Existing Chemical Hazard Assessment Report

Australian Government
Department of Health and Ageing
NICNAS

Diisononyl Phthalate

June 2008

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME
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Preface

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

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NICNAS assessments are carried out in conjunction with the Department of Environment and Heritage, which carry out the environmental assessment for NICNAS. NICNAS has two major programs: the assessment of the health and environmental effects of new industrial chemicals prior to importation or manufacture; and the other focussing on the assessment of chemicals already in use in Australia in response to specific concerns about their health/or environmental effects.

There is an established mechanism within NICNAS for prioritising and assessing the many thousands of existing chemicals in use in Australia.

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Overview

This review of diisononyl phthalate (DINP) is a health hazard assessment only. For this assessment, two key reviews on DINP prepared by the European Chemicals Bureau and the US Centre for the Evaluation of Risks to Human Reproduction were consulted. Information from these reviews was updated with literature surveys conducted up to September 2006.

Structurally, phthalate esters are characterized by a diester structure consisting of a benzenedicarboxylic acid head group linked to two ester side chains. DINP is not a single compound, but a complex mixture containing mainly C8, C9-branched isomers. DINP has two CAS numbers. The composition of DINP (CAS No 68515-48-0) is represented as mixed phthalates with side chains made up of 5-10% methyl ethyl hexanol, 45-55% dimethyl heptanol, 5-20% methyl octanol, 0-1% n-nonanol, and 15-25% isodecanol. The composition of DINP (CAS No 28553-12-0) is represented as mixed phthalates with side chains made up of 5-10% methyl ethyl hexanol, 40-45% dimethyl heptanol, 35-40% methyl octanol, and 0-10% n-nonanol. Thus, DINP [side chains of dimethyl heptanol (i.e. isononanol)] makes up about 50% of the two DINP mixtures available on the market.

According to the European Council of Plasticisers and Intermediates, approximately 95% of DINP is used in polyvinyl chloride (PVC) applications. The remaining 5% is used in non-PVC applications. More than half of the DINP used in non-PVC applications involves polymer related uses (e.g. rubbers) and the remaining DINP is used in non-polymer applications such as inks, adhesives, sealants, paints and lubricants. Current EU legislation restricts the use of DINP in certain toys and childcare articles which can be placed in the mouth.

In Australia, DINP is imported as finished products or mixtures and as a raw chemical for local manufacture. The chemical is used industrially as plasticiser for PVC applications including cable jacketing, automotive products, flooring, sheets, films, carpet backing, laminations, and adhesive tape. It is also used in non-vinyl applications such as adhesives, surfactants, and printing inks. Imported finished articles containing DINP include toys, play and exercise balls.

When orally administered to rats, a large proportion of DINP is rapidly metabolised in the gastrointestinal tract to the corresponding monoester, monoisononyl phthalate (MINP), and absorbed and excreted, primarily in the urine as oxidation products and phthalic acid. DINP is excreted in the faeces as the parent compound as well as MINP and oxidation products. DINP and its metabolites do not accumulate in the body. There is limited absorption by the dermal route (<4% in 7 days).

In animal studies, DINP has a low order of acute oral, dermal and inhalation toxicity. DINP caused minimal skin and eye irritation and is unlikely to cause skin sensitisation.

The target organs for oral repeated dose toxicity are the liver and kidney. A NOAEL of 88-108 mg/kg bw/d was derived from a two-year oral study in rats, based on enzyme changes (increased serum ALT, AST), liver weight changes (increased absolute and relative liver weights) and concurrent histopathological alterations, as well as kidney changes (increased absolute/relative kidney weights) in both sexes. In contrast, in monkeys, oral administration of DINP for 13 weeks with doses up to 2500 mg/kg bw/d produced slight, non-treatment related liver enzyme changes, but no treatment-related changes in liver weights, biochemical parameters or histological findings.

Based on a 6-week dermal study in rabbits, a NOAEL of 0.5 mL/kg (approx 500 mg/kg bw/d) was derived for dermal application, with gross skin alterations seen at 2500 mg/kg bw/d. No studies involving the inhalation route have been conducted.

Based on available data, DINP is considered not to be genotoxic.

Increased incidences of mononuclear cell leukaemia (MCL), kidney and liver neoplasia were observed in long term animal studies. However these tumours in animals were regarded as toxicological effects with limited relevance to humans. Non-carcinogenic liver and kidney effects (including changes in kidney organ weights, urine chemistry and histopathology) observed in rodents from repeated DINP exposure were not replicated in primates.

In general, DINP had no effect on male mating, female fecundity or female gestational indices. A NOAEL for fertility was established at 622 mg/kg bw/d based on decrease in live birth and survival indices at a high dose.

With regards to effects on reproductive organs, effects of DINP on testes weight were inconsistent in studies on rats and mice. Histopathological changes in the testes were not observed in most of these studies. The NOAEL for reproductive effects was 275 mg/kg bw/d from a 2 year carcinogenicity study in mice, with decreased testes weight observed at 742 mg/kg bw/d.

Developmental toxicity studies in rats indicate that exposure to DINP during early gestation induces skeletal and visceral variations but only at materno-toxic doses (LOAEL of 1000 mg/kg bw/d). A NOAEL of 500 mg/kg bw/d was derived. Later gestational exposure was associated with retention of nipples in males and increases in malformation of the male reproductive tract. The LOAEL was 750 mg/kg bw/d. No NOAEL was derived. In a two-generation study in rats, decreased pup weight was noted on PND 21 with a LOAEL of 159-395 mg/kg bw/d. No NOAEL was derived.

