rusyn et al. 2000; Yeldandi et al. 2000). This section summarizes information presented in these reviews.

Peroxisome proliferators are characterized by their ability to induce an increase in the size and number of peroxisomes, and associated pleiotropic responses (reviewed in CPSC 2001; IARC 1995). Peroxisomes are single membrane-bound subcellular organelles that contain fatty acid β-oxidation activity, and which are present in virtually all eukaryotic cells (Alberts et al. 2002). Peroxisome proliferation is also accompanied by hepatomegaly; induction of the peroxisomal β-oxidation system, certain cytochrome P_{450} type (microsomal) isozymes (CYP4A), and microsomal and cytosolic epoxide hydrolase; stimulation of protein kinase C; reduction of the activities of glutathione peroxidase, glutathione S-transferase, and superoxide dismutase; and lipofuscin accumulation (IARC 1995). The hepatomegaly (liver enlargement) is due to both hepatocyte hyperplasia (increased cell number) and hypertrophy (increased cell size). The induction of oxidative enzymes may result in increased hydrogen peroxide production and increased metabolism of fatty acids. Protein kinase C is associated with cell proliferation. Glutathione S-transferase and superoxide dismutase help to protect the cell against oxidative damage.

Table 8-6. Mechanistic data relating to DINP-induced hepatocellular tumorigenesis

Endpoint	Species, strain	Duration	NOEL [a, b]	LOEL	Reference
Hepatomegaly	Rat, F344	21 days	ND [c]	ND	Barber et al. 1987
		4 weeks	0.1%	1.2%	Smith et al. 2000
		13 weeks	0.1%	0.3%	Bird et al. 1986
		13 weeks	0.25%	0.5%	Myers 1991
		2 years	0.03%	0.3%	Lington et al. 1997; Moore 1998a
	Rat, SD	2 years	0.05%	0.5%	Biodynamics 1986
	Mouse, SV129	1 week	ND	0.8%	Valles et al. 2000
	Mouse, B6C3F1	2 weeks	0.05%	0.6%	Smith et al. 2000
		13 weeks	0.4%	1.0%	Moore 2000
		2 years	0.15%	0.4%	Moore 1998b (males only)
	Cynomolgus monkey	14 days	500 mg/kg-d [d]	ND	Pugh et al. 2000
	Marmoset	13 weeks	2500 mg/kg-d [d]	ND	Hall et al. 1999
Hepatocellular enlargement	Rat, F344	6 to 24 months	ND	0.6%	Lington et al. 1997
		24 months	0.6%	1.2%	Moore 1998a
	Mouse, B6C3F1	24 months	0.4%	0.8%	Moore 1998b
Peroxisome proliferation	Rat, F344	2, 4 weeks	0.1% [e]	1.2%	Smith et al. 2000 (DINP-1, DINP-A)
		21 days	0.6% [c, e, f]	1.2%	Barber et al. 1987; Lin 1987
		24 months	0.6% [f]	ND	Lington et al. 1997
		1, 2, 13 weeks	ND	1.2% [e]	Moore 1998a
		104 weeks	ND	0.6%	Moore 1998a (females > males)
	Rat *in vitro*	48 hours	ND [e]	250 μM	Hasmall et al. 1999
		96 hours	ND [e, g]	ND	Benford et al. 1986 (MINP>DINP)
	Mouse, SV129	1 week	ND	0.8% [h]	Valles et al. 2000
	Mouse, B6C3F1	2, 4 weeks	0.05% [e]	0.6%	Smith et al. 2000 (DINP-1 and DINP-A)
		4 weeks	ND [e, f]	0.05%	Bahnemann 2000; Kaufmann et al. 2001
		78, 104 weeks	ND	0.8% [e]	Moore 1998b
	Cynomolgus monkey	14 days	500 mg/kg-d [d, e]	ND	Pugh et al. 2000
	Marmoset	13 weeks	2,500 mg/kg-d [d, e]	ND	Hall et al. 1999
	Marmoset *in vitro*	96 hours	ND [e, g]	ND	Benford et al. 1986 (MINP>DINP)
	Human *in vitro*	48 hours	750 μM	ND	Hasmall et al. 1999

Table 8-6. Mechanistic data relating to hepatocellular tumor induction by DINP (continued)

Endpoint	Species/ strain	Duration	NOEL [a, b]	LOEL	Reference
PPARα activation	Mouse *in vitro*	24 hours	ND [i]	3 µM	Bility et al. 2004
	Human *in vitro*	24 hours	3 µM [i]	10 µM	Bility et al. 2004
Acyl CoA oxidase induction	Rat, *in vitro*	48 hours	ND [j]	10 µM	Bility et al. 2004
	Human, in vitro	48 hours	100 µM [k]	ND	Bility et al. 2004
Hepatocellular proliferation	Rat, F344	1 week	0.6%	1.2%	Moore 1998a
		2 weeks	0.1%	1.2%	Smith et al. 2000 (DINP-1, DINP-A)
		4 weeks	1.2%	ND	Smith et al. 2000 (DINP-1)
		4 weeks	0.1%	1.2%	Smith et al. 2000 (DINP-A)
		2, 13, 104 weeks	1.2%	ND	Moore 1998a
	Rat *in vitro*	48 hours	ND	250 µM	Hasmall et al. 1999
	Mouse, B6C3F1	1, 4 week	ND	0.05%	Kaufmann et al. 2002
		2 weeks	0.05%	0.6%	Smith et al. 2000 (DINP-1)
		2 weeks	0.6%	ND	Smith et al. 2000 (DINP-A)
		4 weeks	0.6%	ND	Smith et al. 2000 (DINP-1, DINP-A)
		78, 104 weeks	0.8%	ND	Moore 1998b
	Human *in vitro*	48 hours	750 µM	ND	Hasmall et al. 1999
Apoptosis	Rat, F344 *in vitro*	48 hours	ND	250 µM	Hasmall et al. 1999 (decrease)
	Mouse, B6C3F1	1 week	0.4%	0.8%	Kaufmann et al. 2002 (increase)
		4 weeks	ND	ND	Kaufmann et al. 2002 (increase)
	Human *in vitro*	48 hours	750 µM	ND	Hasmall et al. 1999 (decrease)
GJIC	Rat, F344	2 weeks	0.1%	1.2%	Smith et al. 2000 (DINP-1, DINP-A)
		4 weeks	1.2%	ND	Smith et al. 2000 (DINP-1)
		4 weeks	0.1%	1.2%	Smith et al. 2000 (DINP-A)
	Mouse, B6C3F1	2 weeks	0.6%	ND	Smith et al. 2000 (DINP-1)
		2 weeks	0.05%	0.6%	Smith et al. 2000 (DINP-A)
		4 weeks	0.05%	0.6%	Smith et al. 2000 (DINP-1, DINP-A)
	Cynomolgus monkey	14 days	500 mg/kg-d [d]	ND	Pugh et al. 2000

Table 8-6. Mechanistic data relating to hepatocellular tumor induction by DINP (continued)

Endpoint	Species/ strain	Duration	NOEL [a, b]	LOEL	Reference
Tumorigenesis	Rat, F344	2 years	0.6%	ND	Lington et al. 1997
		2 years	0.6%	1.2%	Moore 1998a
	Rat, SD	2 years	0.05%	0.5%	Bio/dynamics 1986 (DINP-A) (females)
	Mouse, B6C3F1	2 years	0.05%	0.15%	Moore 1998b (females more sensitive)

[a] GJIC, gap junctional intercellular communication; LOEL, lowest observed effect level; ND, not determined; NOEL, no observed effect level.

[b] Doses levels are percent DINP in feed, unless otherwise specified. Where there are differences between sexes, data are for the more sensitive sex.

[c] Animals were given dietary levels of 0.3, 0.6, 1.2, and 2.5% DINP. Data were presented graphically. NOEL and LOEL are from Klaunig et al. 2003.

[d] By gavage.

[e] By peroxisomal beta-oxidation activity.

[f] By electron microscopy.

[g] Cultures were treated with 0, 0.1, 0.25, or 0.5 mM DINP or MINP. Data were presented graphically; NOEL's/LOEL's were not reported. MINP was a more potent palmitoyl CoA oxidase inducer than DINP in rat hepatocytes. Both compounds were weak inducers in marmoset hepatocytes.

[h] By Western blot analysis of acyl-CoA oxidase and Cyp4a induction.

[l] By a plasmid-based reporter assay using the ligand-binding domain of mouse or human PPARα fused to the DNA binding domain of the yeast transcription factor Gal4. The plasmid was transfected in mouse 3T3-L1 cells.

[j] In rat hepatoma FaO cells.

[k] In human HepG2 cells.

Table 8-7. Palmitoyl-CoA oxidase activity in B6C3F1 mice following DINP exposure for 4 weeks [a]

	Males			Females	
ppm in feed	mg/kg-d	PCOA [b] ± S.D		mg/kg-d	PCOA [b] ± S.D
0	0	4.03 ± 0.91		0	5.3 ± 0.41
500	115	6.05 [c] ± 1.42		142	5.9 ± 0.82
1500	352	9.31 [d] ± 2.00		441 [e]	8.9 [d] ± 0.74
4000	951 [e]	23.14 [d] ± 5.33		1192 [e]	22.1 [d] ± 1.60
8000	2022 [e]	43.37 [d] ± 2.18		2509 [e]	43.9 [d] ± 1.52

[a] Kaufmann et al. 2002.

[b] PCOA, cyanide insensitive palmitoyl-CoA oxidase activity, nmol NADH formed/mg protein/min.

[c] Significantly different from the control, $p \leq 0.02$.

[d] Significantly different from the control, $p \leq 0.002$.

[e] Significant tumor response at this dose level.

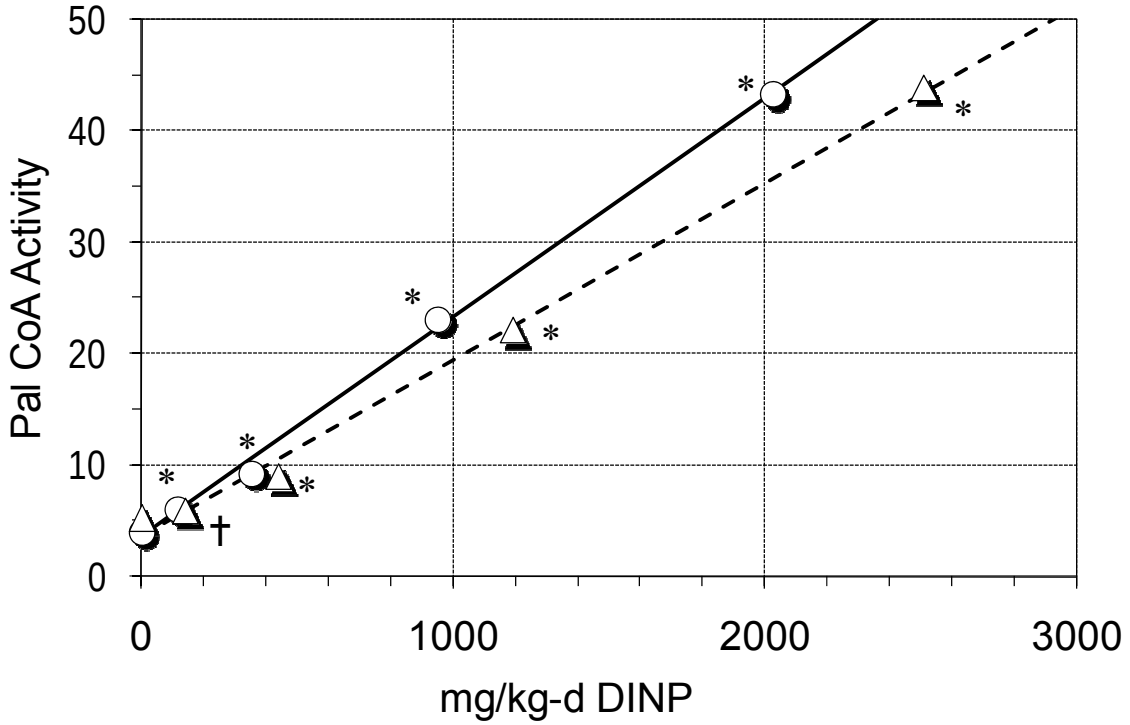

Figure 8-1. Palymitoyl CoA oxidase activity in B6C3F1 mice exposed to dietary DINP for 4 weeks (Kaufmann et al. 2002). Cyanide insensitive Pal CoA oxidase expressed as nmol NADH/mg protein/min: Circles, males, observed; triangles,females, observed; solid line, males, linear regression; broken line, females, linear regression; asterisk, significantly different from controls; dagger, not significantly different from controls.

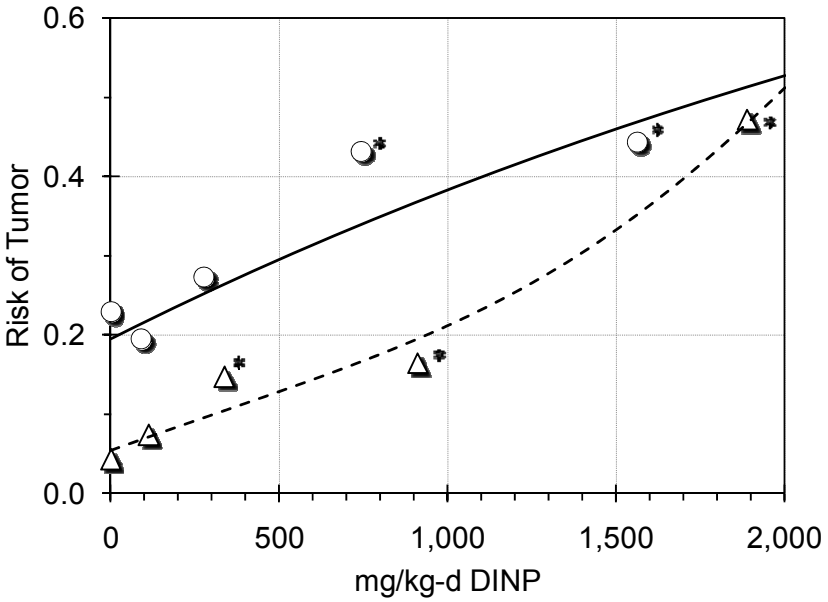

Figure 8-2. Risk of hepatocellular tumor (adenoma or carcinoma) in B6C3F1 mice fed DINP for two years (Moore 1998b): circles, males; triangles, females; solid line, males, multistage model; broken line, females, multistage model; asterisk, significantly different from controls. Multistage model fitted using BMDS 2.0 (EPA 2008). (Adapted from Klaunig et al. 2003, Figure 2.)

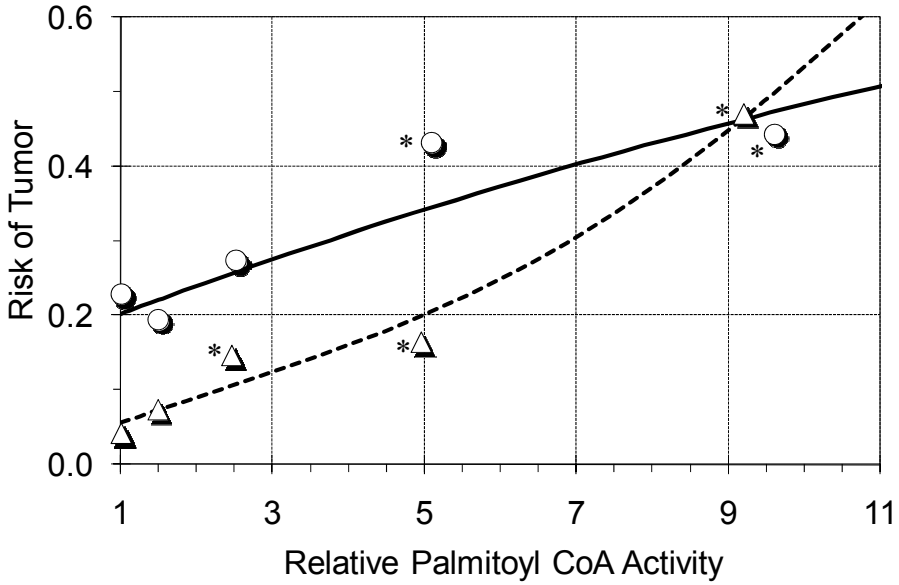

Figure 8-3. Risk of hepatocellular tumor (ademona or carcinoma) in B6C3F1 mice as compared to relative palmitoyl CoA oxidase activity (nmol NADP/mg protein/min). Mice were fed DINP for two years (Moore 1998b). Pal CoA oxidase were measured following 4 weeks of dietary exposure (Kaufmann et al. 2002): circles, males; triangles, females; solid line, males, multistage model; broken line, females, multistage model; asterisk, tumor incidence significantly different from controls. Multistage model fitted using BMDS 2.0 (EPA 2008). (Adapted from Klaunig et al. 2003, Figure 3.)

Peroxisome number and volume density remain fairly constant under various physiological and pathological conditions (Yeldandi et al. 2000). In liver parenchymal cells, peroxisomes normally occupy less than two percent of the cytoplasmic volume. Following exposure to peroxisome proliferators, the number and volume of peroxisomes increase in rat and mouse liver to the extent that they may occupy as much as 25% of the hepatocyte cytoplasmic volume.

Peroxisome proliferators are a diverse group of synthetic and naturally occurring compounds, including hypolipidemic drugs (e.g., clofibrate, nafenopin, ciprofibrate, fenofibrate, gemfibrozil, Wy-14,643), leukotriene antagonists, dialkyl phthalates (e.g., DEHP and DINP), herbicides, solvents, and the naturally occurring steroid dehydroepiandrosterone (Gonzalez et al. 1998; IARC 1995; Kawashima et al. 1983; Lake 1995; Reddy and Lalwani 1983). The only common structural feature of these compounds appears to be the presence of, or the ability to be metabolized to, a carboxylic acid group or derivative (Ashby et al. 1994; Peraza et al. 2006). The pleiotropic responses induced by peroxisome proliferators are qualitatively similar in mice and rats. However, the potency of peroxisome proliferators varies considerably, with the fibrate drugs being among the most potent and phthalate esters being relatively weak (Ashby et al. 1994; IARC 1995; Klaunig et al. 2003). For example, fenofibrate was reported to be 30-fold more potent than DINP (Barber et al. 1987).

Peroxisome proliferators generally exhibit little or no evidence of genotoxicity in standard assays (Galloway et al. 2000). Thus, it has been proposed that the sustained induction of peroxisome proliferation and related responses lead to oxidative stress and the accumulation of indirect mutations (reviewed in Cattley et al., 1998; Conway et al., 1989; CPSC 2001; IARC 1995; Yeldandi et al. 2000). Other responses induced by peroxisome proliferators that may also contribute to carcinogenesis include increased cellular proliferation and a concomitant inhibition of apoptosis. The non-parenchymal (Kupffer) cells of the liver may also play a role in initiating hepatocellular proliferation. These mechanisms are not mutually exclusive; rather, they may act in concert to induce carcinogenesis.

