Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP)

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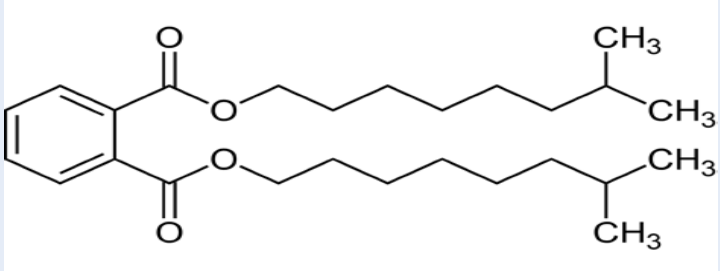
Overview

- DINP: production, use and exposure
- Carcinogenicity studies in animals
- Other relevant data
  - Pharmacokinetics and metabolism
  - Genotoxicity and other mechanistic data
  - Structure activity relationships
- Possible mechanisms of action
- Reviews by authoritative bodies
- Summary of evidence

Diisononyl Phthalate (DINP)



- DINP is produced by multiple processes
- Multiple Chemical Abstract Service Registry (CAS) numbers
- Isomeric mixture consisting of a branched alkyl diester of either eight, nine or ten carbons with the bulk of the mixture (about 70 %) containing nine carbons
- DINP produced by different processes considered commercially interchangeable

DINP Uses

- General purpose plasticizer used in a variety of products
- Highest production volume among the 10 individual phthalates
- Use in toys and child care articles restricted in CA since 2009 under AB 1108

Biomonitoring/Exposure

- Detected in household products, household dust, and food
- Detected in the environment in water, plants, and fish
- Several biomonitoring studies have measured DINP in various populations
  - Metabolites detected in all age groups in a high percentage of samples
  - Higher exposures for workers using DINP-based products

Carcinogenicity Studies

- Human: None
- Animal:
  - Six dietary studies in Fischer 344 rats
  - Two dietary studies in Sprague-Dawley rats
  - Four dietary studies in $B6C3F_1$ mice

Tumor incidence in male Fischer 344 rats. 2-year feeding study (Lington *et al*., 1997)

Organ	Tumor	DINP dietary concentrations (ppm)				Trend test p-value
		0	300	3000	6000	
Male Rats						
Liver	Neoplastic nodules[1]	3/81	1/80	1/80	1/80	NS
	Hepatocellular carcinoma	0/81	0/81	0/80	3/80	p=0.015
	Combined	3/81	1/80	1/80	4/80	NS
Kidney	Transitional cell carcinoma	0/81	0/80	3/80[2]	0/80	NS
	Tubular cell carcinoma	0/80	1/81	0/80	2/80[3]	NS
Spleen	Mononuclear cell leukemia	33/81	28/80	48/80**	51/80**	p<0.01

[1]Since 1986, NTP refers to these tumors as "hepatocellular adenomas".
[2] Renal transitional cell carcinoma historical control incidence : 1/1352, Haseman *et al.*, 1998.
[3] Renal tubular cell carcinoma historical control incidence : 3/1352, Haseman *et al.*, 1998.
Fisher pairwise comparison with control: ** p<0.01; *** p<0.001; NS: not significant

Tumor incidence in female Fischer 344 rats. 2-year feeding study (Lington *et al*., 1997)

Organ	Tumor	DINP dietary concentrations (ppm)				Trend test p-value
		0	300	3000	6000	
Female rats						
Liver	Neoplastic nodules	0/81	2/81	0/81	1/81	ND
	Hepatocellular carcinoma	1/81	0/81	0/80	1/80	ND
	Combined	1/81	2/81	0/80	2/80	NS
Spleen	Mononuclear cell leukemia	22/81	20/81	30/80	43/80***	p<0.001

Fisher pairwise comparison with control; ** p<0.01; *** p<0.001; NS: not significant; ND: not done

Tumor incidence in male Fischer 344 Rats. 2-year feeding study (Moore, 1998a)

Organ	Tumor	DINP dietary concentration (ppm)					Trend test p-value
		0	500	1500	6000	12,000	
Male rats							
Liver	Hepatocellular adenoma	4/65	4/50	2/50	6/65	10/65	$p < 0.05$
	Hepatocellular carcinoma	1/65	0/50	0/50	1/65	12/65***	$p < 0.001$
	Hepatocellular carcinoma or adenoma	5/65	4/50	2/50	7/65	17/65***	$p < 0.001$
Kidney	Transitional cell carcinoma	0/65	0/55	0/55	1/65[1]	0/65	NS
	Renal tubular carcinoma	0/65	0/55	0/55	0/65	2/65[2]	NS
Spleen	Mononuclear cell leukemia	22/65	23/55	21/55	32/65*	30/65*	$p < 0.01$

[1] Renal transitional cell carcinoma historical control incidence : 1/1352, Haseman *et al.*, 1998.