Table of Contents

Acronyms and Abbreviations

ALT	alanine aminotransferase
AR	androgen receptor
AST	aspartate aminotransferase
BBP	butylbenzyl phthalate
bw	body weight
C	Celsius
CAS	Chemical Abstracts Service
CERHR	Centre for the Evaluation of Risks to Human Reproduction
CHO	Chinese hampster ovary
DEHP	diethylhexyl phthalate
DEP	diethyl phthalate
DINP	diisononyl phthalate
DMP	dimethyl phthalate
DNA	deoxyribonucleic acid
ECB	European Chemicals Bureau
EEC	European Economic Community
EPA	Environmental Protection Agency (USA)
ER	oestrogen receptor
EU	European Union
f	female
F0	parental generation
F1	filial 1 (first generation)
F2	filial 2 (second generation)
g	gram
GD	gestation day
GIT	gastro-intestinal tract
GJIC	gap junctional intercellular communication (
GLP	good laboratory practice
h	hour
IgE	immunoglobulin E
IL-4, IL-13	interleukin-4, interleukin-13
kg	kilogram
kPa	kilopascals

L	litre
LC50	median lethal concentration
LD50	median lethal dose
LOAEL	lowest-observed-adverse-effect level
m	male
MCF-7	human breast adenocarcinoma cell line
MCL	mononuclear cell leukaemia
mg	milligram
MINP	monoisononyl phthalate
mL	millilitre
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
nL	nanolitre
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
PND	post-natal day
ppm	parts per million
PVC	polyvinyl chloride
w/w	weight per weight
µ	micro

1. Introduction

This review of diisononyl phthalate (DINP) is a health hazard assessment only. For this assessment, two key reviews on DINP prepared by the European Chemicals Bureau (ECB, 2003) and the Centre for the Evaluation of Risks to Human Reproduction (CERHR, 2003) were consulted. Information from these reviews was updated with relevant studies from more recent literature surveys conducted up to September 2006.

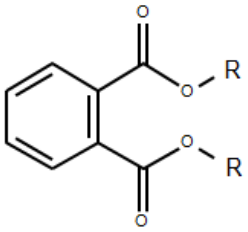
Information on Australian uses was compiled from data supplied by industry in 2004 and 2006.

References not marked with an asterisk were examined for the purposes of this assessment. References not examined but quoted from the key reviews as secondary citations are also noted in this assessment and marked with an asterisk.

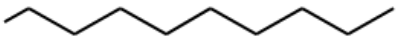
Hazard information from this assessment is published also in the form of a hazard compendium providing a comparative analysis of key toxicity endpoints for 24 *ortho*-phthalate esters (NICNAS, 2008).

2. Identity

2.1 Identification of the substance

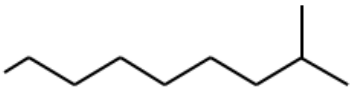
CAS Numbers:	68515-48-0; 28553-12-0
Chemical Name:	1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich (68515-48-0); 1,2-benzenedicarboxylic acid, diisononyl ester (28553-12-0)
Common Name:	Diisononyl phthalate (DINP)
Molecular Formula:	$C_{26}H_{42}O_4$ (average)
Structural Formula:	

CAS Number: 68515-48-0

R = 

(based on C9 length)

CAS Number: 28553-12-0



Molecular Weight:	420.6 (average)
Synonyms:	Di-"isononyl"phthalate
Purity/Impurities/Additives:	Purity >99.5%, in terms of ester content

Note: DINP is not a single compound, but a complex mixture containing mainly C8, C9-branched isomers. The composition of CAS 68515-48-0 is represented as mixed phthalates with side chains made up of 5-10% methyl ethyl hexanol, 45-55% dimethyl heptanol, 5-20% methyl octanol, 0-1% n-nonanol, and 15-25% isodecanol; and the composition of CAS 28553-12-0 is represented as mixed phthalates with side chains made up of 5-10% methyl ethyl hexanol, 40-45% dimethyl heptanol, 35-40% methyl octanol, and 0-10% n-nonanol. Thus, diisononyl phthalate [side chains of dimethyl heptanol (i.e. isononanol)] makes up about 50% of the two 'DINP' mixtures which appear to be available on the market. The above structural formulas are those associated with the CAS numbers given but they do not reflect the complexity of the commercially-available phthalate mixtures.

2.2 Physico-chemical properties

Table 1: Summary of physico-chemical properties

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

Based on CERHR (2003); ECB (2003)

3. Uses

According to European Council of Plasticisers and Intermediates (ECPI, 1997*), approximately 95% of DINP is used in PVC applications. The remaining 5% is used in non-PVC applications. More than half of the DINP used in non-PVC applications involves polymer related uses (e.g. rubbers). The remaining DINP is used in non-polymer applications including inks and pigments, adhesives, sealants, paints and lacquers and lubricants (ECB, 2003). Current EU legislation restricts the use of DINP in certain toys and childcare articles which can be placed in the mouth.