Peroxisome Proliferator-Activated Receptor-α. Reddy and Lalwani (1983) were the first to propose the existence of a specific receptor(s) responsible for the action of peroxisome proliferators. A peroxisome proliferator-activated receptor (PPAR) was first identified and cloned in mice (Isseman and Green 1990). Three isoforms of PPAR (α, β/δ, and γ), which is a member of the steroid hormone nuclear receptor superfamily, have been identified, although only PPARα mediates peroxisome proliferation (Gonzalez 1997). A heterodimeric receptor complex comprised of PPARα and the retinoid X receptor is activated by peroxisome proliferators and 9-*cis*-retinoic acid (Cattley et al. 1998; Peters et al. 2005). The activated complex binds to a specific base sequence, or response element, located in the promoters of peroxisome proliferator-responsive genes. The induction of some of the critical enzymes of the peroxisomal, microsomal, and mitochondrial fatty acid oxidation systems by peroxisome proliferators is transcriptionally controlled by PPARα. The peroxisome proliferator-α response element (PPRE) is recognized by other nuclear hormone receptors, which may modulate the

effects of PPARα on gene expression. Recently, several coactivators or corepressors have been identified that may further modulate the effects of PPARα on gene expression (Reddy 2001; Yeldandi et al. 2000). These cofactors may contribute to the species- and tissue-specific induction of PPARα-responsive genes. Although humans express PPARα at a lower level than mice, the human PPARα was shown to function normally in mouse cells *in vitro* (Cattley et al. 1998) and *in vivo* (Yang et al. 2008).

The mouse PPARα gene has been isolated and a strain of PPARα-null mice, the so-called "knockout" mice incapable of expressing PPARα, has been developed (Lee et al. 1995). The PPARα-null mice were characterized by elevated serum cholesterol levels (Peters et al. 1997a), the presence of lipid-containing vesicles in the liver, increased body fat (Lee et al. 1995), and reduced expression of mitochondrial fatty-acid metabolizing enzymes (Aoyama et al. 1998). The constitutive levels of peroxisomal and microsomal enzymes were similar to those of wild-type mice (Aoyama et al. 1998). However, the pleiotropic effects associated with PPARα induction**,** including the development of liver tumors**,** were not observed in PPARα null mice (Hays et al. 2005; Lee et al. 1995; Peters et al. 1997a; Ward et al. 1998). Thus, the immediate pleiotropic responses, as well as the delayed hepatocarcinogenic effects, are believed to be dependent upon PPARα activation (CPSC 2001; Gonzalez et al. 1998; Klaunig et al. 2003; Peters et al. 2005; Peraza et al. 2006; Roberts et al. 2000; Rusyn et al. 2000).

When PPARα null mice were fed 0.1 percent Wy-14,643for 11 months, no liver tumors were observed, whereas the tumor incidence was 100 percent in homozygous wild-type mice (Peters et al.1997b). A related drug, bezafibrate, also induced tumors in wild-type mice following exposure at 0.5% for 11 months, but not in PPARα-null mice (Hays et al. 2005). These studies provide strong evidence that PPARα is required for the induction of liver tumors by peroxisome proliferation. However, it is unknown whether the PPARα-null mice would have developed tumors if the exposure had been continued until two years.

In contrast, DEHP induced maternal toxicity, embryolethality, and teratogenicity in both PPARα-null and wild-type mice (Peters et al. 1997c). These studies suggest that PPARα is required for the expression of peroxisome proliferation and tumorigenesis, but not reproductive and developmental toxicity.

Potential Modes of Action (MOA's) in Rodent Liver. Three general MOA's been proposed for the induction of liver tumors in rodents exposed to peroxisome proliferators (IARC 1995; Klaunig et al. 2003). These MOA's are not necessarily mutually exclusive.

1. Oxidative Stress. Peroxisome proliferation may lead to as great as 20- to 40-fold induction of fatty acyl-CoA oxidase, the first enzyme in the classical β-oxidation pathway, along with more modest increases in other peroxisomal oxidases and cytochrome P450 CYP 4A isoforms (reviewed in CPSC 2001; Klaunig et al. 2003; Peters et al. 2005; Yeldandi et al. 2000). The substantial increase in oxidase activity is accompanied by small (2- to 3-fold) increases in peroxisomal catalase and decreased glutathione peroxidase. Furthermore, the dicarboxylic acids formed by CYP4A fatty acid

ω-oxidation serve as substrates for fatty acyl-CoA. Thus, disproportionate activation of H_2O_2-generating enzymes and H_2O_2-degrading catalase may lead to a state of increased oxidative stress in liver cells (Reddy 1990; Reddy and Lalwani 1983; Reddy et al. 1980). Undegraded H_2O_2 may react with transition metals, leading to hydroxyl radical formation and oxidative DNA damage.

The general correlation of the magnitude of peroxisome proliferation with hepatocarcinogenicity tends to support this mechanism (Ashby et al. 1994). Furthermore, cell lines overexpressing fatty acyl-CoA oxidase underwent morphologic transformation when they were treated with fatty acyl-CoA substrates and produced tumors in nude mice (Chu et al. 1995; Dadras et al. 1998; Okamoto et al. 1997). However, oxidative DNA damage has not been consistently associated with peroxisome proliferation (Cattley et al. 1998; IARC 1995). Therefore, some investigators have proposed that, while oxidative stress may contribute to carcinogenesis, other mechanisms may be equally as important (Gonzalez et al. 1998; Marsman et al. 1988; Roberts 1996; Rusyn et al. 2000).

2. Cell Cycle Modulation. During the first several days of exposure in rats or mice, peroxisome proliferators stimulate the proliferation of hepatocytes, resulting in an increase in cell number, in what is termed acute cell proliferation. If exposure continues, a chronic cell stimulation may result, in which increased cell proliferation is balanced by a concomitant increase in apoptosis (programmed cell death) (Marsman et al. 1992; Rusyn et al. 2000). Peroxisome proliferators have been demonstrated to inhibit apoptosis (e.g., Roberts 1996; Roberts et al. 2000). Wy-14,643 induced the expression of cyclin-dependent kinases (CDK-1, CDK-2, and CDK-4), proliferating cell nuclear antigen (PCNA), and c-*myc* proteins in wild-type mice, but not in PPARα-null mice (Peters et al. 1998). These proteins are involved in the regulation of cell cycle progression and cell proliferation. It is not known whether the induction of these proteins was under the direct, or indirect, control of PPARα (Peters et al. 1998). The sustained, low level of cell proliferation is considered to increase the probability that spontaneous DNA damage would be converted into mutations (IARC 1995).

Acute cell proliferation (increase in labeling index) was observed with the hypolipidemic drug Wy-14,643, clofibric acid, and DEHP (Marsman et al. 1988, 1992). Chronic cell proliferation was observed with Wy-14,643 (Marsman et al. 1988, 1992). The chronic cell proliferation induced by Wy-14,643 was not limited to preneoplastic foci (see below) and was accompanied by an increase in apoptosis (Marsman et al. 1988). However, chronic cell proliferation was not observed with clofibric acid or DEHP at doses that were tumorigenic in animals (Marsman et al. 1988, 1992). Only the acute phase of hepatocellular proliferation was observed in the cancer bioassay in rats with DINP (Moore 1998a). Therefore, it is likely that chronic cell proliferation is not a prerequisite for the tumorigenicity of peroxisome proliferators.

Enzymatically or histologically altered hepatic foci are believed to be indicative of preneoplastic cells. Some peroxisome proliferators have been shown to promote the growth of specific subtypes of altered hepatic foci. The hypolipidemic drug Wy-14,643 promoted the growth of ATPase-deficient foci in rats previously exposed to the initiator

diethylnitrosamine (Cattley and Popp 1989). The drug nafenopin promoted the growth of liver tumors in rats initiated with aflatoxin B_1 and led to an increase in the number of hepatic foci characterized by weak basophilia and a lack of γ-glutamyltranspeptidase activity (Kraupp-Grasl et al. 1990). DEHP inhibited the formation of γ-glutamyltranspeptidase-positive foci (Carter et al. 1992), but promoted the number of ATPase-deficient foci (reviewed in Bentley et al. 1993). That peroxisome proliferators appear to promote only certain types of altered hepatic foci may explain why several promotion assays gave negative results (Bentley et al. 1993).

3. Kupffer Cell-Mediated Processes. Because peroxisome proliferation is increased to a greater extent *in vivo* than *in vitro*, it has been proposed that cytokines from nonparenchymal (Kupffer) cells may be involved in the generation of intercellular signals leading to hepatocyte proliferation (reviewed in CPSC 2001; Rusyn et al. 2000). Kupffer cells (hepatic macrophages) are a source of mitogens, such as TNF-α. Treatment of cell cultures with antibodies to TNF-α (Bojes and Thurman 1996) or Kupffer cell inhibitors (Rose et al., 1997) prevented the stimulation of hepatocyte proliferation by Wy-14,643. However, TNF-α null mice were not refractory to peroxisome proliferator induced cell proliferation, suggesting that other factors may mediate the proliferative response (Lawrence et al., 2001).

Treatment of Kupffer cells with Wy-14,643 led to superoxide production, leading to the induction of the transcription factor NF-κB by a NADPH oxidase-dependent process (reviewed in CPSC 2001; Rusyn et al. 2001). NF-κB is a regulator of TNF-α. In addition, this process is apparently independent of PPARα, because Kupffer cells do not express PPARα and hydroxyl radicals were induced in PPARα knockout mice (Peters et al. 2000; Rusyn et al. 2006). It is likely that cytokines released from Kupffer cells and PPARα are both required for the proliferative response and cancer (Parzefall et al. 2001). Thus, Kupffer cells appear to be necessary, but not sufficient, for the induction of cancer by peroxisome proliferation.

Overall Mode of Action. Many details regarding the mechanism by which peroxisome proliferators induce hepatocellular tumors in mice and rats remain to be elucidated (Corton et al. 2000; CPSC 2001; Klaunig et al. 2003; Peters et al. 2005). However, there is a substantial amount of evidence that events downstream of PPARα activation—including oxidative stress, cell proliferation (with or without Kupffer cell involvement), and suppression of apoptosis—lead to liver cancer in rodents (CPSC 2001; Klaunig et al. 2003).

Klaunig et al. (1983) proposed an MOA that involves multiple pathways (see Figure 8-4). Activation of PPARα (step 1) may lead to the induction of three pathways: (2a) peroxisome proliferation; (2b) cell cycle, growth, and apoptosis gene expression; and (2c) non-peroxisomal lipid metabolism. Gap junctions are under the control of pathway (2b). The last pathway (2c) is apparently the primary pathway induced in human cells (see below), and does not to contribute to carcinogenesis. The MOA also allows for the possibility of direct activation of Kupffer cells independently of PPARα, which Kupffer cells do not express. Thus, the three possible modes of action—peroxisome proliferation

(2a), cell proliferation (2b), and Kupffer cell-mediated events (6)—may contribute to carcinogenesis in this model. Steps 2a and 2b are clearly under the control of PPARα, whereas Kupffer activation is apparently independent of PPARα.

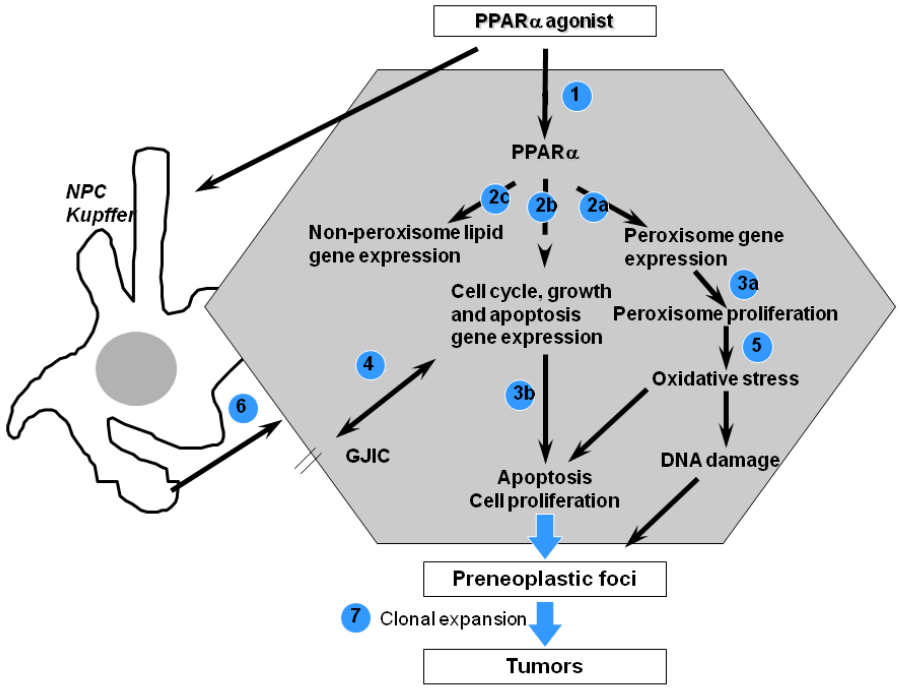

Figure 8-4. Proposed mode of action for tumorigenesis induced by perosixome proliferators in rodent hepatocytes (Klaunig et al. 2003).

Alternative Modes of Action. Peroxisome proliferators are generally non-genotoxic. Therefore, direct genotoxic MOA's are unlikely (Klaunig et al. 2003). However, it is conceivable that other PPARα-independent mechanisms may occur (Melnick 2001, 2002; Peraza et al. 2006; Peters et al. 2005). Kupffer cells are believed to contribute to carcinogenicity induced by peroxisome proliferation through a PPARα-independent process. Some developmental effects of *o*-DAP's occur indendently of PPARα (Peters et al. 1997c). Furthermore, much of our knowledge of the MOA of peroxisome proliferators comes from the fibrate drugs, which are stronger peroxisome proliferators than *o*-DAP's (Klaunig et al. 2003). Most PPARα agonists also induce PPARγ, which may have a role in carcinogenesis in the liver and colon sites (Peraza et al. 2006).

While the fibrate drugs Wy-14,643 (Peters et al. 1997b) and bezafibrate (Hays et al. 2005) failed to induce tumors in PPARα-null mice, these studies were terminated at 11 months. It is not known whether tumors would have developed in the PPARα-null mice following a lifetime exposure. In contrast, relatively low levels of DEHP (0.05% in feed) induced more tumors (mainly adenomas) in PPARα-null mice than in wild-type

mice (Ito et al. 2007). Only males were tested. The authors noted that 8-hydroxy-deoxyguanosine (8-OHdG) levels, which are indicative of oxidative stress, were elevated in the PPARα-null mice, but not in the wild type. The protooncogenes c-jun and c-fos, which are associated with cell proliferation, were also selectively elevated in the PPARα-null mice. Furthermore, cell cycle arrest at the G2/M phase and apoptosis were both suppressed in PPARα-null mice, but were active in wild-type cells (Takashima et al. 2008). The authors suggested that tumors in the PPARα-null mice were induced by a different mechanism that was not operating in wild-type (PPARα expressing) mice (reviewed in Ito and Nakajima 2008).

The study results are surprising in that one would expect more tumors in the wild-type mice. However, the study is limited in that the DEHP doses were less than optimal and the tumor incidences were modest. For comparison, in B6C3F1 mice (David et al. 1999), 0.05% DEHP in feed resulted in a tumor incidence (adenoma or carcinoma) of 32% (21/65) in males and 11% (7/65) in females (Table 8-8). At the high dose of 0.6% in David et al., the tumor incidence was 53% in males and 63% in females. For comparison, in the study by Ito et al., 0.05% DEHP resulted in a tumor incidence of only 10% (2/20) in the wild-type SV129 mice, and 26% (8/31) in PPARα-null mice (only males were tested; tumor incidence includes 1 cholangiocellular carcinoma at 0.05%). The incidence in the wild-type mice was not significantly different from the controls (0/24). In addition, Ito et al. tested only 20—30 animals per dose group, whereas David et al. tested 60—70 animals. The results would be more convincing if the animals were exposed to higher DEHP doses to yield greater tumor incidences.

Species Differences in Response to Peroxisome Proliferators. While rats and mice are highly responsive to peroxisome proliferators, Syrian hamsters are moderately responsive, and guinea pigs, dogs, marmosets, and rhesus monkeys are generally less responsive or nonresponsive (Cattley et al. 1998; CPSC 2001; IARC 1995; Klaunig et al. 2003; Lake 1995; Peters et al. 2005). For example, when Syrian hamsters and rats were exposed to Wy-14,643, peroxisome proliferation was increased in both the rats and hamsters, but hepatocellular proliferation was substantially increased only in rats (Lake et al. 1993). Peroxisome proliferation has been studied in cats, rhesus monkeys, cynomolgus monkeys, marmosets, pigeons, and chickens exposed to peroxisome proliferators. Increased liver weight was accompanied by evidence of peroxisome proliferation in some cases (Reddy et al. 1984; Lalwani et al 1985), but not in others (Tucker and Orton 1993). These observations suggest that the differences between species may be quantitative, rather than qualitative (CPSC 2001).

Liver biopsies from humans taking hypolipidemic drugs generally showed no evidence of peroxisome proliferation (Blumcke et al. 1983; De La Iglesia et al. 1982; Gariot et al. 1987). However, clofibrate was reported to have a statistically significant 50% increase in peroxisome number and a nonsignificant 23% increase in peroxisome density (Hanefield et al. 1983; see also PDR 2000). Furthermore, these studies were limited by the health and nutritional status of controls and treated subjects (CPSC 2001).

Table 8-8. Liver tumor incidence in mice feed DEHP for up to two years

	% in feed	0	0.01%	0.05%	0.15%	0.6%
SV129 wild type, male Ito et al. 2007	No.[a]	24	23	20	ND	ND
	HCA	0	2	2		
	HCC	0	0	0		
	CCC	0	0	0		
	Total (%)	0 (0%)	2 (8.7%)	2 (10%)		
SV129 PPARα-null, Male Ito et al. 2007	No.	25	25	31	ND	ND
	HCA	0	1	6[b]		
	HCC	1	0	1		
	CCC	0	0	1		
	Total	1 (4.0%)	1 (4.0%)	8 (25.8%)[b]		
B6C3F1, male David et al. 1999	No.	70	60	65	65	70
	HCA	4	10[b]	13[b]	14[b]	19[b]
	HCC	4	5	9	14[b]	22[b]
	Total	8 (11%)	14 (23%)	21 (32%)[b]	27 (42%)[b]	37 (53%)[b]
B6C3F1, female David et al. 1999	No.	70	60	65	65	70
	HCA	0	2	4	9[b]	34[b]
	HCC	3	2	3	10[b]	16[b]
	Total	3 (4%)	4 (6%)	7 (11%)	19 (29%)[b]	34 (63%)[b]

[a] No., number of animals examined; HCA, hepatocellular adenoma; HCC, hepatocellular carcinoma; CCC, cholangiocellular carcinoma.

[b] Statistically significant compared to controls, one-tailed Fisher exact test.