[2] Renal tubular cell carcinoma historical control incidence : 3/1352, Haseman *et al.*, 1998.

Fisher pairwise comparison with control:* $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS: not significant

Tumor incidence in female Fischer 344 rats. 2-year feeding study (Moore, 1998a)

Organ	Tumor	DINP dietary concentration (ppm)					Trend test p-value
		0	500	1500	6000	12,000	
Female rats							
Liver	Hepatocellular adenoma	0/65	1/50	0/50	1/65	3/65	$p < 0.05$
	Hepatocellular Carcinoma	1/65	0/50	0/50	1/65	5/65	$p < 0.01$
	Hepatocellular carcinoma or adenoma	1/65	1/50	0/50	2/65	8/65*	$p < 0.001$
Spleen	Mononuclear cell leukemia[1]	17/65	16/49	9/50	29/65*	30/65*	$p < 0.001$

[1] p-Values associated with mononuclear cell leukemia are based on life table analysis, in which tumors in animals that die prior to terminal sacrifice are regarded as being (directly or indirectly) the cause of death.
Fisher pairwise comparison with control: * $p < 0.05$

Tumor incidence in male Fischer 344 rats. 78-week DINP feeding, 26-week recovery period (Moore, 1998a)

Organ	Tumors	DINP Dietary Concentration (ppm)	
		0	Recovery group 12,000 ppm
Male rats			
Liver	Hepatocellular carcinoma	1/65	2/50
	Hepatocellular adenoma or carcinoma	5/65	7/50
Kidney	Renal tubular carcinoma	0/65	4/50*[1]
Spleen	Mononuclear cell leukemia	22/65	31/50***

[1] Renal tubular cell carcinoma historical control incidence : 3/1352, Haseman *et al.*, 1998.
Fisher pairwise comparison with control: * $p<0.05$; ** $p<0.01$; *** $p<0.001$

Tumor incidence in female Fischer 344 rats. 78-week DINP feeding, 26-week recovery period (Moore, 1998a)

Organ	Tumors	DINP Dietary Concentration (ppm)	
		0	Recovery group 12,000 ppm
Female rats			
Liver	Hepatocellular carcinoma	1/65	1/50
	Hepatocellular adenoma or carcinoma	1/65	2/50
Spleen	Mononuclear cell leukemia	17/65	24/50**

Fisher pairwise comparison with control: * $p<0.05$; ** $p<0.01$

Tumor incidence in male Sprague-Dawley rats. 2-year feeding study (Bio\dynamics, 1986)

Organ	Tumor	DINP concentrations (ppm)				Trend test p-value
		0	500	5000	10,000	
Male Rats						
Liver	Hepatocellular neoplastic nodules	2/70	5/69	6/69	5/70	NS
	Hepatocellular carcinoma	2/70	2/69	6/69	4/70	NS
Testis	Interstitial cell carcinoma	2/59	-[1]	-[1]	7/60	-
Pancreas	Islet cell carcinoma	1/70	-[1]	-[1]	4/70[2]	-

[1] No incidence data available.
[2] Islet cell carcinoma historical control incidence : 1/1340, Chandra *et al.*, 1992.

Tumor incidence in female Sprague-Dawley rats. 2-year feeding study (Bio\dynamics, 1986)

Organ	Tumor	DINP concentrations (ppm)				Trend test p-value
		0	500	5000	10,000	
Female rats						
Liver	Neoplastic nodules	1/70	1/70	5/70	2/70	NS
	Hepatocellular carcinoma	0/70	0/70	5/70*	7/70**	p<0.001
Uterus	Endometrial adenocarcinoma	0/70	-[1]	-[1]	2/69[2]	-

[1] No incidence data available.
[2] Endometrial adenocarcinoma historical control incidence: 1/1329, Chandra *et al*., 1992.
Fisher pairwise comparison with control; * p<0.05; ** p<0.01.