In Australia, DINP is imported as finished products or mixtures and as a raw chemical for local manufacture. The chemical is used industrially as plasticiser for PVC applications including cable and wire jacketing, automotive products, flooring, sheets, films, carpet backing, laminations, and adhesive tape. It is also used in non vinyl applications such as adhesives, surfactants, and printing inks. Imported finished articles containing DINP include toys, play and exercise balls.

4. Human Health Hazard

4.1 Toxicokinetics

Previous evaluations

Oral

Two studies on the toxicokinetics, metabolism and distribution of DINP (CAS 68515-48-0) in rats were available (Hazleton, 1972*; Midwest Research Institute, 1983a*).

In the first study (Hazleton, 1972*), 2500mg/kg/d of ^{14}C DINP was administered orally to male rats. Over 80% of the administered dose was excreted in the faeces and most of the radioactivity was excreted within 24-hours after dosing. Of the selected organs (hearts, livers, kidneys, intestines, stomachs, fat and muscle), radioactivity was mainly recovered in the gastrointestinal tract (GIT) and liver. No expired ^{14}C DINP was detected.

In the second study (Midwest Research Institute, 1983a*), a single dose of ^{14}C DINP (50 or 500 mg/kg) was administered orally to both male and female rats. The compound was readily absorbed, with absorption of DINP decreasing as dose increased. The entire administered radioactivity was essentially eliminated in urine (at least 49% at the low dose of 50 mg/kg and 39% at the high dose of 500 mg/kg) and faeces (approx 51% of both the low and high dose) within 72 hours after dosing. DINP was rapidly distributed to major tissues, particularly the liver (4.7% of administered dose), followed by the kidneys (0.31%). DINP was de-esterified to the monoester, which was further metabolised by side-chain oxidation of the ester group or by hydrolysis to phthalic acid. The formation of oxidation products appeared to increase at the higher dose, while hydrolysis to phthalic acid decreased. DINP metabolites reached the testes at the high dose and were detected in fat.

Repeated dosing (for 5 days at 50, 150 or 500 mg/kg) did not result in accumulation of DINP and/or its metabolites in blood and tissues, but increased formation and elimination of the monoester oxidation products were observed. Absorption was incomplete at all dose levels following repeated exposure.

Dermal

The results of a dermal exposure study (1, 3 and 7 days) indicate low dermal absorption of DINP (CAS 68515-48-0) in adult male rats. Most of the applied radioactivity was recovered from the application areas (92-103%) in all treatment conditions tested. One to 2% of radioactivity was recovered in the urine and about 1% or less in the faeces. The presence of radioactivity in the faeces and the GI tract suggested biliary excretion of the absorbed radioactivity. Low levels of radioactivity were measured in the blood and tissues (Midwest Research Institute, 1983b*).

Data not reported in previous evaluations

In vivo human data for toxicokinetics, metabolism and distribution were not available. A study conducted in 129 human volunteer adults with no known exposure to DINP, no hydrolytic monoester monoisononyl phthalate (MINP) was detected in

any urine samples collected. The limit of detection was noted to be 0.36 ng/ml (Silva et al., 2006). This metabolite, however, has been found in human breast milk (Silva et al., 2003*; Main et al., 2006).

Conclusion

When orally administered to rats, DINP is rapidly metabolised in the GIT to the corresponding monoester (MINP) and absorbed and excreted, primarily in the urine as oxidation products and phthalic acid. Generally, at least 50% of an oral dose is absorbed and excreted in the urine. DINP is excreted in the faeces as the parent compound as well as MINP and oxidation products. DINP and its metabolites do not accumulate in the body. There is little absorption by the dermal route.

4.2 Acute toxicity

Table 2: Summary of acute toxicity studies on DINP

Study	Species	Results (LD50/LC50)	Test Substances	References
Oral	Rat	>10000 mg/kg bw	CAS 68515-48-0	Hazleton (1968c*)
		>50000 mg/kg bw	CAS not determined	Hazleton (1980b*)
		>40000 mg/kg bw	CAS 28553-12-0	Midwest Research Institute (1981*)
		>10000 mg/kg bw	CAS 28553-12-0	BASF (1981b*)
		>10000 mg/kg bw	CAS 28553-12-0	Hüls (1985a*)
Inhalation (4-h)	Rat	>4.4 mg/L of air (analytical)	CAS not determined	Hazleton (1980a*)
Dermal	Rabbit	>3160 mg/kg bw	(CAS 68515-48-0)	Hazleton (1968a*)

Based on ECB (2003)
Note: only validated studies were included

Data not reported in previous evaluations

No data

Conclusion

In acute studies, findings consisted of poor state, respiratory difficulties and altered appearance following oral administration of high doses (up to 40000 mg/kg). No mortality, body weight changes, gross lesions or microscopic alterations of the lungs were observed following aerosol exposure of 4.4 mg/l of air during 4 hours (Hazleton, 1980a*).

Most of the animal studies of acute toxicity were either not available for review or performed prior to establishment of Good Laboratory Practice (GLP), OECD or EU Test Guidelines. However, based on the results given, it can be considered that DINP

has a low order of acute oral (LD50 >10000 mg/kg bw), dermal (LD50 >3160 mg/kg bw) and inhalation toxicity (LC50 >4.4 mg/L).