A number of studies have demonstrated effects associated with peroxisome proliferation—peroxisomal enzyme induction, stimulation of DNA synthesis, and suppression of apoptosis—in cultured rodent hepatocytes (reviewed in CSPC 2001; Klaunig et al. 2003; Peters et al. 2005). Such effects generally have not been observed with human or primate hepatocytes. However, acyl-CoA oxidase activity has been reported to be induced in human hepatocytes by clofibrate or ciprofibrate (Perrone et al. 1998; Scotto et al. 1995). In one study, the induction of acyl-CoA oxidase was accompanied by an increase in apoptosis and decreased DNA synthesis, leading the authors to conclude that human cells would be refractory to carcinogenesis induced by peroxisome proliferators (Perrone et al. 1998). *In vitro* studies with cultured hepatocytes are generally limited by the absence of Kupffer cells, which may play a role in signaling hepatocytes to divide (see above).

Endogenous ligands of PPARα include fatty acids and fatty acid derivatives, arachidonic acid-derived prostaglandins, and eicosanoids (reviewed in CPSC 2001). Hypolipidemic drugs are believed to activate PPARα in the liver of humans and rodents. Activation of human PPARα leads to increased levels of apolipoprotein A-II and lipoprotein lipase transcription, and reduced apolipoprotein C-III, which are responsible for their lipid-

lowering activity (Auwerx et al. 1996; Staels et al. 1997; Vu-Dac et al. 1995). Fatty acid transport protein and acyl-CoA synthetase are also induced (Martin et al. 1997). However, peroxisome proliferation does not seem to occur. Therefore, it appears that humans have a functional PPARα receptor, but that the cellular responses to receptor activation are different in comparison to rodents (Klaunig et al. 2003; Peters et al. 2005).

Transient transfection experiments show that human PPARα can transactivate PPRE reporter constructs, further supporting the conclusion that humans possess a functional PPARα (Sher et al. 1993). PPRE's have been described in human genes that are known to be regulated by PPARα in rodents, including: human apo C-III, lipoprotein lipase, apo A-I, apo A-II, carnitine palmitoyltransferase-I, and acyl CoA oxidase (reviewed in CPSC 2001; Klaunig et al. 2003). Since humans apparently have a functional PPARα and PPRE's, it would be interesting to be able to explain the differences between humans and rodents in their ability to induce peroxisome proliferation.

Several possible mechanisms to explain the lack of peroxisome proliferation in human liver have been suggested. 1) Human liver has less than one-tenth as much PPARα mRNA as mouse liver (Palmer et al. 1998). Thus, it has been proposed that the level of PPARα in the human liver may be sufficient to activate target genes involved in lipid homeostasis, but insufficient to induce peroxisome proliferation. 2) Human PPARα is less readily induced, as compared to mouse PPARα (Bility et al. 2004).
3) Polymorphisms in the human PPRE may lead to interindividual differences in responses to PPARα agonsists (Peters et al. 2005). Pseudogenes may compete for coactivators. 4) Finally, several coactivators or corepressors have been identified that may further modulate the effects of PPARα on gene expression (Reddy 2001; Peters et al. 2005; Yeldandi et al. 2000). These cofactors may contribute to the species- and tissue-specific induction of PPARα-responsive genes.

Recently, a transgenic strain of PPARα-null mice expressing the human PPARα gene has been developed (Yang et al. 2008). The so-called PPARα-humanized mouse strain expressed human PPARα at similar levels as mouse PPARα in the wild-type mice (SV129). Interestingly, the humanized mice are capable of inducing peroxisome proliferation and lipid metabolism in response to peroxisome proliferators (fibrates), but do not induce cell proliferation or genes associated with cell proliferation. When the humanized mice were fed 0.1% Wy-14,643 for 38 weeks, the incidence of liver tumors was only 5% (1/20), compared to 71% in the wild-type mice (5/7) (Morimura et al 2006).

To summarize, it is clear that humans possess a functional PPARα that can be activated by endogenous ligands, hypolipidemic drugs, and probably environmental exposures. However, it is also apparent that some of the genes regulated by PPARα in humans differ from those regulated by PPARα in rodents. Thus, peroxisome proliferation is less easily, or not at all, inducible in humans (CPSC 2001; Klaunig et al. 2003; Peters et al. 2005).

A few carcinogenicity studies of peroxisome proliferators in species other than the mouse or rat have been reported (IARC 1995; Cattley et al. 1998). While rats and mice are susceptible to hepatocarcinogenesis following chronic exposure to peroxisome

proliferators (Ashby et al. 1994; Bentley et al. 1993; Lake 1995; Reddy and Lalwani 1983), other species appear resistant. For example, the peroxisome proliferators nafenopin and Wy-14,643 induced liver tumors in rats, but not Syrian hamsters following 60 weeks of exposure (Lake et al. 1993). However, it is possible that with a lifetime exposure tumors might have been observed in the hamster. Some hypolipidemic drugs failed to induce liver tumors in non-human primates (Cattley et al. 1998). However, these experiments were limited by the small number of animals and less than half-lifetime of exposure (e.g., Tucker and Orton 1993). A few studies of limited duration in humans taking hypolipidemic drugs failed to show an increased risk of liver cancer (e.g., Law et al. 1994; Huttunen et al. 1994), although these studies had insufficient power to evaluate the risk of liver cancer (IARC 1995). Furthermore, site concordance across species cannot necessarily be assumed.

Human Relevance

While humans possess a functional PPARα receptor and PPRE-inducible genes, it is apparent that humans respond to PPARα ligands by inducing a different suite of genes, in comparison to rodents (Shah et al. 2007). For example, humans respond to hypolipidemic fibrate drugs by a PPARα-mediated process, yet there is little or no evidence of peroxisome proliferation in humans. This conclusion is further supported by studies involving mouse strains that express human PPARα (Morimura et al. 2006; Yang et al. 2008).

Therefore, although data in humans and other primates are limited, the CHAP on DINP concluded that peroxisome proliferation is a process that is not easily induced in humans (CPSC 2001). Although the possibility that a sufficiently strong peroxisome proliferator or sufficiently high exposure could induce peroxisome proliferation in humans cannot be ruled out, the CHAP further concluded that it is unlikely that DINP could present a cancer hazard to humans under foreseeable conditions of exposure. A committee convened by the International Life Science Institute (ILSI) concluded that peroxisome proliferation is unlikely to lead to cancer in humans (Klaunig et al. 2003). The CPSC staff agrees that peroxisome proliferation is not readily induced in humans and that exposure to DINP, a relatively weak peroxisome proliferator in comparison to the fibrate drugs, is unlikely to present a cancer hazard to humans (Babich 2002).

Phthalates are considered to be very weak PPARα agonists, and even the fibrate drugs are weak agonists (Maloney and Waxman 1999). Several pharmaceuticals have been developed that induce PPARα and/or PPARγ, and others are being developed (Peraza et al. 2006). It is unknown at this time whether a sufficiently strong PPARα or mixed agonist might be found that can induce peroxisome proliferation in humans (Peters et al. 2005). Thus, some authors are reluctant to conclude with certainty that a sufficiently strong PPARα agonist would not pose a cancer risk to humans (CPSC 2001; Klaunig et al. 2003; Peters et al. 2005).

The observation that DEHP induced tumors in PPARα-null mice (Ito et al. 2007) suggests that DEHP, and possibly other phthalates, may induce liver tumors by a non-

PPARα-dependent MOA. This brings into question the conclusion that rodent liver tumors induced by phthalates are not relevant to humans, because the rodent tumors are PPARα-dependent and humans are unresponsive. However, the study is limited by the low doses studied and the resulting low tumor incidence.

Furthermore, the non-PPARα MOA does not appear to occur in wild-type mice. It is unknown whether the non-PPARα MOA can occur in humans. However, since humans express functional PPARα, this might suppress the non-PPARα MOA as it seems to do in mice. Additional research is needed to assess the significance of the study by Ito et al. (Ito and Nakajima 2008).

Kidney

A small number (2/65) of renal tubular cell carcinomas were observed in high dose males in the Covance study in rats (Moore 1998a) (Table 8-9). Although the increase over background (0/65) was not statistically significant, the trend test was positive (p=0.022). In addition, the incidence (4/50) in the recovery group (79-week exposure/26-week recovery) was statistically significant.

Table 8-9. Incidence of renal tubular carcinoma in male Fischer 344 rats in a 2-year dietary study of DINP-1 (Moore 1998a) [a]

	Percent DINP in feed					
Incidence	**0**	**0.05**	**0.15**	**0.6**	**1.2**	**1.2** [b]
Overall incidence	0/65	0/55	0/55	0/65	2/65	4/50
At 79 weeks	0/10	NA	NA	0/10	0/10	–
Poly 3 [c, d]	p=0.022	–	–	–	p=0.219	p=0.03 [e]

[a] Adapted from CPSC 2001.
[b] Recovery group. Animals were exposed for 78 weeks, followed by a 26-week recovery period.
[c] Significance value for trend (excluding recovery group) is given in the column for the control group. The recovery group was not included in the Poly 3 analysis.
[d] Statistical analysis was provided by the National Toxicology Program. Additional statistical tests are found in CPSC 2001, Appendix B, Part A.
[e] Level of statistical significance computed by Fisher Exact test. (Statistical tests were not run by NTP on recovery group.)

A small number of renal tubular cell carcinomas were observed only in males exposed to 1.2 percent DINP. Furthermore, there is experimental evidence that these tumors arose by a mechanism involving the accumulation of α2u-globulin (Caldwell et al. 1999). α2u-Globulin is a protein that is specific to the male rat. Renal tubular cell tumors induced by this mechanism are not considered relevant to human risk assessment

(Schaeffer 1991). The higher incidence of these tumors in the recovery group animals suggests that DINP, as other peroxisome proliferators, may inhibit the synthesis of α2u-lobulin (Alvares et al. 1996).

Mononuclear Cell Leukemia

Significant increases in the incidence of mononuclear cell leukemia (MNCL) were observed in Fischer 344 rats of both sexes exposed to at least 0.3 percent DINP (Lington et al. 1996; Moore 1998a) (Tables 8-10, 8-11). Although the incidence of MNCL was low at 79 weeks in the Covance study, it was significantly elevated in the recovery groups (Table 8-9). MNCL has a high spontaneous incidence in Fischer rats, ranging from 10 to 72 percent in National Toxicology Program studies (Haseman et al. 1990), and has increased over time (Haseman et al. 1998). Elevated incidence of MNCL is a common finding in chronic studies in Fischer rats. Due to its high background rate, MNCL is often considered to be of uncertain relevance in the evaluation of the cancer hazard in humans. Furthermore, no hematopoetic neoplasms were found in Sprague-Dawley CD rats treated with DINP-A (Bio/dynamics 1986) or in mice treated with DINP-1 (Moore 1998b). Therefore, MNCL will not be used to predict cancer risk in humans.

Table 8-10. Incidence of mononuclear cell leukemia in a 2-year dietary study of DINP-1 in Fischer 344 (Lington et al., 1997) [a]

	Percent DINP in feed			
	0	**0.03**	**0.3**	**0.6**
Males	33/81 p=0.00003	28/80 –	48/80 p=0.011	51/80 p=0.0028
Females	22/81 p=0.00001	20/81 –	30/80 p=0.11	43/80 p=0.0005

[a] Adapted from CPSC 2001.
[b] Statistics for pairwise comparison of treated and control incidences by the Fisher exact test are given beneath incidence values for treated animals. Statistics for trend tests are given beneath control incidences.

Other Sites

Other peroxisome proliferators have been reported to induce tumors in the testes and pancreas, in addition to the liver by a mechanism that appears to be independent of peroxisome proliferation (e.g., Biegel et al. 2001). In the Lington et al. (1997) and Covance (Moore 1998a) studies of DINP-1 with Fischer 344 rats, interstitial cell tumors of the testes were observed at a high incidence both in control and treated males. No conclusions regarding this tumor site may be made, due to the high background incidence.

In the Bio/dynamics (1986) study of DINP-A with Sprague-Dawley rats, the incidence of testicular interstitial cell hyperplasia was significantly elevated at the high dose (1.0%) relative to both concurrent and historical controls (see also CPSC 2001). Interstitial cell tumors were non-significantly elevated at the high dose (7/60) relative to concurrent (2/59) and historical (3.4 to 23.4%) controls. Other sites with non-significant increases in hyperplasia and/or tumor incidence were the pancreas and endometrium.

Table 8-11. Incidence of mononuclear cell leukemia in a 2-year dietary study of DINP-1 in Fischer 344 rats (Moore 1998a) [a]

Incidence	Percent DINP in feed					
	0	0.05	0.15	0.6	1.2	1.2 R [b]
Males						
Overall incidence At 79 weeks Life table [c, d]	22/65 1/10 p=0.002	23/55 NA -	21/55 NA -	32/65 0/10 p=0.027	30/65 0/10 p=0.022	31/50 [e] (p=0.0024)
Females						
Overall incidence At 79 weeks Life table	17/65 0/10 p<0.001	16/49 NA -	9/50 NA -	30/65 1/10 p=0.020	29/65 1/10 p=0.021	24/50 [e] (p=0.013)

[a] Adapted from CPSC 2001.
[b] Recovery group. Animals were exposed for 78 weeks, followed by a 26-week recovery period.
[c] Statistical significance computed by life table analysis, since MCL is a relatively lethal disease. Significance value for trend is given in the column for the control group.
[d] Statistical analysis was provided by the National Toxicology Program. Additional statistical tests are found in CPSC 2001, Appendix B, Part A.
[e] Level of significance for the recovery group computed by Fisher Exact test. (Statistical tests were not run by NTP on the recovery group.)

Summary of Carcinogenicity

In chronic dietary studies, DINP treatment was associated with increased incidences of hepatocellular tumors in rats and mice of both sexes, renal tubular cell carcinoma in male rats, and mononuclear cell leukemia in Fischer 344 rats. As discussed above, however, the hepatocellular tumors are believed to arise by a mechanism (peroxisome proliferation and related effects) that is not easily induced in humans (Babich 2002; CPSC 2001; Klaunig 2003). The renal tubule tumors are believed to arise by a mechanism (α2u-globulin) that is unique to male rats (Schaeffer 1991). The MNCL is a neoplasm with a high spontaneous rate in Fischer 344 rats that is considered of questionable relevance to humans (Babich 2002; CPSC 2001). Therefore, the CPSC staff regards DINP to be possibly carcinogenic in humans (rather than probably or known to be), based on limited evidence of carcinogenicity in experimental animals, as defined under the FHSA and implementing regulations (CPSC 1992). The finding that DINP is possibly carcinogenic

means that the CPSC staff will not consider carcinogenicity in evaluating the potential risks of DINP exposure to humans (Babich 2002).

9. Dose Response

Studies of non-cancer health effects from chronic exposure to DINP are summarized in Table 5-8. Liver is the most sensitive organ site. Previous CPSC risk assessments have considered liver the critical endpoint. Other endpoints are also discussed, as they may be important for cumulative risk assessment. Kidney was also a target of DINP toxicity. Reproductive effects are summarized in Table 6-3. Developmental effects from prenatal and perinatal exposures are summarized in Table 6-5 and 6-6, respectively. ADI levels for non-cancer effects are derived below. Estimates of cancer unit risk (slope factors) are not derived here, because the tumors induced by DINP are of questionable relevance to humans (section 8). Estimated ADI levels are summarized in Table 9-1.

Liver

Liver is the most sensitive organ site for the non-cancer effects of DINP (Table 5-8), being more sensitive than the kidney and reproductive/developmental effects. Rats are also more sensitive than mice. Thus, liver effects in the rat have been considered to be the critical endpoint for assessing the chronic effects of DINP (CPSC 1998a; CPSC 2001; Wilkinson and Lamb 1999). The NOAEL for non-cancer effects is 15 mg/kg-d, which is based on increased incidence of spongiosis hepatis and increased serum enzyme levels in male rats (Lington et al. 1997).

The acceptable daily intake (ADI) is an estimate of the amount of chemical a person can be exposed to on a daily basis over an extended period of time (up to lifetime) with a negligible risk of suffering deleterious effects. An uncertainty factor approach is used to derive ADI values for non-cancer endpoints (CPSC 1992). The default procedure is to divide the NOAEL by a net uncertainty factor of 100. This 100-fold factor is the product of a 10-fold factor for interspecies differences and another 10-fold factor for interindividual differences. If a NOAEL has not been established, then the LOAEL is divided by an uncertainty factor of 1,000. In this case, the net uncertainty factor includes an additional 10-fold factor. Previously, the CPSC staff applied a 100-fold uncertainty factor to the NOAEL (15 mg/kg-d) in the Lington study, resulting in an acceptable daily intake (ADI) value of 150 μg/kg-d (CPSC 1998).

The benchmark dose (BMD) may be used as an alternative to the NOAEL in setting ADI values (Crump, 1984). The principal advantage of using the BMD is that it is less sensitive to the selection of experimental doses and the number of animals per dose group. Thus, the CHAP derived a benchmark dose (D_{05}) estimate of 12 mg/kg-d by fitting the Lington data to a quantal polynomial model (CPSC 2001; see also Babich 2002)[*]. In this case, the benchmark dose is the maximum likelihood estimate (MLE) of the dose at which the extra risk of spongiosis hepatis is 5 percent.

[*] Calculated with Benchmark Dose Software version 1.20 (EPA 2000).

The polynomial model is described by (Crump 1984; EPA 2000):

$$P = C + (1 - C) \cdot (1 - e^{-\sum_{i=1}^{n} B_i D^i}) \qquad (9\text{-}1)$$

where: P is the probability of lesion; D_i is the dose for the *i*-th group; B_i and C are parameters to be estimated; and $B_i \geq 0$.

The CHAP applied a net uncertainty factor of 100 to the BMD estimate (12 mg/kg-d) from the Lington study to give an ADI of 120 µg/kg-d (CPSC 2001). This ADI was adopted by the European Commission (CSTEE 2001).

No data on the relative susceptibility of children or immature animals to the chronic liver toxicity of DINP are available. In general, children are more susceptible to some chemicals and less susceptible to others (Guzelian et al. 1992; NRC 1993). The National Research Council recommended the use of an additional 10-fold uncertainty factor as a default assumption to account for the possible increased susceptibility of children (NRC 1993). In some cases, the use of an additional safety factor is mandated under the Food Protection Act. In 1991, CPSC proposed the use of an additional 10-fold uncertainty factor for children, to be used as a default assumption in the absence of information to the contrary. The proposal was part of the Commission's proposed guidelines for assessing chronic toxicity. However, public comments, including comments from other regulatory agencies, argued that the default 10-fold uncertainty factor for sensitive populations was sufficient to protect children and that the overall risk assessment process was conservative. Therefore, the additional uncertainty factor for children was dropped from the final chronic hazard guidelines (CPSC 1992). The CHAP (CPSC 2001) and the CPSC staff (Babich 2002) did not apply an additional uncertainty factor for children. However, as noted by the CHAP, the lack of data on the effects of DINP in children or immature animals is a potentially significant source of uncertainty.