Tumor incidence in male $B6C3F_1$ mice. 2-year feeding study (Moore, 1998b)

Organ	Tumor	DINP dietary concentrations (ppm)					Trend test p-value
		0	500	1500	4000	8000	
Male mice							
Liver	Hepatocellular adenoma	10/70	7/67	8/66	15/65	13/70	p<0.05
	Hepatocellular carcinoma	10/70	8/67	10/66	17/65	20/70**	p<0.001
	Hepatocellular adenoma or carcinoma	16/70	13/67	18/66	28/65**	31/70**	p<0.001

p-Values associated with pairwise comparison are based on logistic regression (CPSC, 2001); * p<0.05, ** p<0.01, *** p<0.001

Tumor incidence in female $B6C3F_1$ mice. 2-year feeding study (Moore, 1998b)

Organ	Tumor	DINP dietary concentrations (ppm)					Trend test p-value
		0	500	1500	4000	8000	
Female mice							
Liver	Hepatocellular adenoma	2/70	4/68	5/68	4/67	18/70***	p<0.001
	Hepatocellular carcinoma	1/70	2/68	5/68	7/67*	19/70***	p<0.001
	Hepatocellular adenoma or carcinoma	3/70	5/68	10/68*	11/67**	33/70***	p<0.001
Pancreas	Islet cell carcinoma	0/70	-	-	-	2/70	-

[1] Pancreatic islet cell carcinoma historical control incidence : 3/1347, Haseman *et al.*, 1998.

p-values associated with pairwise comparison are based on logistic regression (CPSC, 2001); * p<0.05; ** p<0.01; *** p<0.001

Tumor incidence in male $B6C3F_1$ mice. 78-week DINP feeding, 26-week recovery period (Moore, 1998b)

Organ	Tumors	DINP Dietary concentrations (ppm)	
		0	Recovery group 8000 ppm
Male Mice			
Liver	Hepatocellular adenoma	10/70	8/50
	Hepatocellular carcinoma	10/70	12/50
	Hepatocellular adenoma or carcinoma	16/70	19/50***

Fisher pairwise comparison with control; ** p<0.01; *** p<0.001

Tumor incidence in female $B6C3F_1$ mice. 78-week DINP feeding, 26-week recovery period (Moore, 1998b)