4.3 Irritation

4.3.1 Skin irritation

Previous evaluations

A GLP complied study (Exxon Biomedical Sciences, 1996a*) was carried out using undiluted DINP (CAS 68515-48-0) applied for 4 hours to the clipped intact skin of six male New Zealand white rabbits with a semi-occlusive dressing, followed by an observation period of 72 hours. One rabbit showed very slight erythema at 1 hour and another at 24 hours. Otherwise, all rabbits were free of erythema and oedema.

Two other studies on DINP (CAS 28553-12-0) were carried out in rabbits, including a study involving a 24 hours exposure to abraded skin. Only slight erythema and oedema were observed (BASF, 1981a*; Hüls, 1985b*).

In humans, DINP (CAS 68515-48-0) was applied undiluted for 24 hours to the skin of volunteers, followed by an observation period of 24 hours (Hill Top Research, 1995*). Positive and negative controls were included. Mild to moderate erythema was observed with the positive control, but not with the test substance.

Data not reported in previous evaluations

No data

Conclusion

DINP caused minimal irritation to the skin of animals, which was reversible, and no skin irritation in humans.

4.3.2 Eye irritation

Previous evaluations

Following single application of DINP (CAS 68515-48-0) to the eye in 6 male and 6 female albino rabbits, irritation was confined to the conjunctivae and generally consisted of marked redness and slight discharge at 1 and 4 hours (score of 3), and slight redness only (moderate in one case) at 24 hours (score of 1). By 48 or 72 hours the irritation had completely subsided in all cases (Hazleton, 1968b*).

Single application of DINP (CAS 28553-12-0) to 2 male and 4 female white Vienna rabbits caused slight conjunctival redness (mean score 0.83) at 24 hours only and slight corneal opacity (mean score 0.5) at 72 hours only. The iris was unaffected. The reversibility of the corneal effects was not determined (BASF, 1981b*).

Another study (Hüls, 1985c*) on DINP (CAS 28553-12-0) was carried out on small white Russian rabbits (3 males and 3 females). There was no effect on the cornea and iris but, at 1 hour post-exposure, slight to medium redness was noted for the conjunctivae, accompanied by some discharge. The absolute score was 4.33 at this time, but returned to 0.33 at 24 hours and 0 at later times. The irritation index was calculated as 1.17/110.

Data not reported in previous evaluations

No data

Conclusion

DINP caused minimal eye irritation in animals, which was reversible.

4.4 Sensitisation

Previous evaluations

Two Buehler tests on female guinea pigs using DINP (CAS 68515-48-0) were reported. The earlier study produced some evidence of sensitisation, with score 2 erythema being observed at day 37 in 3/20 animals (cf. 4/10 control animals had score 1 on the same day) (Exxon Biomedical Sciences, 1992*).

The second Buehler test conducted in conformity with GLP and with EEC Method B6, was carried out in 20 control and 20 DINP (CAS 68515-48-0) treated animals. No evidence of skin sensitisation was observed (Huntingdon Research Centre, 1994*).

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

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

Data not reported in previous evaluations

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

Conclusion

The results of one of the two Buehler tests suggested potential for DINP (CAS 68515-48-0) to cause skin sensitisation. However, another animal study conducted accordingly to Buehler (one challenge) gave negative results.

No positive reactions were reported in a Repeated Insult Patch test using CAS 68515-48-0 in humans. Taken as a whole, DINP (CAS 68515-48-0) is unlikely to cause skin sensitisation.

It should be noted that no experimental data are available for CAS 28553-12-0.

4.5 Repeated dose toxicity

Previous evaluations

Numerous animal studies have been conducted to determine repeat dose toxicity effects.

Oral

Oral, repeat dose studies include: a four-week study, a thirteen-week study and a two-year carcinogenicity study in mice; one-week study, 2-week study, six 13-week studies, three 2-year toxicity and carcinogenicity studies in rats; 13-week study in dogs; 2-week study and a 13-week study in monkeys. A number of studies, including 14-day, 21-day and 28-day studies were conducted in rats to assess the effect of DINP on peroxisomal proliferation. Short-term studies to investigate the hepatic effects of DINP were also carried out in both rats and mice. A study on monkeys was done to elucidate the human relevance of liver effects observed in rats and mice. Overall conclusions from some of these studies are outlined below and summarised in Table 3.

These studies reveal that repeated doses of DINP have effects mainly on the liver, kidney and testes. In the case of liver and kidney, increased absolute and/or relative organ weights and biochemical changes consistent with organ toxicity were observed repeatedly with oral DINP administration in rats and mice.

A 13-week dietary study in Fischer 344 rats (15/sex/dose) showed statistically significant increases in liver and kidney weights with organ discolouration, liver enzyme, triglyceride and cholesterol and urine chemistry changes consistent with organ toxicity (Bio/Dynamics 1982b*). The doses used were 0, 0.1, 0.3, 0.6, 1, and 2% of DINP in the diet (approx 77, 227, 460, 767, 1554 mg/kg bw/d). The NOAEL was 0.1% (77mg/kg bw/d) based on the increase of kidney and liver weights, and the decrease of cholesterol level noted at 0.3% (227 mg/kg bw/d).