Kidney

The kidney is another target for the chronic effects of DINP exposure. Although the kidney is less sensitive than the liver, kidney effects may be relevant in assessing cumulative exposures. The two-year studies in rats and mice are most relevant for assessing chronic effects. Increases in kidney weight are likely to be adaptive responses and, therefore, are not considered adverse effects. In F344 rats, there was mineralization of renal papillae, pigmentation of tubular cells, increased urine volume, decreased electrolytes, increased blood urea nitrogen, and increased kidney weight in both sexes at 0.6% in feed (Table 5-6) (Moore 1989a). The NOAEL was 0.15%, or 88-109 mg/kg-d. In B6C3F1 mice, increased progressive neuropathy was observed in females at 0.8% in feed or 1,888 mg/kg-d (Moore 1998b). Data suitable for dose response modeling are not available. However, the rat NOAEL of 88 mg/kg-d can be used to derive an ADI for kidney. Uncertainty factors of 10 for animal-to-human extrapolation and 10 for inter-individual differences (CPSC 1992) can be applied. Dividing the NOAEL of 88 mg/kg-d by an overall uncertainty factor of 100 results in an ADI for chronic effects in the kidney of 0.88 mg/kg-d or 880 µg/kg-d.

Table 9-1. Summary of estimated acceptable daily intake (ADI) values for DINP

Site	Endpoint	Species, strain, sex	ADI (mg/kg-d)	Method	Reference
Liver	Spongiosis hepatis	F344 rat, M	0.12	BMD [a]	Lington et al. 1997
Kidney	Mineralization and other effects	F344 rat, M+F	0.88	NOAEL	Moore 1998a
Reproductive	Fertility	SD rat, M+F	6.6	NOAEL	Waterman et al. 2002
Developmental	Reduced pup weight	SD rat, M (pups)	1.0	BMD	Masutomi et al. 2003

[a] BMD, benchmark dose; NOAEL, no observed adverse effect level.

Reproductive Toxicity

There were no effects on fertility or other reproductive endpoints in the two-generation study in SD rats (Waterman et al. 2000). Thus, the NOAEL for reproductive effects is the highest dose tested, or 0.8% in feed (Table 6-3). Effects on parental testicular and ovarian weights were observed at 1.5% in the preliminary one-generation study (Waterman et al. 2000). The data are not suitable for dose response modeling due to the lack of effects. However, the NOAEL of 0.8% in feed (665—802 mg/kg-d) can be used to derive an ADI. Applying an overall uncertainty factor of 100 results in an ADI for reproductive effects (parents) of 6.6 mg/kg-d.

Developmental Toxicity

Hellwig et al. (1997) reported a NOAEL of 200 mg/kg-d in Wistar rats exposed prenatally to DINP (Table 6-5). Effects at the LOAEL of 1,000 mg/kg-d included dilated renal pelves, lumbar ribs, and cervical ribs. Waterman et al. (1999) reported a NOAEL of 100 mg/kg-d and LOAEL of 500 mg/kg-d, but they did not test the 200 mg/kg-d dose.

Gray et al. (2000) and Ostby et al. (2001) exposed SD rats perinatally at doses of 750 and 1,000 mg/kg-d, respectively, and reported areolas, nipples, testicular malformations, epididymal agenesis, and reduced AGD in male pups (Table 6-6). Masutomi et al. (2003) reported a NOAEL of 30—66 mg/kg-d for decreased male pup weight, with a LOAEL of 307—657 mg/kg-d. In the two-generation study, Waterman et al. (2000) reported a LOAEL of 143—285 mg/kg-d for reduced pup weight. The NOAEL of 30—66 mg/kg-d for decreased pup weight can be used to derive a developmental ADI. Applying an overall uncertainty factor of 100 would result in an ADI of 0.3 mg/kg-d or 300 µg/kg-d. However, the corresponding LOAEL is 10 times greater than the NOAEL. Thus, there is a considerable gap between the LOAEL and NOAEL.

Alternatively, one could derive a benchmark dose by modeling the data on pup body weights. This would require selecting a response level, such as percent decrease in body weight that is

considered to be adverse. The dose response data (Table 9-2) were modeled using a reduction of one standard deviation as the response level.[†] The continuous Hill model provided an adequate fit to the data (p=0.57) (Figure 9-1). The unrestricted continuous polynomial model was also able to fit the data (p=0.52), but it resulted in a U-shaped dose response curve because the second-order slope term was positive. No other models were able to fit the data, including the polynomial model restricted to negative slopes. The continuous Hill model can be described by:

$$BW = \gamma + \frac{v \cdot D^n}{k^n + D^n} \quad (9\text{-}2)$$

where: BW, body weight; γ, intercept; D, dose; n, power; and v and k are parameters to be estimated.

Table 9-2. Male pup body weight at PND 27 following prenatal and lactational exposure to DINP [a]

Dose (ppm in feed)	N [b]	Body Weight (g) Mean	±	S.D.
0	5	88.5	±	6.4
400	5	84.4	±	3.8
4000	5	72.6 [c]	±	7.9
20000	5	50.6 [c]	±	7.9

[a] Masutomi et al. 2003, Table 4.
[b] N, number of animals; S.D., standard deviation.
[b] Significantly different from the control

Based on the maximum likelihood estimate, the benchmark dose is 1309 ppm in feed or 100—216 mg/kg-d (Table 9-3). The two dose estimates are the maternal dose during gestation and lactation, respectively. Applying an overall uncertainty factor of 100 to the benchmark dose estimate of 100 mg/kg-d gives an ADI of 1.0 mg/kg-d.

[†] Calculated with Benchmark Dose Software version 2.0 (EPA 2008).

Table 9-3. Benchmark dose estimates for a one-standard deviation reduction in male pup weight

	ppm	mg/kg-d
BMD [a]	1508	116—249 [c]
BMDL [b]	995	76—164
Probability	0.52	

[a] BMD, benchmark dose, maximum likelihood estimate of the dose at which mean body weight was reduced by one standard deviation. Estimated with the polynomial model, BMDS 2.0 (EPA 2008).
[b] 95% Lower confidence limit of BMD.
[c] Dose estimates are for maternal exposure during gestation and lactation, respectively.

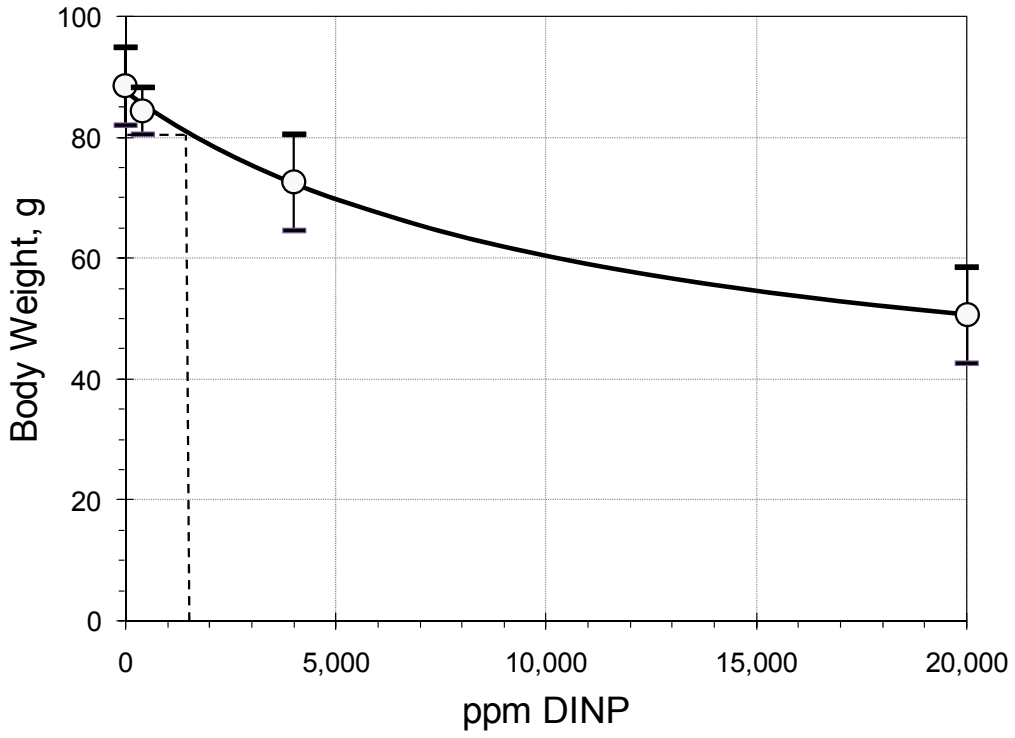

Figure 9-1. Effect of maternal DINP exposure on male pup body weight (g) on postnatal day 27 (Masutomi et al. 2003). Circles, mean body weights; solid line, continuous Hill model fitted with BMDS 2.0; error bars are standard deviations (n=5 per dose group). Broken line indicates the dose at which the average pup weight is reduced by one standard deviation (1508 ppm). Pups were exposed *in utero* and via lactation.

10. Exposure

Exposure to DINP has been reviewed by others (Babich 2002; CERHR 2003; ECB 2003; Schettler 2006). A number of new studies have become available since 2002. Food is believed to be the primary source of exposure to most *o*-DAP's (CERHR 2000b; Schettler 2006; Wormuth et al. 2006). Compared to other *o*-DAP's, however, DINP has been reported less frequently and at lower levels in food and environmental media. The relative lack of data on DINP levels may be due to either low DINP levels or that investigators chose to study other *o*-DAP's. While most *o*-DAP exposure is believed to be from food, other exposure sources such as ingestion of household dust and infant mouthing activity may account for a greater proportion of total DINP exposure, as compared to other *o*-DAP's (Wormuth et al. 2006). It is possible that the patterns of *o*-DAP use differ among the U.S., Europe, and Asia (Wittasek and Angerer 2008). *o*-DAP use patterns also change over time (Wittasek et al. 2007). Therefore, one should exercise caution in interpreting data from different countries and different time periods.

Production and Use

DINP-1 is manufactured in the U.S. by four companies: ExxonMobil Company, Baton Rouge, LA; Ferro Corporation, Bridgeport, NJ; Sunoco, Inc., Pittsburgh, PA; and Teknor Apex, Brownsville, TN (EPA 2006). Sunoco also reports manufacturing DINP-2 at the Pittsburgh site. DINP is reported to be used for electrical and electronic products; fabrics, textiles and apparel; and rubber and plastic products. The latter two categories include products used by children. A total of eleven companies report that they import DINP-2; five companies report that they import DINP-1.

Domestic consumption of DINP was estimated to be 178,000 metric tons (392 million pounds) in 1998. DINP represents approximately 10 to 15 percent of total dialkyl phthalate plasticizer production (Madison et al. 2000). DINP production in the U.S. currently exceeds that of DEHP (ExxonMobil 2009). DINP production is expected to increase at the expense of DEHP. Worldwide, more DEHP is produced than any other plasticizer (ExxonMobil 2009).

Over 90% of DINP is used as a plasticizer for PVC (ECB 2003; ExxonMobil 2009). DINP is used as a plasticizer in a variety of products manufactured from PVC, including vinyl flooring, wire and cable insulation, stationery, coated fabrics, gloves, toys, tubing, garden hoses, artificial leather, footwear, automobile undercoating, and roofing (CERHR 2003; ECB 2003; ExxonMobil 2009). The use of DINP in toys represents less than 1% of total DINP consumption. Most of the DINP in toys imported into the U.S. is manufactured by Asian companies (ExxonMobil 2009). DINP has limited use in food packaging in the U.S. and is not used in medical devices (CERHR 2003). Non-PVC uses of DINP include rubbers, inks, paints, lacquers, adhesives, and sealants (ECB 2003).

DINP Levels in the Environment, Food, and Products

o-DAP's—including DEHP, dibutyl phthalate, and butyl benzyl phthalate—may be found in water (ATSDR 2002; CERHR 2000; Yin and Su 1996), ambient air (ATSDR 2002; CERHR 2000b), indoor air (CERHR 2000; Øie et al. 1997; Tienpont et al. 2000; Wechsler et al. 1984),

and soil (ATSDR 1993). *o*-DAP's may also be found in food (ATSDR 2002; Giam and Wong 1987; MAFF 1996a; Tsumura et al. 2001; Yano et al. 2002; Yin and Su 1996), infant formula (Baczynskyj 1996; MAFF 1996b, 1998; Yano et al. 2005), medical devices (Barry et a. 1989; Calafat et al. 2004a; FDA 2002; Karle et al. 1997; Plonait et al. 1993; Sjoberg et al. 1985a,b), pharmaceuticals (Hauser et al. 2004; Hernández-Diaz et al. 2009), and parenteral nutrition products (Kambia et al. 2001). Food is believed to be the primary source of exposure to most *o*-DAP's (CERHR 2000; Wormuth et al. 2006). *o*-DAP's are not generally used in food packaging in the U.S. (ATSDR 2002). The primary source of *o*-DAP's in food is believed to be general environmental contamination, rather than food packaging (ATSDR 2002; MAFF 1996a, b; Schettler 2006). However, much of the data on *o*-DAP exposure are old and there are a number of data gaps regarding environmental transport and fate (ATSDR 2002; Hauser and Calafat 2005).

Environmental Media

DINP and DIDP were detected in urban storm water in Sweden more frequently (69%) than other phthalates, including DEHP (23%) (Björklund et al. 2009). DINP concentrations ranged from 0.3 to 90 ng/L. DINP accounted for an average of 72% of total phthalate levels. Phthalate concentrations in storm water sediment ranged from 90 to 200 µg/g dry weight. Björklund et al. estimated that a total of 400 g of DINP is released per hectare per year (Table 10-1).

DINP and other *o*-DAP's were also found in seawater and sediment in False Creek Harbor, Vancouver (Mackintosh et al. 2006). The mean total (free and particle-bound) DINP concentration in seawater was 90.9 ng/L (range 61.2 - 135), while the total concentration of all phthalates was 735 mg/L. The average free (unbound) DINP concentration was 43.0 ng/L (range 28.9 – 63.9). The average DINP concentration was 483 ng/g (dry weight) in suspended sediment and 27,200 ng/g in bottom sediment. The authors also measured *o*-DAP's in marine organisms ranging from algae to invertebrates to fish in the same harbor (Mackintosh et al. 2004). DINP levels ranged from 2.41 to 4.04 ng/g of lipid. The DINP levels were roughly comparable to those of DEHP and DIDP. After analyzing these data, the authors concluded that *o*-DAP's are not bioconcentrated as they ascend the food chain. Rather, they undergo trophic dilution, which means that the predators have lower *o*-DAP levels than their prey. Trophic dilution is due to relatively efficient *o*-DAP's metabolism by predators.

Lertsirisopon et al. (2009) studied the abiotic degradation of a series of *o*-DAP's in water. *o*-DAP's can degrade by hydrolysis, which is enhanced by low or high pH, or by photolysis. Photolysis leads to decarboxylation, resulting in a benzoic acid ester. The half-life of DINP in sunlight was estimated to be 140 days at pH=7, as compared to 1600 days for DEHP. The half-life of DINP in sunlight was 32 days at pH=5 and 61 days at pH=9. No degradation of DINP or DEHP was observed in the dark at pH=7. The authors suggested that the more rapid degradation of DINP, relative to DEHP, could result in lower DINP concentrations in aquatic environments.

o-DAP's were also detected in the flue gas from a hazardous waste incinerator (Wienecke et al. 1995). DINP was present at 344 and 462 ng/Nm^3 (ng/m^3 at standard temperature and pressure) in two samples. Diisooctyl phthalate and DBP were detected at comparable levels, while BBP was present at lower levels. DEHP was not reported.

Several investigators have detected DINP and other *o*-DAP's in surface dust from residences (Abb et al. 2009; Bornehag et al. 2004; Øie et al. 1997). DEHP was generally present at the greatest concentrations. DBP, DIBP, and BBP were also detected frequently. DINP accounted for 3.6 to 11.1% of total *o*-DAP's (Table 10-2). DINP levels were highly skewed. Mean DINP concentrations ranged from 100 to 639 µg/g, while median concentrations ranged from 41 to 129 µg/g. In one study, DINP was not detected in suspended dust, although the other *o*-DAP's were present (Øie et al. 1997).

Table 10-1. Environmental Concentrations of DINP

Medium	N [a]	Units	LOD	DF (%)	Mean	Range	Reference
Storm water	39 [b]	µg/L	0.1	69%	NR	0.3 – 90	Björklund et al. 2009
Storm water sediment	39	µg/g (dry weight)	NR	NR	NR	90 – 200	
Sea water (total)	9	ng/L	200 – 530	25%	90.9	61.2 – 135	Mackintosh et al. 2006
Sea water (free)	9	ng/L	200 – 530	25%	43.0	28.9 – 63.9	
Marine bottom sediment	3	µg/g (dry weight)	4.4	92%	27,200	14,700 – 50,400	
Marine suspended sediment	12	µg/g (dry weight)	4.4	92%	483	259 – 900	
Marine organisms	141 [c]	ng/g (lipid)	1.04	89%	NR	2.41 – 4.04	Mackintosh et al. 2004
Flue gas [d]	2	ng/Nm^3		100%	403	344 – 462	Wienecke et al. 1995

[a] DF, detection frequency (%);LOD, limit of detection; N, number of samples tested; NR, not reported.
[b] 13 samples collected at each of three sites.
[c] 141 samples of 20 species.
[d] ng/m^3 at normal conditions, i.e., standard temperature and pressure.

Food

Sørensen (2006) measured *o*-DAP levels in milk and milk products. DEHP was present at levels from 7 to 138 µg/kg (ppt) in milk (n=22), yogurt (n=3), and infant formula (n=8). DINP was not detected (LOD=5 µg/kg). DBP, BBP, and DIDP also were not detected.

Tsumura et al. (2001) measured the levels of *o*-DAP's and di(2-ethylhexyl)adipate (DEHA) in one-week total dietary samples (21 meals) from three Japanese hospitals. The study was repeated after the use of DEHP-containing PVC gloves for food handling was prohibited

(Tsumura et al., 2003). Prior to the prohibition (Tsumura et al. 2001a), DINP was detected in one-third of meals tested (Table 10-3). DEHP was detected in all meals in all hospitals, while DEHA was detected in 50% of meals. DBP and BBP were present less frequently and at lower levels. Levels of DEHP, DEHA, and DINP were highly correlated (r^2 from 0.94 to 0.99). Following the prohibition of DEHP-containing gloves, DINP was detected in only 14% of meals and the average concentration was less that the detection limit (4 to 6 ng/g). DEHP was present in 98% of meals, while DEHA was present in 68% of meals. The authors estimated average daily intakes by assuming that non-detects were equal to one-half the detection limit. Assuming a 70 kg body weight, the average daily intake of DINP was estimated to be 0.92 μg/kg-d in 1999 and 0.07 μg/kg-d in 2001. This study suggests that the gloves were not a significant source of dietary DEHP, but they could have contributed to dietary DINP and DEHA.

Table 10-2. DINP concentrations (μg/g) in household dust.