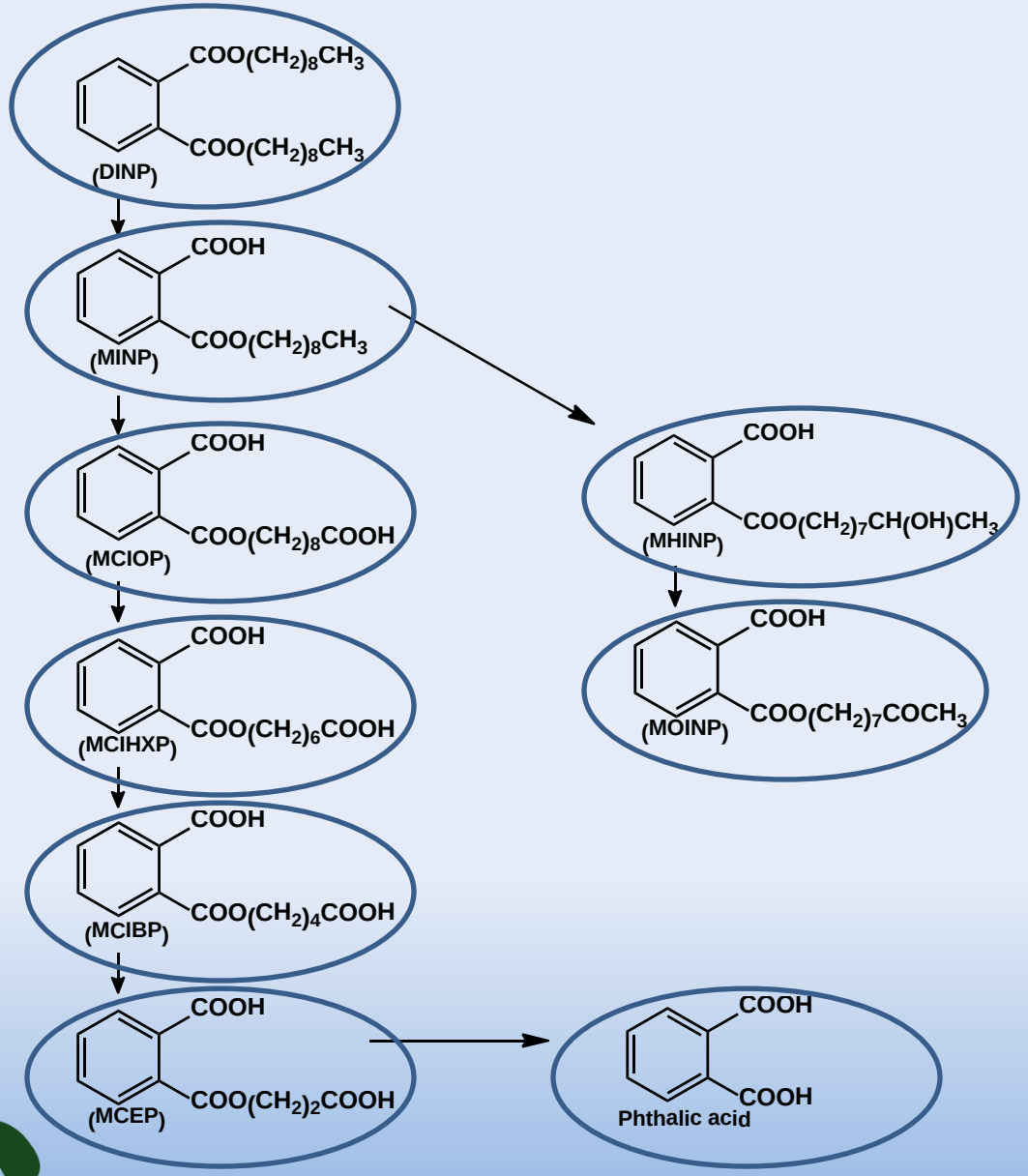
Organ	Tumors	DINP Dietary concentrations (ppm)	
		0	Recovery group 8000 ppm
Female Mice			
Liver	Hepatocellular adenoma	2/70	8/50
	Hepatocellular carcinoma	1/70	13/50**
	Hepatocellular adenoma or carcinoma	3/70	18/50**

Fisher pairwise comparison with control; ** p<0.01; *** <0.001

Pharmacokinetics and Metabolism in Humans

- In single oral dose studies multiple metabolites were observed
- More than ninety percent of the metabolites were excreted in the first 24 hours
- Biphasic elimination pattern
- Elimination half-life was 3-5 hours in the first phase and 12-18 hours in the second phase
- Essentially similar pharmacokinetics and metabolism in animals

Proposed metabolism of DINP



DINP:	Diisononyl phthalate
MINP:	Monoisononyl phthalate
MCIOP:	Monocarboxy isooctyl phthalate
MHINP:	Monohydroxy isononyl phthalate
MCIHXP:	Monocarboxy isohexyl phthalate
MOINP:	Monooxoisononyl phthalate
MCIBP:	Monocarboxy isobutyl phthalate
MCEP:	Monocarboxyethyl phthalate

(adapted from Saravanabhavan and Murray, 2012)

Genotoxicity

Genotoxicity Assay	Metabolic activation	
	With	Without
Reverse gene mutation in *Salmonella typhimurium* (multiple test strains)	-	-
Forward mutation in L5178Y TK +/- in mouse lymphoma cells	-	-
Chromosomal aberrations in CHO cells	-	-
Unscheduled DNA synthesis in primary rat hepatocytes	-	
In vivo micronucleus assay in rats and mice	-	

In vitro cell transformation

- DINP has been tested in seven studies using Balb/c-3T3 A31 mouse cells
- DINP was found to be positive in one study
- Negative in three studies
- A non-significant increase in transformed foci in three studies

DINP Effects on Steroidogenesis

- Multiple perinatal DINP exposure studies in rats indicate:
  - Reduced testosterone levels in male pups and reduced *ex vivo* testosterone production
  - Reduced mRNA expression of genes involved in steroid production, e.g., insulin-like-3, CYP11A, steroidogenic acute regulatory protein (StAR)
  - Reduced anogenital distance in male pups
  - Reduced absolute weight of seminal vesicles
- Disturbances of testosterone production in humans are associated with testicular dysgenesis syndome (TDS) in children
- TDS is associated with germ cell cancer