Another 13-week dietary study in Fischer 344 rats (10/sex/dose) using doses of 0, 2500, 5000, 10000, 20000 ppm (approx 0, 176-218, 354-438, 719-823, 1545-1687 mg/kg bw/d males-females, respectively) showed significantly increased absolute and/or relative liver and kidney weights with statistically significant changes in haematological and urine chemistry parameters. Hepatocellular changes in liver and increases in granular casts and regenerative/basophilic tubules in kidney were noted. No NOAEL was identified. The LOAEL was 176-218 mg/kg bw/d based on the increases of liver and kidney weights in males and females (Hazleton, 1991a*). This study was performed according to GLP procedures and EPA guidelines.

A 13-week dietary study in B6C3F1 mice fed diets containing 0, 1500, 4000, 10000, 20000 ppm (approx 0, 365, 972, 2600, 5770 mg/kg/d) showed increases in absolute and relative liver weights from 4000 ppm. Absolute and relative kidney weights were decreased in males at the same dose levels with significant decreases in urinary sodium, chlorides and creatinine seen in high dose animals. At high doses (20000 ppm), moderate to severe hepatocellular enlargement, pigmented Kupffer cells and bile canaliculi and minimal to slight liver degeneration/necrosis were observed. Tubular necrosis in the kidney as well as immature/abnormal sperm, lymphoid depletion in spleen and thymus were also seen at this dose. At 10000 ppm and higher, decreased (absolute) epididymis and testes weights were observed. The NOAEL was 1500 ppm (approx 365 mg/kg/d), based on increase in liver weights at 4000 ppm

(approx 972 mg/kg bw/d) (Hazleton 1992*). This study was also performed according to GLP procedures and EPA guidelines.

A 2-year dietary carcinogenicity study using doses of 0, 500, 1500, 4000 and 8000 ppm (approx 0, 90-112, 275-335, 742-910, 1560-1887 mg/kg bw/d males-females, respectively) in B6C3F1/Crl BR mice (70/sex/dose) revealed statistically significant decreases in absolute and/or relative kidney weights and statistically significant increases in absolute and/or relative liver weights. At study termination, the most substantial gross changes were lung masses in all groups (primarily males), liver masses (most frequently seen in mid-high and high dose groups), enlarged spleen in all groups, granular pitted/rough kidneys in high dose females (corresponding to increased incidence/severity of treatment-related nephropathy) and distended urinary bladder (most frequently seen in mid-high and high dose males). Diffuse hepatocellular enlargement and pigment were also observed histologically in high dose groups. At mid-study and study termination, mean liver palmitoyl-CoA oxidase activities were statistically significantly increased in all high dose animals compared to controls suggesting significant peroxisome proliferation. A NOAEL of 500 ppm (90-112 mg/kg bw/d) was derived, based on decreased absolute kidney weights and increased incidence of liver masses in males, and increased absolute liver weights in females at the mid-low doses of 1500 ppm (275-335 mg/kg bw/d) (Aristech Chemical Corporation, 1995*; Moore 1998*).

In Beagle dogs, renal tubular cell and hepatocytic hypertrophy were observed at a dose of 2% (2000 mg/kg/d) in a 13-week feeding study (Hazleton, 1971*). In contrast, kidney effects were absent in Marmoset monkeys gavaged with DINP at up to 2500 mg/kg bw/d in a 13-week study (Huntingdon Life Sciences, 1998*). In this study, using doses of 100, 500 and 2500 mg/kg bw/d (4 monkeys/sex/group), no changes in biochemical parameters, hormonal concentrations or organ histology were found that were considered treatment-related. Body weight losses or low body weight gains were observed for both sexes at the highest dose. Also, there was a slight increase in palmitoyl CoA oxidase and lauric acid 11- and 12-hydroxylase activity. However, these were not considered biologically significant due to the wide range of individual variations, absence of statistical significance and absence of concomitant increases in liver weights. A NOAEL of 500 mg/kg bw/d and LOAEL of 2500 mg/kg bw/d based on decreases in body weight and body weight gain were assigned from this 13-week gavage study. No effects were noted also in a subsequent shorter 2-week study in adult Cynomolgus monkeys administered up to 500 mg/kg bw/d DINP by gavage (Pugh et al., 2000*).

In a 2-year dietary study employing Fischer 344 rats, DINP was administered at 0, 500, 1500, 6000 and 12000 ppm (approx 0, 29-36, 88-108, 358-442, 733-885 mg/kg bw/d males-females, respectively) (Aristech Chemical Corporation, 1994*). The ECB (2003) review noted this study from amongst three chronic 2-year studies in rats as the most recent and employing GLP. The liver and kidney were target organs for DINP. In both sexes, livers were enlarged with granular appearances. Statistically significant increases in mean absolute and/or relative liver weights were observed during the study and at termination. However, liver enlargements appeared reversible with absolute and relative liver weights in the high dose recovery group (78 weeks treatment followed by 26 weeks untreated recovery) comparable to control values. Increased serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were observed from 1500 ppm in females and from 6000 ppm in males.

Palmitoyl-CoA oxidase activity was also increased significantly in all 12000 ppm treated males and females and 6000 ppm treated females.

Kidney effects were also observed, consisting of increased absolute and/or relative kidney weights in both sexes and some related effects, more marked in the males (increased serum urea, increased urine volume and decreased urine potassium, calcium, creatinine and chloride suggesting compromised tubular function). Histologically, there was an increase in the frequency and severity of chronic progressive nephropathy in males. These histological features were consistent with a specific male rat alpha 2μ globulin nephropathy. A NOAEL of 1500 ppm (88-108 mg/kg bw/d males-female respectively) was established from this study based on liver and kidney toxicity consisting of increased liver and kidney weights, and hepatic biochemical changes (increased serum ALT and AST) associated with histopathological findings at a higher dose (LOAEL = 358-442 mg/kg bw/d). These observations were unrelated to peroxisome proliferative effects.