Locale	Sample	N [a]	Mean	Median	GM	*o*-DAP [b] (%)	Range	Reference
Germany [c]		30	--	129	--	11.1	50—1050	Abb et al. 2009
Sweden [d]	All homes	346	639	41	--	3.6	--	Bornehag et al. 2004
	Cases	87	671	0	453		352—583	
	Controls	90	589	47	446		351—566	
Norway [e]	Total dust	38	100	--	--	10.4	0—01,380	Øie et al. 1997
	Organic fraction		120	--	--		0—1,610	

[a] GM, geometric mean; N, number of residences sampled.
[b] Average DINP concentration as percentage of total *o*-DAP's; based on median (Abb et al., Bornehag et al.) or mean (Øie et al.).
[c] The range includes the 5th and 95th percentile values as read from a box plot.
[d] Cases are physician-diagnosed asthma, rhinitis, or eczema. The range is the 95% confidence interval of the GM. Concentrations were converted from mg/g to μg/g. The DINP detection limit was not reported.
[e] Reported as "heavier phthalates, mixture of isomeric dinonyl phthalates." Concentrations were converted from μg/100 mg to μg/g. The DINP detection limit was not reported.

Tsumura et al. (2001b) also measured plasticizer levels in packaged lunches before (1999) and following (2000) the prohibition of DEHP-containing gloves for food handling. The lunches were sold in convenience stores. DINP was present in three of 10 samples prior to the ban and one sample following the ban. All of the samples tested contained DEHA and DEHP, both before and following the prohibition. However, concentrations were lower following the prohibition (Table 10-4).

Pedersen et al. (2008) measured plasticizer levels in fatty goods sold in glass jars with screw-top lids. DINP was found in one sample of peanut butter at 0.0099%.

Table 10-3. Plasticizers in Hospital Food (Tsumura et al. 2001a, 2003) [a]

Compound	Year	DF [b]	Concentration [c]		Average Intake [d]	
		%	ng/g		μg/d	μg/kg-d [e]
DINP	1999	33	32.0	(2.0—92)	64.6	0.92
	2001	14	0.0		4.7	0.07
DEHP	1999	100	302.7	(46—478)	519	7.41
	2001	98	94.3	(77—103)	160	2.29
DBP	1999	46	4.3	(0—7.0)	14.3	0.20
	2001	32	4.4	(2.0—7.1)	13.1	0.19
BBP	1999	71	2.7	(0.10—5.0)	4.7	0.07
	2001	13	2.0	(0.6—2.8)	3.4	0.05
DEHA	1999	86	50.2	(6.5—78.2)	86.4	1.23
	2001	68	7.5	(4.7—12.7)	12.5	0.18

[a] Measured in 21 meals in each of three Japanese hospitals in 1999 (published in 2001) and 2001 (published in 2003).
[b] DF, detection frequency in 63 meals.
[c] Mean and range of 3 hospitals, as reported by the authors.
[d] Assuming non-detects are equal to one-half the detection limit.
[e] Calculated by the reviewer, based on 70 kg body weight.

Table 10-4. Plasticizers in Packaged Lunches (Tsumura et al. 2001b) [a]

Plasticizer	LOD [b]	1999			2000		
	(ng/g)	N [c]	Concentration (ng/g) [d]		N	Conc. (ng/g)	
DINP	40	3	198	(<40—598)	1	76	(<20—76)
DEHP	14.9	10	4420	(803—8,930)	10	198	(45—517)
DBP	18.6	6	36	(<18.6—64)	0	<18.6	
BBP	0.5	10	37	(2.1—277)	6	3.5	(<0.5—5.3)
DEHA	1.8	10	540	(13—1494)	9	21	(<4—90)

[a] Measured in 10 packaged lunches in 1999 and 2000.
[b] LOD, limit of detection.
[c] N, number of samples of 10 tested with detectable plasticizer.
[d] Mean concentration (detects only), calculated by the reviewer, and range (including non-detects), ng/g dry weight.
Abbreviations: BBP, butylbenzyl phthalate; DBP, dibutyl phthalate; DEHA, di(2-ethylhexyl) adipate; DEHP, di(2-ethylhexyl) phthalate.

Household Products

The Household Products Database listed DINP-1 as an ingredient in only one product, an aerosol clear lacquer finish (NLM 2009b). DINP was present at 3.9%. The Household Products Database is not comprehensive. It does not include all products and does not include ingredients in articles such as toys and home furnishings.

The Danish Ministry of the Environment has studied the chemical ingredients of many household articles. They found DINP in a variety of vinyl household products such as shower curtains, floor coverings, and gloves (DME 2001, 2009). They also found DINP in pet toys (DME 2006b) and adult toys (DME 2006c). DINP was present at levels from 0.6 to 58.5%. In some cases it was found in combination with other phthalates (see Table 10-5).

Table 10-5. DINP Levels in Household Products

Product	DF [a]	Concentration (%) [b]		Other Plasticizers [c]	Reference
Bath mat	1/5	80.0		--	DME 2009
Carpet tile (backing)	1/2	27.0		DEHP	DME 2001
Food jar (gasket)	2/19	22.0	(20—24)	DIDP, ESBO, DEHP,	Pedersen et al. 2008
Glove, lightweight	1/4	58.5		DEHP, BBP	DME 2001
Pet toys	10/13	27.7	(6.9—54)	DEHP	DME 2006b
Shoulder bag	1/3	11.0		DEHP, BBP	DME 2001
Shower curtain	1/3	8.6		DEHP	DME 2001
Vibrator, soft vinyl	2/7	0.55	(0.5—0.6)	--	DME 2006c
Vinyl floor	3/5	12.0	(0.6—30.5)	DEHP, DBP, BBP	DME 2001
Vinyl wallpaper	2/4	24.5	(23—26)	DEHP	DME 2001

[a] DF, detection frequency, number with DINP over number of products tested.
[b] Concentration of DINP, % (w/w), mean and range.
[c] Other plasticizers found in similar products or in combination with DINP.
Abbreviations: BBP, butylbenzyl phthalate; DBP, dibutyl phthalate; DEHP, di(2-ethylhexyl) phthalate; DIDP, diisodecyl phthalate; ESBO, epoxidized soybean oil.

Children's Products

DINP and other *o*-DAP's have been used in children's articles such as teethers, soft plastic toys, and occasionally even in pacifiers. Until 1985, DEHP was commonly used in children's products made from PVC. Manufacturers voluntarily removed DEHP from pacifiers, teethers, and rattles after the NTP (1992) found that DEHP caused liver tumors in rodents and CPSC began regulatory proceedings. In 1998, DINP was the predominant plasticizer in PVC teethers and soft plastic toys sold in the U.S. (Chen 1998). DINP is generally used at levels from 15% to 50% (Table 10-6). In 1998, the CPSC staff found only one pacifier made from PVC (Chen 1998). However, several different *o*-DAP's were reported in teethers toys, and some pacifiers, sold in Europe and Japan.

Table 10-6. DINP Levels in Children's Pacifiers, Teethers, and Toys [a]

Product	DF [b]	Concentration (%) [c]		Other Plasticizers [d]	Reference
Pacifiers	1/1	58.0			Sugita et al. 2001
	1/1	58.3			Niino et al. 2003
Pacifiers, teethers, and toys	NR/27 [b]	NR	(3.9—44)		Health Canada 1998
Pacifier shield	2/5	0.105	(0.05—0.16)	DEHP	DME 2009
Teethers	1/1	36.0			Fiala et al. 2000; Steiner et al. 1999
	8/9	36.6	(19.3—54.4)	DNP	Chen 1998
	1/1	39.0			Sugita et al. 2001
	1/1	45			Bouma and Schakel 2002
	1/1	38.9			Niino et al. 2003
Teethers and toys	10/10	NR	(21.0—46.6)		Rijk and Ehlert 1999; Rijk et al. 1999
	5/5	NR	(26.0—41.7)		Simoneau et al. 2001
Toys, foam	4/6	0.024	(0.00051—0.094)	DIDP, DIBP, DBP	DME 2006a
Toys, soft plastic	24/25	36.3	(15.1—51.0)	DEHP	Chen 1998
	36/85	30.0 [e]	(12.9—39.4)	ATBC, DEHA, DEHP	Chen 2002
	5/9	27.9	(16.0—58.3)	DEHP, DBP	Niino et al. 2003
Toys, soft PVC:					Sugita et al. 2001
Toy food	20/20	32.3	(15.0—45.0)	DEHP, DBP	
Balls	2/7	23.5	(21.0—26.0)	DEHP, DBP, DHpP, DEHA	
Inflatable toys	1/4	40.0		DEHP	
Rattle	1/1	38.0			
Toys, soft PVC:					Sugita et al. 2001
China	19/19	30.4	(14.0—41.0)		
Japan	3/6	11.4	(1.5—30.0)	DEHP, DNP, DHpP	
Toys, soft PVC:					Sugita et al. 2001
China	39/42	32.2		DEHP, DBP	
Japan	8/25	23.3		DEHP, DBP, DNP, DHpP, DEHA	
Thailand	1/1	39.0			
Total	48/68	30.8			
Toys, soft PVC	33/62	35.3	(16—48)	DEHP, ATBC, etc.	Bouma and Schakel 2002

[a] The studies listed here include products from several countries. All of the studies predate the CPSIA (2008), and some predate the European Commission temporary ban (1999) and permanent ban (2005).
[b] DF, detection frequency, number with DINP / number of products tested; NR, not reported.
[c] Concentration of DINP (%), mean and range (detects only).
[d] Other plasticizers used in similar products or in combination with DINP.
[e] DINP was detected in 36 samples, but quantified in only 24.

Abbreviations: ATBC, acetyl tributyl citrate; BBP, butylbenzyl phthalate; DEHA, di(2-ethylhexyl) adipate; DEHP, di(2-ethylhexyl) phthalate; DEP, diethyl phthalate; DHpP, di-heptyl phthalate; DIBP, di-isobutyl phthalate; DNP, di-*n*-nonyl phthalate.

In 1999, manufacturers voluntarily removed all phthalates from pacifiers, teethers, and rattles due to concerns about the health effects of DINP. DINP and other *o*-DAP's could still be used in soft plastic toys. In 2002, only about one-third of soft plastic toys contained DINP, and no phthalates were found in teethers (Chen 2002) (Table 10-6). In addition to teethers and toys, the Danish Ministry of the Environment found a variety of *o*-DAP's in various children's articles, such as outerwear, non-slip bath surfaces, shampoo bottles, and art materials (Table 10-7).

In 1999, the European Commission banned the use of six phthalates (BBP, DBP, DEHP, DINP, DIDP, and DNOP) in children's products. The CPSIA of 2008 permanently banned BBP, DBP, and DEHP in children's toys and child care articles. In addition, the CPSIA temporarily banned the use of DINP, DIDP, and DNOP in child care articles and children's toys that can be placed in a child's mouth, pending review by the CHAP. In 2009, the CPSC staff found that very few of the toys tested contain phthalates, even where phthalates are allowed by the CPSIA (in preparation). The currently available data on the presence of DINP and other phthalates in children's products reflect the prevalence of these compounds prior to the European and American regulations limiting their use. Therefore, the data presented in Tables 10-6 and 10-7 do not reflect current use patterns.

Table 10-7. DINP Levels in Other Children's Products

Product	DF [a]	Concentration (%)		Other Plasticizers [c]	Reference
Bath mat	1/5	80.0		DEHP	DME 2009
Body wash/bubble bath bottle	4/9	21.5	(1—31)	DEHP, *iso*-DEHP	DME 2007b
Body wash/bubble bath bottle	2/5	15	(10—20)	DEHP, DNOP, DEP	DME 2009
Eraser	6/10	47.7	(32—70)	DEHP	DME 2007a
Mitten label/ reflector	2/5	8.2	(7.8—8.6)	DEHP	DME 2009
Nursing pillow:	1/1				DME 2008
Outer cover		14.4			
Inner cover		22.0			
Foam		0.38			
Polymer clay	1/4	9.8		BBP, DEHP, *iso*-EHP, et al.	DME 2002

[a] DF, detection frequency, number with DINP / number of products tested.
[b] Concentration of DINP (%), mean and range (detects only).
[c] Other plasticizers used in similar products or in combination with DINP.

Abbreviations: BBP, butylbenzyl phthalate; DEHP, di(2-ethylhexyl) phthalate; *iso*-DEHP, di(2-ethylhexyl) *meta*-phthalate; DEP, diethyl phthalate; DNP, di-*n*-nonyl phthalate.

DINP Migration from PVC Products

Researchers and regulatory agencies have used migration studies to estimate the rate at which DINP migrations from products and, in turn, for estimating human exposure. Most of the migration studies were intended to assess oral exposure from the mouthing of teethers and soft plastic toys by small children. Initially, a variety of unvalidated laboratory migration methods were used, which resulted in a wide range of migration rate estimates (reviewed in Babich 2002). Migration rates ranged from about 0.3 to 400 µg/10 cm^2/h for teethers and soft plastic toys (Babich 2002). Method development was confounded by the lack of a standard reference material. Laboratory migration methods involved immersing a test sample in a saliva simulant or saline solution and applying some form of mechanical or ultrasonic agitation, such as shaking or tumbling (Axford et al. 1999; Chen 1998; Earls et al. 1998; Fiala et al. 2000; Niino et al. 2003; Rastogi et al. 1997; Vikelsoe et al. 1997).

Eventually, a tumbling method, dubbed the "head-over-heels" method was adopted as the method of choice. The head over heels method was first developed by the TNO Nutrition and Food Research Institute (Rijk and Ehlert 1999; Rijk et al 1999) and later modified by the European Commission's Joint Research Center (JRC) (Simoneau and Rijk 2001; Simoneau et al 2001). Development of the method was facilitated by a standard disk, a 10 cm^2 disk containing 40% DINP. The JRC method involves placing a 10 cm^2 sample in a 250 mL screw cap bottle with 50 mL of saliva simulant (Simoneau and Rijk 2001). (The original method used 25 mL of synthetic saliva.) The bottles are placed in the apparatus and tumbled at a rate of 60 rpm for 30 minutes (Figure 10-1). The sample is then extracted with fresh simulant for an additional 30 minutes. The two 50 mL extracts are analyzed, and the results presented as µg/10 cm^2/h. The sample size of 10 cm^2 is roughly the surface area of a pacifier nipple (CPSC 1985). Migration rates by head over heels methods range from about 1.0 to 11 µg/10 cm^2/h (Table 10-8).

Table 10-8. DINP migration rate (µg/10 cm^2/min) measured by the tumbling method.[a]

Product tested	% DINP	N[b]	Mean	SD	Range	Reference
Soft plastic toys	12.9—39.4	24	4.1	± 2.7	(1.0—11.1)	Chen 2002
Soft plastic toys	16—48	32	2.2	± 1.1	(0.3—4.9)	Bouma and Schakel 2002
Teethers and soft plastic toys	21.0—46.6	10	2.4	± 1.38	(0.9—5.6)	Rijk and Ehlert 1999; Rijk et al. 1999
Teethers and soft plastic toys	26—41.7	5	4.0	± 1.45	(1.9—5.4)	Simoneau et al. 2001
Teether	NR	1	3.1	± 0.5	(2.5—4.2)	RIVM 1998
Teether	45	1[c]	1.8	± 0.29		Bouma and Schakel 2002

[a] Method of Simoneau et al. 2001 or similar method.
[b] N, number of products tested; NR, not reported; SD, standard deviation.
[c] Total of 5 measurements from one teether.

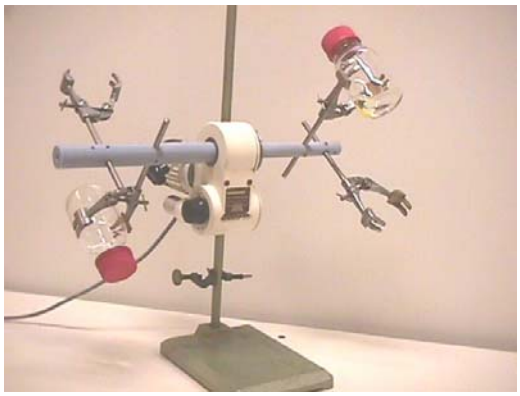

Figure 10-1. Apparatus for measuring *o*-DAP migration rates by the head over heels (tumbling) method (Chen 2002).

It is noteworthy that there does not appear to be a direct relationship between the DINP concentration in the product and the migration rate (Figure 10-2). Aside from DINP content, there are other factors that may affect the migration rate, including manufacturing method, thickness, and the presence of fillers and pigments.

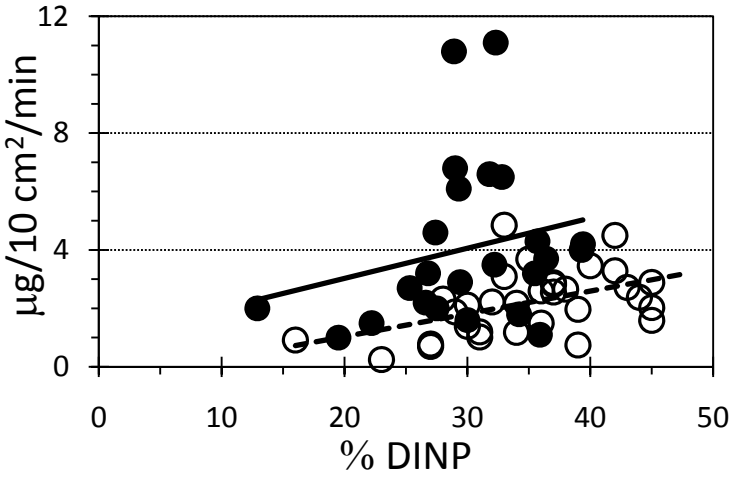

Figure 10-2. DINP migration rates measured by the head-over-heels (tumbling) method. Filled circles, solid line, Chen 2002; open circles, broken line, Bouma & Schakel 2002. Lines are linear trends.

The JRC laboratory method was validated by comparison of the laboratory method to *in vivo* studies using adult volunteers (Simoneau et al. 2001). These were dubbed the "chew and spit" studies. In a typical experiment, volunteers are given a 10 cm^2 sample, such as the standard DINP disk or a disk cut from a toy, and asked to mouth, suck, or gently chew on the sample for 15 minutes, during which all saliva was collected in a flask. This is repeated for a total of four 15-minute sessions. The four saliva samples collected from each volunteer are pooled and analyzed to determine the migration rate. There was also a 15-minute "blank" consisting of a polytetrafluoroethylene (PTFE) disk. Migration rates measured in human subjects studies ranged

from 0.5 to 10 µg/cm^2/min (Table 10-9). The JRC laboratory method was developed to approximate the 90th percentile migration rate from the human subjects studies (Simoneau et al. 2001). Thus, the JRC method over-predicts the mean *in vivo* migration rate by about 3.6-fold (Babich 2002).