Structure Activity Comparisons: Tumor Types

Chemical	Liver		Pancreas		Testicular interstitial cell		MNCL		Renal and Bladder		Uterus	
	Mice	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice	Rats
DINP	M F	M F	F[2]	M[2]		M		M F		M[4]		F[5]
DEHP	M F	M F		M[1]		M		M				
BBP				M[1]				M F		F[3]		

[1]Pancreatic acinar cell adenoma or carcinoma
[2]Pancreatic islet cell carcinoma
[3]Bladder transitional epithelium hyperplasia, papilloma and carcinoma
[4]Renal transitional epithelial cell carcinoma and renal tubular carcinoma
[5]Endometrial adenocarcinoma

Structure Activity Comparisons: Other Parameters

Chemical	Genotoxicity			In vitro cell transformation	PPAR α+γ	ER	AhR	PXR	CAR	GJIC inhibition	Anti-androgenic & anti-steroidogenesis
	DNA damage	Gene mutations	Chromosomal damage								
DINP	NE	-	-	+/-[1]	+	+	-	+	+	+	+
DEHP	+	+	+	+	+	+	+	+	+	+	+
BBP	+	-	+	+	+	+	+	+	NE	NE	+

[1]One of seven assays positive.
PXR: Pregnane X receptor; CAR: Constitutive androstane receptor; GJIC: Gap junction intercellular communication, NE: Not evaluated.

Possible Mechanisms of Action

- Genotoxicity
  - Available data are negative
  - Oxidative DNA damage - not adequately tested
- Inhibition of steroidogenesis
- Induction of Tumor Necrosis Factor α (TNFα)
- Decreased gap junction intercellular communication (GJIC)
- Activation of CAR and PXR

Possible Mechanisms of Action (Continued)

- Activation of PPARα
  - *Hypothesis*- Activation of PPARα is necessary event in liver tumor induction in rat and mice, and the liver tumors induced are not relevant to humans
  - Findings inconsistent with PPARα MOA hypothesis:
    - DEHP induces liver tumors in PPARα null mice (Ito, 2007)
    - Receptor activation alone in a mouse model does not produce liver tumors (Yang et al. 2007)
- DINP-specific data related to PPARα activation
  - Suggest this hypothesized MOA may not be operative in DINP-induced liver tumors
    - Inconsistent observations of short-term hepatocellular proliferation
    - Lack of sustained long-term hepatocellular proliferation

Possible Mechanisms of Action
(continued)

- α2u-Globulin nephropathy
  - acute exposure does not exacerbate hyaline droplet formation (not observed until 12 months of exposure)
  - subchronic histopathological changes including granular cast formation and linear papillary mineralization not observed
  - renal histopathological changes in female rats observed (renal tubule regeneration) (Lington *et al.*, 1997)
  - does not meet all IARC criteria for α2u-globulin nephropathy mechanism

Review by Authoritative Bodies

- DINP has not been classified as to its carcinogenicity by:
  - U.S. EPA, U.S. FDA, NTP, NIOSH, or IARC

Summary of Carcinogenicity Evidence

Human Evidence: No data

Animal Evidence:

- Statistically significant increases in tumor incidence
  - Liver tumors (male and female rats and mice)
  - Mononuclear cell leukemia (male and female rats)
  - Renal tubular cell carcinomas (male rats)(rare)
- Tumor incidence increase not statistically significant, but tumor types considered to be rare or uncommon
  - Pancreatic islet cell carcinomas (rare)
  - Uterine adenocarcinomas (rare)
  - Renal transitional cell carcinomas (rare)
  - Leydig cell carcinomas (uncommon)

Summary of Carcinogenicity Evidence

Other Relevant Data:

- DINP activates several nuclear receptors (PPARα, PPARϒ, CAR, PXR)
- DINP has anti-androgenic activity and causes steroidogenesis disruption
- DINP induces tumor necrosis factor α (TNFα)
- DINP inhibits gap junction intercellular communication (GJIC)
- Common tumor sites/types observed in animal studies of DINP and structurally related phthalates