Several studies performed specifically to assess the peroxisomal proliferation potential of DINP revealed biochemical evidence of peroxisomal proliferation in rodents (Hüls, 1992*). In contrast, there was no evidence of peroxisome proliferation in Cynomolgus or Marmoset monkeys following oral administration of DINP for 2 and 13 weeks respectively.

Dermal

A six-week dermal study was undertaken in New Zealand White rabbits with groups of 4 animals each receiving doses of 0.5 or 2.5 mL/kg bw DINP or 2.5 mL/kg bw mineral oil as control (Hazleton, 1969*). Applications were made for 24 hours on abraded and intact skin, five days a week for a total of 30 exposures. DINP effects were confined to gross alterations of the skin. At the lowest dose, mild dermal irritation occurred with slightly more severity than mineral oil vehicle alone. At the high dose, slight or moderate erythema and slight desquamation were observed. There were no systemic effects. A NOAEL of 0.5 mL/kg (approx 500 mg/kg) was established.

Data not reported in previous evaluations

No data

Conclusion

The main target organs for repeat dose effects of DINP are the liver and kidney. A NOAEL of 88-108 mg/kg bw/d was determined from a 2-year repeat dose toxicity/carcinogenicity study in Fischer 344 rats conducted according to GLP (Aristech Chemical Corporation, 1994*). A LOAEL of 358-442 mg/kg bw/d was derived based on kidney and liver toxicity. At these higher doses, absolute and/or relative kidney weights in both sexes were increased and liver effects consisting of hepatic biochemical changes (increased serum ALT, AST), increased liver weight and histopathological liver enlargement and granular appearances were observed. Palmitoyl-CoA oxidase activity was also increased in high dose animals. This NOAEL is based on kidney changes unrelated to peroxisome proliferative effects.

A NOAEL of 0.5 mL/kg (approx 500 mg/kg) was established for repeat dose dermal toxicity based on a 6-week study in rabbits. Gross skin alterations were observed at a higher dose (2500 mg/kg).

Table 3: Summary of key repeated dose toxicity studies

Species, study duration and test substances	Doses (mg/kg bw/d) and administration mode	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) and Effects observed	References
Oral				
Rat Fischer 344; 1-week study; CAS 68515-48-0	0, 2% (0, 1700) in diet	NE	1700; ↑ kidney, liver weights, macroscopic liver changes, ↓ cholesterol, triglycerides	Bio/Dynamics (1982a*)
Rat Fischer 344 females; 2-week study; CAS 28553-12-0	0, 25, 75, 150, 1500 gavage	25	1500; ↑ liver weights	Hüls (1992*)
Rat Fischer 344; 13-week study; CAS 68515-48-0	0, 0.1, 0.3, 0.6, 1, 2% (0, 77, 227, 460, 767, 1554) in diet	77	227; ↑ kidney, liver weights, ↓ cholesterol	Bio/Dynamics (1982b*)
Rat Sprague Dawley; 13-week study; CAS 68515-48-0	0, 0.3, 1% (0, 201-251, 690-880 (m-f)) in diet	NE	201-251; ↑ kidney, liver weights, urine chemistry changes, ↓ triglycerides	Bio/Dynamics (1982c*)
Rat Fischer 344; 13-week study; CAS 28553-12-0	0, 2500, 5000, 10000, 20000 ppm (0, 176-218, 354-438, 719-823, 1545-1687 (m-f)) in diet	NE	176-218; ↑ kidney, liver weights	Hazleton (1991a*)
Rat Fischer 344; 2-year study; CAS 68515-48-0	0, 0.03, 0.3, 0.6% (0, 15-18, 152-184, 307-375 (m-f)) in diet	15-18	152-184; ↑ kidney, liver weights; ↑ incidence of non-neoplasic changes in kidney & liver	Exxon Biomedical Sciences (1986*)
Rat Fischer 344; 2-year study; CAS 68515-48-0	0, 500, 1500, 6000, 12000 ppm (0, 29-36, 88-108, 358-442, 733-885 (m-f)) in diet	88-108	358-442; ↑ kidney, liver weights in both sexes; ↑ AST & ALT with histopathological findings	Aristech Chemical Corporation (1994*)
Mouse B6C3F1; 13-week study; CAS 28553-12-0	0, 1500, 4000, 10000, 20000 ppm (0, 365, 972, 2600, 5770) in diet	365	972; Enlarged liver; ↑ absolute and relative liver weights	Hazleton (1992*)