Table 10-9. DINP migration rate (µg/10 cm^2/min) measured with human subjects.[a, b]

Product tested	% DINP	N[c]	Mean	SD	Range	Reference
Soft plastic toy	43	10	4.5	± 2.64	1.1—10.0	Chen 1998[d]
Soft plastic toys	39	37	1.5	± 0.95	0.2—4.0	Sugita et al. 2003
	58	15	1.8	± 1.19	0.5—4.5	
	38	12	1.4	± 1.38	0.2—4.1	
Standard disk	40	20	1.8	NR	1.4—2.4	RIVM 1998
Teether, sucking	36	9	1.4	NR	0.5—2.4	Fiala et al. 2000; Steiner et al. 1998[d]
Teether, chewing	36	9	2.2	NR	1.3—9.7	

[a] Adapted from Babich 2002.
[b] Method of Simoneau et al. 2001 or similar method.
[c] N, number of subjects; NR, not reported; SD, standard deviation.
[d] Migration rates were converted from their original units.

Scenario-Based Exposure Assessments

Children's Exposure from Mouthing Teethers and Toys

Several government agencies conducted scenario-based exposure assessments of children's exposure to DINP. Some of these exposure assessments have employed conservative assumptions, such as the use of upper bound estimates of mouthing times. As a result, the estimated exposures vary widely. However, as new information became available, such as data on children's mouthing behavior, the estimated exposures have generally declined (Table 10-10).

Dutch Consensus Group. The Dutch Consensus Group (RIVM 1998) performed a risk assessment for the European Union. They used DINP migration data from a human subject study combined with mouthing duration data from Groot et al. (1998). They used probabilistic methods (bootstrap procedure) to estimate exposure distributions for the use of teethers (toys intended to be mouthed) in children of various age groups. The mean (9.66 µg/kg-d) and 95th percentile (26.0 µg/kg-d) exposures were greatest in the 6 to 12-month-old age group. The *in vivo* migration data were likely more reliable than the *in vitro* data available at the time of this study. However, the *in vivo* data did not encompass a broad range of products, as only one teether and one standard disk were included. The use of mouthing data for "toys intended to be mouthed" may exclude other soft plastic (PVC) toys that children routinely mouth. While the Groot et al. data were the only mouthing data available at the time, they are based on a relatively small (n=42) sample size.

Health Canada. Health Canada (1998) used migration rates from the first human subjects study by the Dutch Consensus Group. They assumed mouthing durations of 1 to 3 hours per for toys and 2 to 6 hours per day for pacifiers. They also used 5th percentile body weights to calculate the average daily exposure.

CHAP on DINP. The CHAP on DINP (CPSC 2001) estimated oral DINP exposure using the upper bound (estimated 95th percentile value) migration rate obtained in the CPSC human subject study (Chen 1998). The CHAP assumed mouthing durations of 3 hours and 1 hour per day for children 0 to 18 and 19 to 36 months old, respectively. Thus, upper bound measures of migration and exposure were combined to produce an upper bound estimate of oral exposure of 280 μg/kg-d in children 0 to 18 months old. CPSC migration (Chen 2002) and mouthing (Greene 2002a) data were not available at the time that the CHAP report was published.

CPSC . The first CPSC risk assessment (CPSC 1998) was based on migration rates using an impaction method that was previously used for DEHP (CPSC 1985). Exposure durations were estimated from a reanalysis of the Dutch Consensus group study. The impaction method, which was not previously validated, was believed to underestimate exposure. Thus, the migration rate was multiplied by a correction factor to adjust for the difference in migration rates between the impaction method and human subjects.

The updated CPSC risk assessment (Babich 2002; Babich et al. 2004) used mouthing duration data from the CPSC observational study (Greene 2002a; Kiss 2002). Mouthing data included hourly mouthing times, as well as exposure times (time awake and not eating). DINP migration data for 24 soft plastic children's articles (primarily toys) were obtained using the JRC laboratory method (Chen 2002). DINP migration rates were adjusted by an *in vivo*: *in vitro* ratio, because the JRC method slightly over predicts the *in vivo* migration rate. Distributions of the daily DINP exposures were estimated by probabilistic methods (bootstrap procedure), as described by Greene (2002b).

The basic case—soft plastic toys, adjusting for the prevalence of DINP—represents the best estimate of circa 2000 oral exposure to DINP in children's products. For the basic case, the greatest exposure was obtained with children aged 12-to-24 months (Greene 2002b). The estimated mean DINP exposure was 0.08 (95% confidence interval (CI) 0.04-0.14) μg/kg-d (Table 10-10). The median (50th percentile) exposure was 0.00 (0.00-0.00) μg/kg-d. The 95th percentile exposure was 0.53 (0.24-0.89) μg/kg-d. The 99th percentile exposure (not shown) was 1.5 (0.89-2.3) μg/kg-d.

The hypothetical cases represent exposure parameters in 1998 or that could result if the use of DINP in children's products were to increase in the future. For soft plastic toys (100% containing DINP), the greatest exposure was with children aged 12-to-24 months. The estimated mean DINP exposure was 0.22 (0.11-0.32) μg/kg-d (Table 10-10). The median (50th percentile) exposure was 0.00 (0.00-0.05) μg/kg-d. The 95th percentile exposure was 1.1 (0.62-1.6) μg/kg-d.

For "all toys, teethers, and rattles," exposure was greatest among 3 to 12-month-olds. The estimated mean exposure was 2.9 μg/kg-d, while the median was 1.4 μg/kg-d. The estimated 95th percentile exposure was 10.7 (6.5-16.1) μg/kg-d.

As with previous studies, the greatest estimated exposures were obtained with pacifiers, due to the relatively long mouthing duration.* Exposure was greatest among 3 to 12-month-olds. The estimated mean exposure was 4.8 μg/kg-d, while the median was 0.00 (0.0-0.64) μg/kg-d. That the median was zero reflects the observation that 57 percent of children did not mouth pacifiers. The 95th percentile exposure was 24.6 (11.7-41.4) μg/kg-d. The estimated 99th percentile exposure (not shown) was 62.4 μg/kg-d (28.4-101.5) (Greene 2002b).

European Commission. The most recent European Commission risk assessment used the maximum *in vivo* migration rate from the Dutch Consensus Group study (RIVM 1998) and assumed 3 hours of exposure per day (ECB 2003). This resulted in an upper bound exposure estimate of 200 μg/kg-d in children 3 to 12 months old.

Japanese NIH. Sugita et al. (2003) used probabilistic methods, based on migration data from human subjects and mouthing durations from the Dutch Consensus Group.

Dermal Exposure

Some risk assessments of DINP exposure from children's products such as teethers and toys considered only oral exposure (CPSC 1998; Health Canada 1998; RIVM 1998; Wilkinson and Lamb 1999). However, the CPSC risk assessment of DEHP exposure from children's products considered dermal exposure from products such as playpens and baby pants, as well as oral exposure (CPSC 1983). Recently, the CHAP (CPSC 2001) and the European Commission risk assessments (ECB 2003) also considered dermal DINP exposure from PVC products such as rainwear, footwear, gloves, and toys (Table 10-11).

To estimate dermal exposure to DEHP, CPSC scrubbed PVC products with a wood block covered with lanolin-impregnated cotton cloth (CPSC 1983). CPSC also assumed that 10% of the DEHP migrating to the skin would be absorbed. By this method, CPSC estimated that the dermal intake (i.e., absorbed dose) from vinyl playpens would be 96 mg DEHP per year, or roughly 33 μg/kg-d (CPSC 1983). CPSC also estimated a dermal dose from vinyl baby pants of 120 mg over 2 years, or roughly 21 μg/kg-d. Percutaneous absorption studies published since the 1983 risk assessment suggest that percutaneous absorption of DEHP is about 5 percent over a 5-day period (Elsisi et al. 1989). Thus, the dermal dose from DEHP is probably lower than what CPSC estimated.

* In 1998, only one pacifier tested by CPSC contained a phthalate, and this was voluntarily withdrawn by the manufacturer.

Table 10-10. Estimates of oral DINP exposure (μg/kg-d) from children's products [a, b]

Agency	Product(s)	Age	Mean	Median	95%	Range	Reference
Dutch Consensus Group [c, d]	Teethers	3 - 6 months	9.66	7.17	26	– 70.7	RIVM 1998
		6 - 12 months	7.79	4.8	25.5	– 142	
		12 - 18 months	2.33	1.06	10.5	– 51.1	
		18 - 36 months	1.13	0.52	4.32	– 23	
Health Canada [d]	Teethers, toys	3 - 12 months	44	--	--	4 – 320	Health Canada 1998
		13 - 26 months	39	--	--	5 – 228	
	Pacifiers	3 - 12 months	120	--	--	18 – 640	
		13 - 26 months	62	--	--	5 – 458	
CPSC (1998) [e]	Teethers, toys	3 - 12 months	5.7	--	94.3	--	CPSC (1998)
		13 - 26 months	0.7	--	7.6	--	
Chronic Hazard Advisory Panel [f]	Toys	0 - 18 months	--	--	280	--	CPSC 2001
		19 - 36 months	--	--	66	--	
CPSC (2002) [g]	Soft plastic toys (42% with DINP)	3 - 12	0.07	0.00	0.44		Babich 2002; Babich et al. 2004
		12 – 24	0.08	0.00	0.53		
		24 – 36	0.03	0.00	0.12		
	Soft plastic toys [h] (100% with DINP)	3 - 12	0.17	0.00	0.94		
		12 – 24	0.22	0.01	1.1		
		24 – 36	0.07	0.00	0.27		
	All teethers, toys, & rattles [h] (100% with DINP)	3 - 12	2.9	1.4	10.7		
		12 – 24	0.84	0.33	3.4		
		24 – 36	0.28	0.08	1.2		
ECB [c, i]	Toys	3 – 12 months	--	--	200	31 – 226	ECB 2003
Japanese NIH [j]	Toy	6 – 12 months	14.8	12.8	35.7		Sugita et al. 2003
	Toy, pacifier	6 – 12 months	21.4	14.1	65.8		

[a] Adapted from CPSC 2001, Babich 2002.
[b] All units are in micrograms per kilogram per day.
[c] For the European Commission.
[d] Based on the migration rates measured with human subjects by the Dutch consensus group, 5th percentile body weights, and assuming mouthing times of 1 to 3 hours for teethers and toys and 2 to 6 hours for pacifiers.
[e] The migration rate by the impaction method was multiplied by a scaling factor to adjust for the differences between impaction and human subjects.

[f] Estimate for "relatively highly exposed children" based on the estimated 95th percentile migration with human subjects (CPSC 1998) and assuming mouthing durations of 3 hours/day for 0 – 18-month-olds and 1 hour/day for 19 – 36-month-olds.

[g] Probabilistic estimates based on migration rates of 24 toys (Chen 2002) measured by the JRC method (Simoneau and Rijk 2001), mouthing data on 169 children age 3 to 36 months (Kiss 2002), and the observation that only 42% of toys contained DINP.

[h] Hypothetical cases assumed 100% prevalence of DINP and used migration data from toys as surrogates for teethers and rattles.

[i] Based on the maximum *in vivo* migration rate (8.9 μg/cm^2/min) in the Dutch Consensus Group study (RIVM 1998), 10 cm^2 surface area, 3 hour exposure duration, and 8 kg body weight (ECB 2003, Table 4.1.1.2.1). The low value in the range is based on the average migration rate in Steiner et al. 1998. The high value is the 95 percent upper confidence interval of the 95th percentile exposure, as calculated by CPSC (1998).

[j] Probabilistic estimates based on migration rates from an i*n vivo* study study and mouthing times from RIVM 1998.

The European Commission (ECB 2003) considered dermal exposure in its risk assessment for the European Union. Deisinger et al. (1988) studied the percutaneous absorption of DEHP in rats from a PVC film containing 40 percent DEHP. DEHP was absorbed at a rate of 0.24 μg/cm^2-h. However, in the risk assessment, it was assumed that DINP would be absorbed more slowly than DEHP (Elsisi et al. 1989). It was further assumed that an 8 kg child would handle soft plastic toys for 3 hours per day, and that the surface area contacting the toys would be 100 cm^2. The dermal intake was estimated to be 1 μg/kg-d. They also estimated that the intake for adults wearing PVC gloves for two hours per day, with a surface area (both hands) of 840 cm^2, would be 0.7 μg/kg-d.

The CHAP used the percutaneous absorption rate from the study with DEHP-containing PVC film (Deisinger et al. 1998), and assumed that the absorption of DINP would be no greater than the absorption of DEHP. Using this method, which the CHAP termed the CF (Contact-Flux) method, the CHAP estimated dermal doses for children (19 to 36 months) and adults using rainwear of 3.2 to 3.9 μg/kg-d, respectively. The CHAP estimated exposure from vinyl sandals of 14 μg/kg-d in children (19 to 36 months) and 3.9 μg/kg-d in adults, assuming that significant absorption would only occur through the dorsal surface of the foot.

Table 10-11. Estimates of dermal intake (μg/kg-d) of dialkyl *o*-phthalates from household products

Agency	Product	Age	Exposure (μg/kg-d)	Methodology	Reference
CPSC (DEHP)	Playpen liner	Child	33 [a]	Migration estimated by scrubbing; assumes 10% dermal absorption	CPSC 1983
	Baby pants	Child	21 [b]		
CHAP (DINP)	Rainwear	19-36 months	3.2	Migration and dermal absorption from Deisinger et al. (1998)	CPSC 2001, Appendix A
		Adult	0.45		
	Sandals [c]	19-36 months	14		
		Adult	3.9		
	Rainwear	19-36 months	79	Migration from human subjects study (Chen 1998). Dermal absorption modeled (Bogen 1994).	
		Adult	11		
	Sandals [c]	19-36 months	340		
		Adult	98		
European Commission (DINP)	Soft plastic toys	Child	1	Migration and dermal absorption from Deisinger et al. (1998)	ECB 003
	Vinyl gloves	Adult	0.7		

[a] CPSC estimated a dermal intake of 96 mg DEHP per year. Converted to μg/kg-d by assuming an 8 kg child.

[b] CPSC estimated a dermal intake of 120 mg DEHP in two years. Converted to μg/kg-d by assuming an 8 kg child.

[c] For absorption through the dorsal surface of the foot only.

The CHAP also used an alternative method to estimate dermal doses. The estimated 95th percentile DINP migration rate from the CPSC human subjects study was used as an upper bound estimate of migration. Percutaneous absorption was estimated by means of an empirically derived model developed for dilute aqueous solutions (Bogen 1994). However, this model was based on data with compounds having log K_{ow} values up to 4.11 and molecular weights up to

197.5. A similar type of model was based on data with compounds having log K_{ow} values up to 6 (Potts and Guy 1992). In comparison, DINP has a log K_{ow} of 9, and molecular weight of 418.6. The model Bogen and Potts-Guy models predict high permeability coefficients, based on the high log K_{ow} values. These are not consistent with the low absorption rates measured *in vivo* (Elsisi et al. 1989; Stoltz and El-hawari 1983; Stoltz et al. 1985; Wester et al. 1998) and low permeability constants measure *in vitro* (Barber et al. 1992; Scott et al. 1987). Furthermore, it is likely that intermediate values of log K_{ow} favor absorption, because the skin includes both hydrophobic and hydrophilic barriers. Therefore, the applicability of the modeled permeability constants is doubtful (Babich 2002).

Total Exposure

The European Commission also assessed DINP exposure from multiple media and pathways (ECB 2003). They assessed consumer, occupational, and environmental scenarios. Consumer scenarios included food, building materials and furniture, transportation, apparel, and children's teethers and toys. Worst-case estimates of inhalation exposure to DINP indoors and in automobiles were based on the vapor pressure. Dermal exposure from apparel and toys was based on a study with DEHP (Deisinger et al. 1998). Food exposures for adults and infants were based on detection limits in food or infant formula, as DINP was generally not detected.

The ECB estimated children's exposure (newborn to 36 months) from soft plastic teethers and toys to be 241 μg/kg-d, including 200 μg/kg-d from mouthing and 1 μg/kg-d from dermal exposure (Table 10-12). The children's exposure from all consumer exposures was estimated to be 250 μg/kg-d. They estimated consumer exposure to adults to be 10.8 μg/kg-d. Estimated total exposures, including occupational and environmental sources, were 1,200 μg/kg-d for adults, 200 μg/kg-d for children age 3 to 15, and 410 μg/kg-d for infants.

Table 10-12. Estimated total exposures (μg/kg-d) to DINP (ECB 2003) [a]

Scenario	**Adults**	**Children 3-15** 3—15 years	**Infants** [b] 6—36 months	**Newborn** [b] 0—6 months
Consumer	10	10	250	250
Environment [c]	10	10	160	--
Occupational [d]	1,100	--	--	--
Total	**1,120**	**20**	**410**	**ND**

[a] Adapted from ECB 2003, Table 4.17.
[b] Includes exposure from soft plastic toys (ECB 2003, Table 4.13).
[c] Indirect exposure from the environment.
[d] Maximum estimated occupational exposure.

Wormuth et al. (2006) performed a detailed exposure assessment for eight *o*-DAP's, including DINP. Results were presented graphically as the mean, minimum, and maximum plotted on a logarithmic scale. For teenagers and adults, they estimated an average exposure of <0.01 μg/kg-d, with a range from <0.001 μg/kg-d to <1 μg/kg-d. For infants, the average exposure was

estimated to be >10 μg/kg-d, ranging from <0.1 μg/kg-d to <10 μg/kg-d. Toddler and children's exposures were intermediate between adults and infants. The authors noted that the average exposures for infants and toddlers were greater than the maximum exposures for teenagers and adults. For infants, toddlers, and children, mouthing was the primary exposure route, accounting for >90% of total exposure. Ingestion of dust also contributed to toddler and infant exposures. For teens and adults, multiple sources contributed to the estimated total exposure: air ~dust, > aerosol paints, > gloves.

Biomonitoring Studies

Biomonitoring generally provides the most reliable estimates of total exposure to pollutants. However, biomonitoring generally cannot be used to identify sources or routes of exposure. Urinary metabolites have become the preferred analytes for biomonitoring studies of *o*-DAP exposure. Measuring metabolites, rather than the parent compound reduces errors, because *o*-DAP's are ubiquitous contaminants that are found in laboratory equipment and laboratory flooring, for example. Urine is relatively easy to collect and store. Sampling programs, such as the National Health and Nutrition Examination Survey (NHANES) and National Children's Study collect urine samples from defined populations.

Initial biomonitoring studies measured only the monoesters, which are the initial metabolites of the *o*-DAP's. However, the monoesters of the longer-chain *o*-DAP's such as DINP and DEHP undergo further oxidative metabolism (see section 4, Toxicokinetics). Thus, while the monoesters are the major metabolites for lower molecular weight *o*-DAP's, they are minor metabolites of the higher molecular weight *o*-DAP's. Thus, the early studies were limited by low sensitivity. Biomonitoring studies of *o*-DAP's have been reviewed (Fromme et al. 2007; Wittassek and Angerer 2007; Wittassek et al. 2007).