Species, study duration and test substances	Doses (mg/kg bw/d) and administration mode	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) and Effects observed	References
Mouse B6C3F1; 2-year study; CAS 68515-48-0	0, 500, 1500, 4000, 8000 ppm (0, 90-112, 275-335, 742-910, 1560-1887 (m-f)) in diet	90-112	275-335; ↑ absolute liver weights in females, ↓ absolute kidney weights in males	Aristech Chemical Corporation (1995*); Moore (1998*)
Dog Beagle; 13-week study; CAS 68515-48-0	0, 0.125, 0.5, 2% (0, 37, 160, 2000) in diet	NE	37; ↑ AST in females,↑ liver weights	Hazleton (1971*)
Monkey Cynomolgus males; 2-week study; CAS not specified.	0, 500 gavage	500	NE	Pugh et al. (2000*)
Monkey Marmoset (16-25-month old); 13-week study; CAS not specified.	0, 100, 500, 2500	500	2500; ↓body weight; ↓ body weight gain	Huntington Life Sciences (1998*)
Dermal				
Rabbit New Zealand White; 6-week study; CAS 68515-48-0	0, 0.5, 2.5 mL/kg bw	500 (0.5 mL/kg bw)	2500 (2.5 mL/kg bw); slight or moderate erythema, and slight desquamation	Hazleton (1969*)

Based on ECB (2003)
NE = not established; ↓ = decreased; ↑ = increased

4.6 Genetic toxicity

Previous evaluations

Several studies have been conducted on DINP (CAS 68515-48-0 and 28553-12-0) involving *Salmonella* typhimurium (the Ames test), including a most recent GLP study (Exxon Biomedical Sciences, 1996b*). No positive responses were observed in any study with any of the bacterial strains tested (TA98, 100, 1535, 1537, 1538), either in the presence or absence of metabolic activation.

A mouse lymphoma forward mutation assay (Hazleton, 1986*), performed according to GLP procedures found that DINP (CAS 68515-48-0) did not induce increases in mutant frequency at any dose, either in the presence or absence of metabolic activation.

DINP (CAS 68515-48-0) was also tested for clastogenic activity in cultured Chinese hamster ovary (CHO) cells in the presence or absence of metabolic activation (Exxon Biomedical Sciences, 1996c*). There was a statistically significant increase in the percentage of aberrant cells in the absence of metabolic activation. However, the percentage of aberrant cells fell within the normal range of the vehicle control, was

not dose related, and did not exceed 5%, which is the defined threshold to be considered as a positive result. Therefore, DINP was considered negative for clastogenicity in this study.

A primary rat hepatocyte unscheduled DNA synthesis assay (Litton Bionetics, 1981*) was conducted. DINP (CAS 28553-12-0) was found to be inactive in the assay.

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

Major studies and results are summarised in Table 4.

Data not reported in previous evaluations

No data

Conclusion

DINP tested negative in in vitro bacterial mutation assay, in an in vitro mammalian gene mutation assay and a cytogenetic assay in CHO cells. DINP was also not clastogenic in an in vivo bone marrow assay in Fisher 344 rats.

DINP is considered not genotoxic.

4.7 Carcinogenicity

Previous evaluations

DINP has been subjected to several in vitro cell transformation assays using Balb/c-3T3 mouse cells (clone 1-13) under different conditions. Some of these studies have been described repeatedly (Barber et al., 2000). Three of 7 test results were negative and 3/7 tests were doubtful (slight increases in transforming activity without statistical significance). A single study with concentrations of DINP ranging from 0.03 to 1 µL/mL found statistically significant and dose-dependent type III transforming activity in 3T3 cells in the absence of metabolic activation (Microbiological Associates, 1981b*).

Table 4: Summary of gene mutation and cytogenetic assays on DINP

Genetic toxicity tests and test substances	Test system	Doses	Results	References
In vitro				
Bacterial test (gene mutation) CAS 68515-48-0	*Salmonella typhimurium* TA 98, 100, 1535, 1537, 1538	From 0.5 to 5000 µg/plate ± S9	Negative	Exxon Biomedical Sciences (1996b*)
Mouse lymphoma assay CAS 68515-48-0	L5178 TK ±	From 1500 to 8000 nL/mL without metabolic activation and from 500 to 6000 nL/mL with metabolic activation	Negative	Hazleton (1986*)
Cytogenetic assay CAS 68515-48-0	CHO cells	5, 10, 20, 40, 80, 160 µg/mL ± S9	Negative	Exxon Biomedical Sciences (1996c*)
Mammalian test (Unscheduled DNA synthesis assay) CAS 28553-12-0	Rat hepatocytes	From 0.625 to 10 µg/mL	Negative	Litton Bionetics (1981*)
In vivo				
Cytogenetic assay	Fischer 344 rat bone marrow cells	5-1.7 and 0.5 mg/kg/d during 5 days via oral route	Negative	Microbiological Associates (1981a*)

Note: only validated studies were included

In vivo carcinogenicity studies in animals include a 2-year dietary study in mice and three 2-year dietary studies in rats. A 2-year dietary study in B6C3F1 mice (Aristech Chemical Corporation, 1995*; Moore 1998*) resulted in the establishment of a NOAEL of 112 mg/kg bw/d, with a LOAEL of 335 mg/kg bw/d for females based on an observed increase in total combined hepatocellular neoplasms (adenomas and carcinomas combined). Similarly, a NOAEL of 275 mg/kg bw/d and a LOAEL of 742 mg/kg bw/d for males were established based on increased incidences of hepatocellular neoplasms (adenomas and carcinomas). Ancillary studies showed high levels of peroxisome proliferation in high dose animals as indicated by significant increases in palmitoyl-CoA oxidase activity. This suggested that the liver carcinogenicity was linked to peroxisome proliferative effects.