General Population

Blount et al. (2000) measured urinary monoalkyl phthalate levels in 289 adults. Monoesters with the highest levels were monoethyl, monobutyl, and monobenzyl phthalates, which were attributed to exposure to diethyl, dibutyl, and benzyl butyl phthalates (Table 10-13). Women from 20 to 40 years of age had significantly higher levels of monobutyl phthalate, which was thought to result from the use of dibutyl phthalate in cosmetics (Stock et al. 2001). MINP was not detected in most of the population.

Using the data on urinary metabolites by Blount et al. (2000), Kohn et al. (2000) estimated the daily exposure to *o*-DAP's (Table 10-13). They estimated median exposures as high as 8.5 μg/kg-d for diethyl phthalate. The median exposure for DEHP was estimated to be 0.71 μg/kg-d, with DINP below the detection limit. The 95th percentile DINP exposure was estimated to be 1.7 μg/kg-d. The total median exposure to the seven phthalates assayed was 15.1 μg/kg-d.

David (2001) also estimated exposure from the data of Blount et al. The MAFF measured the levels of urinary monoesters in volunteers given known amounts of dialkyl phthalates (Anderson et al. 2000). Using the data of Anderson et al. and Blount et al., David estimated geometric mean exposures of 12.3 μg/kg-d for diethyl phthalate, 0.60 μg/kg-d for DEHP, and 0.21 μg/kg-d

for DINP. The 95th percentile exposure for DINP was estimated to be 1.08 μg/kg-d (Table 10-13). The total geometric mean exposure for the seven phthalates assayed was 15.4 μg/kg-d.

Table 10-13. Urinary MINP levels and estimated DINP exposure

		Median	GM [a]	95 %	Maximum	Reference
Urinary MINP	ng/mL urine	<0.8 [b]	1.5	7.3	79.7	Blount et al. 2000
	μg/g creatinine	<LOD	1.3	6.8	90.3	
DINP intake [c]	μg/kg-d	<LOD	NR	1.7	22	Kohn et al. 2000
		NR	0.21	1.08	14.35	David 2000

[a] 95%, 95th percentile; GM, geometric mean; LOD, limit of detection; NR, not reported.
[b] The detection limit was 0.8 μg/L urine.
[c] Estimated from urinary MINP levels.

The U.S. Centers for Disease Control and Prevention (CDC) measured urinary monoester levels in over 2,500 individuals age 6 and older in 1999-2000 and 2001-2002 (CDC 2005). MINP was detected in few samples. The 95th percentile levels were below the detection limit, which varied from 1.2 to 30.2 μg/L.

Silva et al. (2006b) measured multiple DINP metabolites in the urine of 129 adults in the U.S. Single urine sample from each subject were collected at various times during the day. MINP was not detected in any samples (LOD=0.36 ng/mL). However, the oxidative metabolites CO_2-MINP, OH-MINP, and oxo-MINP were detected at higher frequencies. OH-MINP was detected in all samples. The median concentration of OH-MINP was 13.2 ng/mL, with a 95th percentile of 43.7 ng/mL (Table 10-14). Creatinine-adjusted levels were not reported.

Metabolites of DINP and other *o*-DAP's were measured in morning urine samples over eight days in 27 women and 23 men in Germany (14-60 years old) (Fromme et al. 2007). Median OH-MINP levels were 5.4 and 4.8 μg/g creatinine in females and males, respectively (Table 10-14). Oxo-MINP levels were slightly lower. Levels of the two metabolites were highly correlated (unadjusted values: r=0.97, $p<0.0001$; creatinine-adjusted values: r=0.96, $p<0.0001$). There were no sex or age related differences found in the levels of either metabolite. Substantial intra-individual variations were observed during the 8-day study, although there were no increasing or decreasing trends.

Koch et al. (2007) measured DINP metabolites in 25 German subjects (Koch et al. 2007). Three oxidized DINP—oxo-MINP, OH-MINP, and CO_2-MINP—metabolites were detected in 80 to 96% of samples. Median metabolite levels were 1.3 μg/L oxo-MINP, 2.5 μg/L OH-MINP, and 5.0 μg/L CO_2-MINP (Table 10-14). Details relating to sample collection were not reported.

In another study, urinary metabolites of five *o*-DAP's were measured in 102 subjects between 6 and 80 years old (Wittassek and Angerer 2008). Median metabolite levels were 1.3 μg/L oxo-MINP, 2.0 μg/L OH-MINP, and 4.0 μg/L CO_2-MINP (Table 10-14). The median DINP intake was estimated to be 0.6 μg/kg-d, with a maximum of 36.8 μg/kg-d.

Table 10-14. Distribution of oxidized DINP urinary metabolite levels in the general populations of the U.S. and Germany.[a]

Metabolite		N [b]	Mean	Percentiles				Max	DF	Reference
				10	50	90	95		(%)	
CO_2-MINP		129		2.0	8.4	27.3	46.2		97	Silva et al. 2006b
OH-MINP				2.6	13.2	40.2	43.7		100	United States
Oxo-MINP				<0.25	1.2	5.0	6.6		87	
CO_2-MINP		25	16.4		5.0			260.2	96	Koch et al. 2007
OH-MINP			14.9		2.5			287.1	96	Germany
Oxo-MINP			8.9		1.3			174.1	80	
OH-MINP	M	23	8.6	3.0	5.5	13.0	18.7	49.4		Fromme et al. 2007
			(6.7)	(2.9)	(4.8)	(9.5)	(9.5)	(37.4)		Germany
	F	27	6.0	1.6	5.7	11.1	11.5	12.1		
			(6.4)	(2.8)	(5.4)	(11.5)	(13.2)	(21.0)		
Oxo-MINP	M	23	4.4	1.6	3.0	8.8	9.3	20.3		
			(3.6)	(1.6)	(2.6)	(5.9)	(7.3)	(17.9)		
	F	27	3.7	1.0	3.1	7.3	8.1	8.2		
			(3.8)	(1.5)	(2.8)	(7.1)	(8.9)	(11.4)		
OH-MINP		634			2.0		11.9	85.4		Wittassek et al. 2007
					(1.9)		(11.1)	(131)		Germany
Oxo-MINP					1.0		5.6	63.8		
					(1.0)		(5.4)	(72.7)		
CO_2-MINP		102			4.0					Wittassek & Angerer 2008
OH-MINP					2.0					Germany
Oxo-MINP					1.3					

[a] Values expressed as µg/L or (µg/g creatine).
[b] DF, detection frequency (%); M/F, male/female; Max, maximum; N, number of subjects.

Table 10-15. Levels of oxidized DINP urinary metabolites in German students from 1988 through 2003 (Wittassek et al. 2007) [a]

Year	N	OH-MINP						Oxo-MINP					
		µg/L			µg/g creatinine			µg/L			µg/g creatinine		
		50th	95th	Max	50th	95th	Max	50th	95th	Max	50th	95th	Max
1988	60	1.5	8.8	11.4	1.2	7.6	12.0	0.6	3.5	4.3	0.5	3.9	4.9
1989	60	1.8	14.9	53.4	1.7	11.0	80.9	0.8	7.3	32.5	0.7	6.6	49.2
1991	60	2.2	31.5	85.4	1.6	38.1	131	0.8	3.2	38.4	0.7	3.7	72.7
1993	60	1.8	10.0	12.3	1.8	11.3	13.0	0.8	5.3	9.0	0.8	5.0	9.6
1996	146	2.0	12.0	27.9	1.9	10.6	25.4	1.0	5.6	15.2	1.1	5.2	16.1
1998	68	2.1	47.9	59.3	2.1	43.3	56.9	1.1	26.6	50.6	1.1	29.2	48.9
1999	60	1.9	11.6	17.9	1.9	11.2	17.7	1.0	9.6	11.5	1.1	6.8	12.5
2001	60	2.1	13.9	57.7	2.2	13.8	26.4	1.1	5.7	63.8	1.3	5.6	26.7
2003	60	2.3	13.3	29.0	2.6	7.9	12.6	1.6	10.4	19.1	1.7	5.8	8.3
Total	634	2.0	11.9	85.4	1.9	11.1	131	1.0	5.6	63.8	1.0	5.4	72.7
Males	326	2.1	13.4	59.3	1.8	11.5	40.8	1.0	5.8	50.6	0.8	4.9	34.3
Females	308	1.9	11.5	85.4	2.1	10.3	131	1.0	5.0	63.8	1.2	6.8	72.7

[a] 24-hour urine samples. Median (50th percentile), 95th percentile, and maximum values were reported as µg/L and µg/g creatinine.

Wittassek et al. (2007) measured *o*-DAP metabolites in archived urine samples that were collected over a 15-year period. Subjects were German university students, 20 to 29 years old. At least 60 students (30 per sex) were sampled at 1- to 3-year intervals. Average DINP metabolite levels (Table 10-15) were comparable to those in other studies (compare Table 10-14). However, median DINP metabolite levels roughly doubled between 1988 and 2003, whereas DEHP metabolite levels declined to roughly one-half (Wittassek et al 2007, Tables 4A-4B). The authors estimated daily intake levels, which reflect the changes in metabolite levels (Figure 10-3; Table 10-16). The authors noted that the changes in *o*-DAP exposures over time may reflect changes in *o*-DAP use patterns, including substitution by non-phthalate plasticizers. BBP, DBP, and diisobutyl phthalate metabolites were also studied.

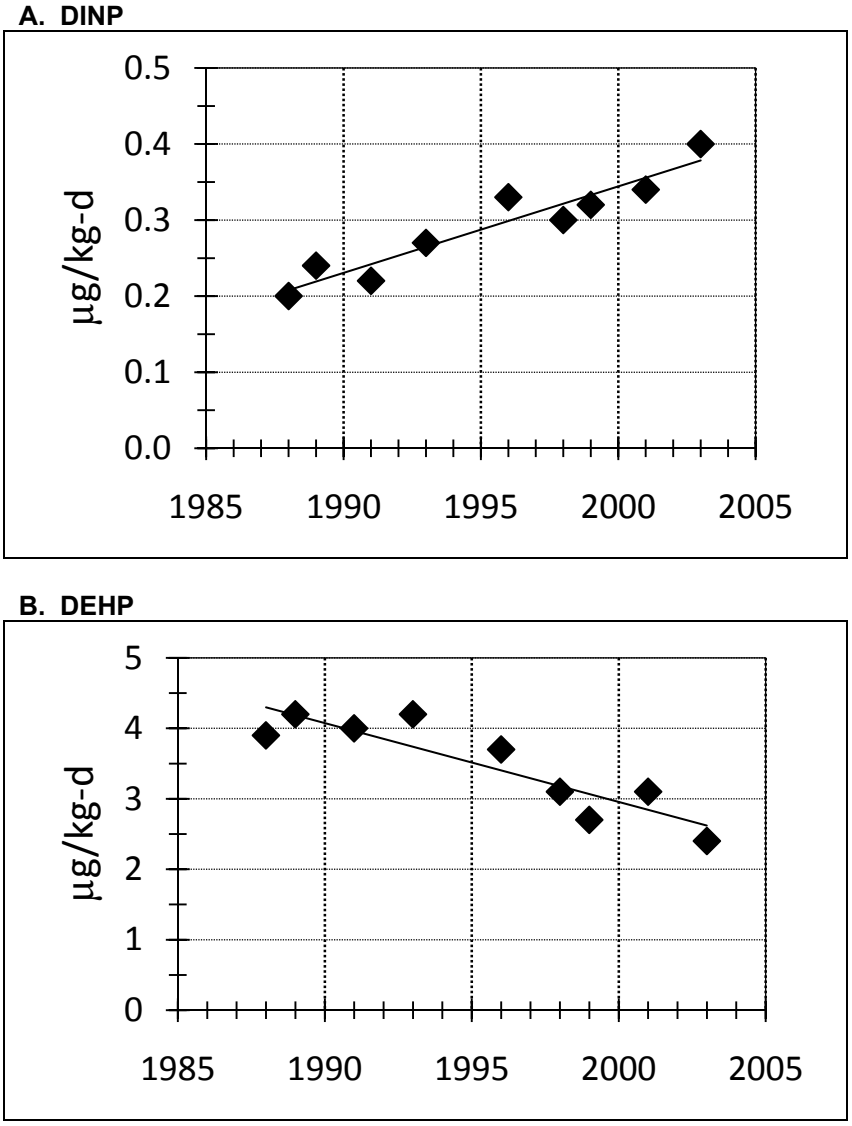

Figure 10-3. Estimated DINP and DEHP intakes in German university students 1988—2003 (Wittassek et al. 2007). (A) DINP. (B) DEHP. Lines are linear trends.

Special Populations

Brock et al. (2002) measured monoester levels in the urine of 19 infants ranging from 11 to 17 months of age. Samples were collected in early 2000. Mean urinary monophthalate levels in the infants were higher than the median levels previously found in adults (Blount et al. 2000). Daily intake levels of dialkyl phthalates were not estimated. As with the adults, the highest urinary levels were for monoethyl and monobutyl phthalates. Levels of MEHP were relatively low. MINP was below the detection limit in all samples.

Two studies measured urinary DINP metabolites in populations of pregnant women. CO_2-MINP was detected in the urine of 84% of subjects (n=19) in Jerusalem (Berman et al. 2009). The median CO_2-MINP level was 3.0 µg/L (3.8 µg/g creatinine), with a geometric mean of 2.3 µg/L (3.1 µg/g) and maximum of 8.0 µg/L (16.2 µg/g). In Rotterdam, OH-MINP was detected in 98% of subjects (n=100), while oxo-MINP was detected in 96% of subjects (Ye et al. 2008). The median OH-MINP level was 2.5 µg/L, with a 95th percentile of 38.3 µg/L. The median CO_2-MINP level was 2.2 µg/L, with a 95th percentile of 30.0 µg/L (Table 10-17).

Human Milk

Calafat et al. (2004b) detected mono-3-methyl-5-dimethylhexyl phthalate (an isomer of MINP), MEHP, and other *o*-DAP metabolites in human milk. The mean level of MINP in three pools was 15.9 ± 7.7 µg/L (total MINP). The mean MEHP level was 7.8 ± 6.8 µg/L. The metabolites in milk were almost entirely unbound, whereas in urine they are largely glucuronidated. The authors suggested that contamination during sample collection might have contributed to the MEHP and MINP levels.

In comparison, the median MINP concentration in human milk was reported to be 101 µg/L (range 27—469 µg/L) in Denmark and 89 µg/L (28—230) in Finland (Main et al. 2006). The median MEHP concentrations were 9.5 µg/L (1.5—191) and 13 µg/L (4.0—1,410) in Denmark and Finland, respectively. The authors estimated that the median MINP intake for infants to be 12.1 µg/kg-d in Denmark and 11.0 µg/kg-d in Finland. The authors suggested that the high levels of MINP in comparison to MEHP and other monoesters may be due to hydrophobicity. DINP is more hydrophobic than DEHP, and DEHP is more hydrophobic than lower molecular weight *o*-DAP's. Thus, MINP should preferentially partition into lipid-rich milk, rather than urine.

In contrast, Högberg et al. (2008) reported that the median concentration of MEHP in human milk in Sweden was only 0.49 µg/L (0.49—6.5). Thus, the average MEHP level varied widely among three studies (Calafat et al. 2004b; Högberg et al. 2008; Main et al. 2006), and the MINP level varied widely among two studies. Högberg et al. did not measure MINP. It is not clear whether these differences are due to real differences in exposure in various countries, or other reasons. Högberg et al. suggested that the relatively high levels reported by Main et al. could be due to contamination during sample collection and storage, or to an error in units.

Table 10-16. Estimates of DINP intake (µg/kg-d) based on urinary metabolites

Population	N [a]	Median	GM	95 %	Max	Reference
U.S. general population [b]	289	<LOD	--	1.7	22	Kohn et al. 2000
U.S. general population [b]	289	--	0.21	1.08	14.35	David 2000
German students (1988—2003) [c]	632	0.29	--	1.7	20.2	Wittassek et al. 2007
Germany, age range 6 to 80	102	0.6	--	--	36.8	Wittassek & Angerer 2008

[a] GM, geometric mean; Max, maximum; N, number of subjects; 95%, 95th percentile.
[b] Based on MINP data from Blount et al. (2000).
[c] Downward trend from 1988 to 2003.

Table 10-17. Distribution of oxidized DINP urinary metabolite levels in special populations [a]

Population	Metabolite	N [a]	Median	GM	95 %	Max	DF %	Reference
Infants, U.S. [b]	MINP	19	<9.0	<9.0	<9.0	<9.0	0	Brock et al. 2002
Pregnant women, Jerusalem	CO_2-MINP	19	3.0 (3.8)	2.3 (3.1)	-- --	8.0 (16.2)	84	Berman et al. 2009
Pregnant women, Rotterdam	OH-MINP	100	2.5	3.0	38.3	122.0	98	Ye et al. 2008
	Oxo-MINP		2.2	2.5	30.0	152.0	96	

[a] Values expressed as µg/L or (µg/g creatine).
[b] DF, detection frequency (%);Max, maximum; N, number of subjects.

Summary of Exposure

DINP is present in a variety of PVC products including including vinyl flooring, wire and cable insulation, stationery, coated fabrics, gloves, toys, tubing, garden hoses, artificial leather, footwear, automobile undercoating, and roofing. DINP has supplanted DEHP as the primary platicizer in the U.S. The use of DINP in toys has declined following its voluntary removal from teethers in 1999 and the enactment of the Consumer Product Safety Improvement Act of 2008.

Early scenario-based exposure assessments focused on the mouthing of teethers and soft plastic toys by children age 3 or younger. Various governmental agencies estimated average children's exposures from <1 to 120 μg/kg-d (Table 10-10). The CPSC staff estimated mean exposures between 0.03 and 2.9 μg/kg-d for various scenarios, based on *in vitro* migration data and an observational study of children's mouthing behavior (Babich 2002; Babich et al. 2004).

The European Chemicals Bureau estimated an average non-occupational exposure of 20 μg/kg-d for adults and children age 3-15 years (ECB 2003). Wormuth et al. (2006) estimated the average exposure to adults and teenagers as <0.01 μg/kg-d, with a range from <0.001 to <1 μg/kg-d.

Biomonitoring data are somewhat limited for DINP because not all investigators measured DINP metabolites and early studies did not measure the most abundant metabolites. In German, general population exposure to DINP is estimated to average <1 μg/kg-d, with a maximum of 36.8 μg/kg-d (Wittassek and Angerer 2008). DINP exposures in pregnant women appeared to be somewhat greater (Table 10-17). Additional biomonitoring data for the U.S. general population, children under age 6, and pregnant women are needed.

In one study of German university students, the average *o*-DAP exposure declined in the order: DBP > DEHP > DIBP > DINP > BBP (Wittassek et al. 2007). In the same study, DINP exposures appeared to increase over time, while DEHP exposure decreased. This trend might be due to the gradual replacement of DEHP with DINP.

11. Discussion

Multiple Forms of DINP

DINP is a complex substance comprised of multiple isomers. In addition, DINP is currently available in two forms (DINP-1 and DINP-2) manufactured by different processes. Other forms are either no longer or were never produced commercially. The two forms are considered commercially interchangeable, that is, they can be used interchangeably in products. The composition of each type is considered to be stable from batch-to-batch, although minor variations may occur. Some manufacturers add small amounts of DIDP or other plasticizers to their products.

Only a few studies have directly compared the toxicities of different DINP types. Hellwig et al. (1997) reported minor differences in the ability of DINP-1 and DINP-2 to induce malformations in offspring following prenatal exposure. There were also minor differences in the prenatal effects of the precursor alcohols (Hellwig and Jackh 1997), although the alcohols were much less potent than the phthalate esters. Smith et al. (2000) compared DINP-1 and DINP-A (never produced commercially) in a number of assays in rat and mouse liver. DINP-1 and DINP-A were essentially similar in their ability to induce peroxisomal beta oxidation in either rat or mouse. However, there were minor differences with regard to induction of hepatomegaly, increased hepatic labeling index, and inhibition of gap junction intercellular communication. DINP-A had a lower NOEL for the induction of liver histopathology in rats following chronic exposure, as compared to DINP-1. However, DINP-A was tested in SD rats (Bio/dynamics 1986), whereas DINP-1 was tested in F344 rats (Lington et al. 1997; Moore 1998a). Overall, any differences in toxicology between DINP-1 and DINP-2 appear to be relatively minor. However, additional comparative studies may be helpful. No two-year biassays with DINP-2 have been reported.

Availability of Toxicity Data and Data Gaps

A considerable amount of data on DINP are available (Table 11-1). DINP-1 has been tested in two-year bioassays in rats (Lington et al. 1997; Moore 1998a) and mice (Moore 1998b). No two-year bioassays of DINP-2 are available. DINP-1 has been subjected to a two-generation study of reproductive toxicity (Waterman et al. 2000). Both DINP-1 and DINP-2 have been tested in standard prenatal developmental toxicity screens (Hellwig et al. 1997; Waterman et al. 1999). Genotoxicity has been well-studied. Numerous mechanistic studies relating to carcinogenesis are also available. DINP-1 has been tested in perinatal developmental studies, although additional dose response data would be informative (Gray et al. 2000; Ostby et al. 2000, 2001). Except for developmental studies, there are few data on the relative susceptibility of immature animals to DINP.

DINP has not been tested in neurotoxicity or developmental neurotoxicity assays. However, there is little evidence to suggest that DINP affects the nervous system. Toxicity data in humans are quite limited, and the available studies are confounded by exposure to multiple phthalates. Biomonitoring data in humans age 6 years and over are available. However, much of these data are limited in that the more abundant metabolites were not measured in the earlier studies.

Biomonitoring data in children under 6 years old are much less abundant, and are subject to the same limitations as the adult data. Overall, there are no major data gaps that would prevent conducting an informative hazard and risk assessment.

One form of DINP (DINP-A) that was not commercialized produced chronic liver toxicity (necrosis) in rats at lower doses (Bio/dynamics 1986) than commercial DINP-1 (Moore 1998a). Although it is not clear, DINP-A may be similar to DINP-2 in isomeric composition (Harmon 2000). It would be helpful to elucidate the identity of DINP-A. However, there are issues with the DINP-A study (discussed in the previous section).

PPARα

Many, but not all, of the toxicological effects of DINP in animals are probably mediated by the nuclear receptor PPARα. DINP also activates PPARγ, although of the significance of PPARγ activation is largely unknown (Peraza et al. 2006). The roles of PPARα, β, and γ in toxicity are reviewed in Peraza et al. (2006).

There is a considerable amount of data suggesting that PPARα activation is required for the pleiotropic effects in rodent liver that include hepatomegaly, increased cell proliferation, peroxisome proliferation, and hepatocellular neoplasms (Ashby et al. 1994; CPSC 2001; Klaunig et al. 2003). The principle evidence for the role of PPARα is that these effects are not observed in PPARα-null mice (Hays et al. 2005; Lee et al. 1995; Peters et al. 1997a, b; Ward et al. 1998). Recently, however, a PPARα-independent carcinogenic mode of action has been proposed for DEHP (Ito et al. 2007).

Other toxic effects were reduced in severity or delayed in appearance in PPARα-null mice, including effects in the kidney and testes (Ward et al. 1998).

In contrast, DEHP induced malformations in both PPARα-null and wild-type mice, following pre-natal exposure (Peters et al. 1997c). The role of PPARα in causing the malformations following perinatal exposure, which results in the "phthalate syndrome," has not been studied with the PPARα-null model. However, structure activity relationships suggest that these effects are also independent of PPARα.

Some liver effects, principally spongiosis hepatis, may be independent of PPARα. Spongiosis hepatis is a degenerative lesion found in rats and medaka. Increased incidence of spongiosis hepatis has been reported in rats chronically exposed to a number of compounds, including both genotoxic and non-genotoxic carcinogens, non-carcinogens, PPARα agonists, and non- PPARα agonists. Thus, the structure activity relationships suggest that spongiosis hepatis occurs independently of peroxisome proliferation.

Table 11-1. Availability of toxicity data on DINP

Study Type	Available Studies	Reference
Acute	Oral, rat	ECB 2003; HSDB 2009; NICNAS 2008
	Inhalation (aerosol), rat, mouse, guinea pig	
	Dermal, rabbit	
Dermal	Skin & eye irritation, rabbit	ECB 2003; HSDB 2009; NICNAS 2008
	Sensitization, guinea pig	
	Sensitization, irritation, human	Medeiros et al. 1999
Toxicokinetics	Rat	Hazleton 1972; El-hawari et al. 1983, 1985; Silva et al. 2006a; Smith et al. 2000
	Rat, percutaneous	Stoltz and El-hawari 1983; Stoltz et al. 1985
	Mouse	Smith et al. 2000
	Human	Fromme et al. 2007; Koch and Angerer 2007; Koch et al. 2007; Silva et al. 2006a
Genetic Toxicity	Salmonella, mutagenicity	McKee et al. 2000; Zeiger et al. 1985
	Rat, mouse cells, *in vitro*, mutagenicity	Barber et al. 2000;
	Phenotypic transformation, *in vitro*	Barber et al. 2000
	Chromosome aberrations, *in vitro* and *in vivo*	McKee et al. 2000
Subchronic	Mouse (feed)	Bankston 1992; Moore 2000
	Rat (feed)	Bird et al. 1996; Myers 1991
	Marmoset (gavage)	Hall et al. 1999
Chronic	Mouse (feed)	Moore 1998b
	Rat (feed)	Biodynamics 1987; Lington et al. 1997; Moore 1998a
Developmental	Prenatal, rat	Hellwig et al. 1997; Waterman et al. 1999
	Perinatal, rat	Borch et al. 2003, 2004; Gray et al. 2000; Hass et al. 2003; Ostby et al. 2000, 2001
Reproductive	2-Generation, rat	Waterman et al. 2000
Neurotoxicity	NA [a]	
Human	Lactational exposure and hormone levels in male infants	Main et al. 2006

[a] NA, not available.

Toxic Effects of DINP

The first step in the risk assessment process is hazard identification, that is, to review the available toxicity data for each chemical under consideration and determine whether the chemical is "toxic" under the FHSA. Acute toxicity is defined by LD_{50} (dose at 50 percent lethality) values in regulations issued under the FHSA. 16 CFR 1500.3 (c) (2) (i). However, reliable human experience data take precedence over animal data. 16 CFR 1500.4. In 1992, the Commission issued guidelines for assessing chronic hazards under the Federal Hazardous Substances Act (FHSA), including carcinogenicity, neurotoxicity, reproductive and developmental toxicity, exposure, bioavailability, risk assessment, and acceptable risk (CPSC, 1992; summarized at 16 CFR 1500.135).

The guidelines include criteria for evaluating the weight of the evidence for chronic endpoints. Based on a review of all of the available evidence, a substance is considered "toxic" under the FHSA due to chronic toxicity, if it is either known to be, or probably, toxic in humans. 16 CFR 1500.3 (c)(2)(ii). Under the FHSA, a substance or mixture is classified as "known to be toxic" in humans only if there is sufficient evidence in humans. It is considered "probably toxic" if there is either limited evidence in humans or sufficient evidence in animals (Table 11-2). These criteria may be applied independently to each chronic endpoint.

Table 11-2. Classification of chronic hazards under the FHSA

	Human studies	Animal studies
Sufficient evidence	Known *	Probable *
Limited evidence	Probable *	Possible
Inadequate evidence	Possible	---

* Considered "toxic" under the FHSA.

DINP does not satisfy the FHSA definition of acutely toxic. Several end points may be considered with respect to chronic toxicity.

Liver

There is sufficient evidence of chronic toxicity in the liver. Effects in the liver, including effects attributed to peroxisome proliferation (hepatomegaly, increased cell number and size) were observed in rats and mice. In addition, degenerative lesions including spongiosis hepatis and necrosis were observed in both sexes of rats in three chronic bioassays (Bio/dynamics 1987; Lington et al. 1997; Moore 1998a). These degenerative effects are not associated with PPARα activation (see above). The CPSC staff concludes that there is sufficient evidence of chronic liver toxicity in animals. Therefore, DINP is considered to be "probably toxic in humans" (Table 11-3). The CPSC staff has regarded chronic liver toxicity as the "critical endpoint" in assessing the chronic effects on DINP (Babich 2002; CPSC 1998; CPSC 2001; Lee 1997). The CHAP (CPSC 2001) derived an ADI of 120 µg/kg-d, based on the incidence of spongiosis hepatis in male rats (Lington et al. 1997).

Kidney

Chronic kidney toxicity was observed in two-year bioassays in rats (Biodynamics 1987; Lington et al. 1997; Moore 1998a) and mice (Moore 1998b). Some effects such as increased kidney weight, focal tubular degeneration, and cystic tubules may be due, in part, to PPARα activation. The appearance of kidney lesions was delayed in PPARα-null mice, but they appeared by 24 weeks and were essentially similar to the lesions in wild-type mice (Ward et al. 1998). The CPSC staff concludes that there is sufficient evidence of chronic kidney toxicity in animals and, therefore, that DINP is "probably toxic" in humans with respect to chronic kidney toxicity. The staff derived an ADI of 0.88 mg/kg-d (880 μg/kg-d), which is greater (i.e., less potent) than the ADI for liver toxicity (Table 11-3).

Reproductive and Developmental Toxicity

The reproductive toxicity of DINP was studied in a two-generation assay in rats (Waterman et al. 2000). There was inadequate evidence of effects on reproduction in this study.

The developmental effects of DINP have been assessed in multiple studies in rats. There is sufficient evidence of developmental effects of DINP following either prenatal (Hellwig et al. 1997; Waterman et al. 1999) or perinatal (Borch et al. 2003, 2004; Gray et al. 2000; Hass et al. 2003; Ostby et al. 2000, 2001) exposure. These effects are believed to be independent of PPARα activation. The CPSC staff concludes that DINP is "probably toxic" to humans, based on sufficient evidence in multiple studies in rats. The CPSC staff derived an ADI of 1.2 mg/kg-d. The developmental toxicity of DINP is less potent as compared to chronic liver toxicity.

Endocrine Effects

Certain of the developmental effects in animals, specifically effects on male sexual development, are believed to be due largely to the inhibition of testosterone synthesis (Gray et al. 2000; Parks et al. 2000). The effects are discussed under Reproductive and Developmental Toxicity (see above). DINP exposure also led to reduced testicular weights following chronic or subchronic exposure in mice and rats. These antiandrogenic effects of DINP on testosterone synthesis are not due to direct interaction of DINP with the androgen receptor. There is no evidence that DINP binds significantly to the estrogen receptor (Harris et al. 1997; Zacharewski et al. 1998).

One study in humans suggests that lactational exposure to DINP metabolites in male humans may be associated with increases in levels of luteinizing hormone and decreased testosterone (Main et al. 2006). This study is confounded by exposure to multiple phthalates. The CPSC staff concludes that there is inadequate evidence of endocrine effects in humans.

Apart from certain developmental effects, there are few studies on the possible endocrine effects of DINP. There were certain testicular effects in mice and rats, including reduce testicular weight and abnormal sperm (Bankston 1992; Moore 1998a, b; Moore 2000; Myers 1991). It is not clear whether the testicular effects are caused by, or are a cause of, endocrine effects in the animal. A Herschberger assay in rats suggests that DINP may cause antiandrogenic effects in

developing males (Lee and Koo 2007). The CPSC staff concludes that there is limited evidence of endocrine effects in animals.

Overall, DINP is considered "possibly toxic" in humans with regard to endocrine effects, based on inadequate evidence in humans and limited evidence in animals. "Possibly toxic" means that the evidence does not satisfy the regulatory definition of "toxic" with regard to endocrine effects. The CPSC staff will not consider endocrine effects in risk assessment or risk reduction activities. However, the staff will continue to monitor new information as it becomes available.

Table 11-3. Chronic health effects of DINP

Endpoint	WOE [a]	Evidence [b]	ADI [c] (mg/kg-d)	Key reference
Liver histopathology	Probable [d]	Sufficient, animals	0.12 [e]	Lington et al. 1997
Kidney histopathology	Probable [d]	Sufficient, animals	0.88	Moore 1998b
Reproductive toxicity	NA	Inadequate, animals	6.6 [f]	Waterman et al. 2000
Developmental toxicity	Probable [d]	Sufficient, animals	1.0	Masutomi et al. 2003
Endocrine effects	Possible	Inadequate, humans	ND	Main et al. 2006
Immunogenicity	NA	Inadequate, animals	ND	ECB 2003
Hepatocelluar tumor	Possible	Limited, animals	ND	Lington et al. 1997; Moore 1998a, b
Renal tubular cell carcinoma	Possible	Limited, animals	ND	Moore 1998a
Mononuclear cell leukemia	Possible	Limited, animals	ND	Lington et al. 1997; Moore 1998a

[a] WOE, weight of the evidence classification based on the CPSC chronic hazard guidelines (CPSC 1992). See text and Table 11-2 for explanation.
[b] Evidence means the evidence on which the WOE is based. For example, "sufficient, animals" means "sufficient evidence in animal studies." See text for explanation.
[c] ADI, acceptable daily intake (mg/kg-d); NA, not applicable, that is, not classifiable (Table 11-2); ND, not determined.
[d] Satisfies the regulatory definition of "toxic."
[e] Liver is the critical endpoint used for most risk assessments. Additional endpoint-specific ADI's were estimated to support cumulative risk assessments.
[f] This is based on the lower bound of reproductive effects. Normally, the CPSC staff would not derive an ADI unless the WOE for that endpoint is "probable" or "known."

Immunotoxicity

It has been proposed that residential exposure to phthalates may contribute to allergic asthma, rhinitis, or dermatitis symptoms. However there was no association between the presence of DINP in house dust and the incidence of atopic disease in one study in humans (Bornehag et al. 2004). DINP was not a dermal irritant or sensitizer in humans. Animal studies have given mixed results regarding the ability of DINP to be a sensitizer or an adjuvant.

Overall, there is inadequate evidence that DINP affects the immune response in animals, and no evidence in humans.

Carcinogenicity

DINP is associated with hepatocellular adenoma and carcinoma in rats and mice, kidney tubular cell carcinoma in male rats, and mononuclear cell leukemia in rats. DINP is not genotoxic. While it is clear that DINP induces hepatocellular tumors, these tumors are believed to arise through a PPARα-dependent mode of action (Babich 2002; CPSC 2001; Klaunig et al. 2003). This conclusion is supported by a plethora of data, especially studies in PPARα-null mice (Hays et al. 2005; Peters et al. 1997b). However, activation of human PPARα does not lead to hepatocellular proliferation or tumorigenesis (Morimura et al. 2006). The CHAP and the CPSC staff did not derive a unit risk (slope factor) for DINP and did not consider liver carcinogenicity in assessing the chronic health effects of DINP (Babich 2002; CPSC 2001). Recently, the possibility of a PPARα-independent mode of action for the induction of liver tumors has been proposed for DEHP (Ito et al. 2007). However, it appears that the PPARα-independent MOA does not occur in whild-type (i.e., PPARα-expressing) mice. Additional investigation is needed to determine the significance of this result with DEHP. Considering all of the evidence, the CPSC staff concludes that DINP induces hepatocellular tumors in mice and rats. However, this occurs by a mode of action that is not likely to occur in humans. Therefore, the animal evidence for the carcinogenicity of DINP, based on hepatocellular tumors, is downgraded from sufficient to limited.

DINP induced a small number of renal tubular cell carcinomas at the high dose (2/65) in male F344 rats (Moore 1998a). There is experimental evidence sufficient to conclude that these tumors are due to α2u-globulin, which is unique to male rats (Caldwell et al. 1999). The CPSC staff concludes that there is "limited evidence of carcinogenicity" in animals, based on renal tubular carcinoma, due to the low incidence of tumor and that the tumors occur through a mode of action that is considered not to be relevant to humans.

DINP was also associated with increased incidence of mononuclear cell leukemia (MNCL) in male and female F344 rats (Lington et al. 1997; Moore 1998a), but not in SD rats (Bio/dynamics 1987). MNCL has a high background incidence in F344 rats and elevated incidence of MNCL is a common finding in bioassays. Therefore, the CPSC staff concludes that the increased incidence of MNCL provides "limited evidence of carcinogenicity" in animals.

Overall the CPSC staff concludes that DINP is "possibly carcinogenic" in humans, based on "limited evidence" of carcinogenicity in animals. All three tumor types associated with DINP—heaptocellular adenoma and carcinoma, renal tubular cell carcinoma, and mononuclear cell leukemia—occur by modes of action that are not likely to occur in humans. The conclusion that DINP is possibly carcinogenic in humans means that the evidence does not satisfy the regulatory definition of "toxic" with regard to carcinogenicity. The CPSC staff will not consider carcinogenicity in risk assessment or risk reduction activities. However, the staff will continue to monitor new information as it becomes available.

Exposure and Risk

Several governmental agencies have assessed the potential human health risks from exposure to DINP. The CHAP on DINP concluded that the risk to children from mouthing teethers and toys containing DINP was "minimal to non-existent" (CPSC 2001). The CPSC staff concluded that exposure to DINP from mouthing teethers and soft plastic toys did not present a hazard to children (Babich 2002; CPSC 2002).

The CERHR (2003) concluded that there was "minimal risk" of developmental or reproductive effects from current exposure levels. The European Chemicals Bureau performed a risk assessment of total DINP exposure from consumer, environmental, and occupational sources. They concluded that "there is at present no need for further information or testing or risk reduction measures beyond those which are being applied already."

However, a considerable amount of new data has emerged since the previous risk assessments were completed. These include studies on the cumulative risks of exposure to multiple phthalates in rats (e.g., Howdeshell et al. 2008) and biomonitoring data (e.g., Silva et al. 2006b). Even if the conclusions reached in the risk assessments performed to date are still valid for DINP exposure alone, none of these risk assessments considered the cumulative effects of exposure to multiple phthalates or other antiandrogens. It has become clear that the cumulative effects of phthalates must be considered (NRC 2008). The information compiled in this report will contribute to a cumulative risk assessment of exposure to multiple phthalates to be performed by the Chronic Hazard Advisory Panel on phthalates.

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