DINP was found to induce non-neoplastic lesions in liver and kidney at high dose (307-375 mg/kg bw/d) after 2-year oral administration of DINP in Fischer 344 rats (110/sex) (Exxon Biochemical Sciences, 1986*). A NOAEL of 15-18 mg/kg bw/d was established, based on increased incidence of mononuclear cell leukaemia (MCL) at 152-184 mg/kg bw/d. MCL is regarded as a common neoplasm in aged Fischer

344 rats with no comparable tumour type in humans (Caldwell, 1999b). A retrospective evaluation of kidney tissue was conducted using immunohistochemical techniques (Caldwell et al., 1999a*). Results showed a dose-dependent alpha 2µ-globulin accumulation in specific regions of male kidneys where increases in cellular proliferation were noted. These findings were attributed to a gender and species-specific alpha 2µ-globulin tumouregenic mechanism male rat kidneys that is not regarded as relevant to humans (Caldwell et al., 1999a*).

Another study using Sprague Dawley CD rats (70/sex/dose) was performed with a non-commercial, branched DINP (CAS no. 71549-78-5) in the diet at dose levels of 0, 500, 5000, 10000 ppm for a period of 2 years. An increased incidence of hepatocellular carcinomas was found in both sexes of the mid and high dose groups leading to a NOAEL of 500 ppm (27-33 mg/kg bw/d males-females, respectively). Also, increased incidence of testicular cell hyperplasia, slightly increased incidences of pancreatic islet cell tumours and parathyroid gland hyperplasia were observed in high dose males. Endometrial hyperplasia was observed in high dose females (Bio/Dynamics, 1986*).

In a 2-year rat study of DINP (CAS no. 68515-48-0) (Aristech Chemical Corporation, 1994*), a NOAEL for carcinogenicity was established at 88-108 mg/kg bw/d male-female Fischer 344 rats respectively, based on increased incidence of MCL observed at higher doses (LOAEL of 358-442 mg/kg bw/d). Increased liver tumours in both sexes and kidney tumours in males were also noted at the highest dose of 733-885 mg/kg bw/d.

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

Metabolites of two types of DINP (CAS 68515-48-0 and CAS 71549-78-5) were studied for their effects on gap junctional intercellular communication (GJIC) effects in the hepatocytes of various species including humans, rats and mice (Baker et al., 1996*). Metabolites of both forms of DINP inhibited GJIC in rat hepatocytes. In contrast, none of the metabolites of either form of DINP inhibited GJIC in human hepatocytes at non-cytotoxic doses. The GJIC assay has been claimed to have good cancer predictive potential for phthalates (Kalimi et al., 1995*).

Data not reported in previous evaluations

No data

Conclusion

Only one of seven cell transformation studies using Balb/c-3T3 mouse cells found in vitro evidence of transforming activity due to DINP. Incidence of MCL, kidney and liver neoplasia were observed in in vivo carcinogenicity animals studies. However, these tumours were regarded as of limited relevance to humans.

Table 5: Summary of key in vivo carcinogenicity studies

Species, study durationand test substances	Doses (mg/kg bw/d) and administration mode	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) and Effects observed	References
Rat Fischer 344; 2-year study; CAS 68515-48-0	0, 0.03, 0.3, 0.6% (0, 15-18, 152-184, 307-375 (m-f)) in diet	15-18	152-184; ↑ MCL, no neoplasic lesion	Exxon Biochemical Sciences (1986*)
Rat Sprague Dawley; 2-year study;; CAS 71549-78-5	0, 500, 5000, 10000 ppm (0, 27-33, 271-331, 553-672 mg/kg bw/d (m-f)) in diet	27-33	271-333; hepatocellular carcinomas (No MCL)	Bio/Dynamics (1986*)
Rat Fischer 344; 2-year study; CAS 68515-48-0	0, 500, 1500, 6000, 12000 ppm (0, 29-36, 88-108, 358-442, 733-885 (m-f)) in diet	88-108	358–442; ↑ MCL 733–885; hepatocellular neoplasia, renal tubule cell carcinomas	Aristech Chemical Corporation (1994*)
Mouse B6C3F1; 2-year study; CAS 68515-48-0	0, 500, 1500, 4000, 8000 ppm (0, 90-112, 275-335, 742-910, 1560-1887 (m-f)) in diet	112 (f); 275 (m)	335 (f) and 742 (m); ↑ total hepatocellular neoplasms (adenomas and carcinomas combined)	Aristech Chemical Corporation (1995*); Moore (1998*)

Based on ECB, 2003
↑ = increased

Liver carcinogenicity observed in both sexes was linked to peroxisome proliferative effects. On this basis, it is considered that DINP-induced hepatomegaly in animals is not a toxicological effect that is relevant for humans (NICNAS, 2008).

Kidney tumours in male rats are consistent with a specific male rat alpha 2µ-globulin accumulation mechanism. Retrospective histological studies of kidneys in male rats exposed to DINP noted accumulation of alpha 2µ-globulin in areas of cellular proliferation accompanying renal tubular nephropathy. Alpha 2µ-globulin nephropathy is regarded as a species-specific effect in male rats (Caldwell, 1999a).

MCL was observed in Fischer 344 rat studies. However, as MCL is a common neoplasm in Fischer 344 rats with no comparable tumour type in humans, its increased incidence after chronic exposure to some substances is considered to be a strain specific effect (Caldwell 1999b; ECB, 2003). This neoplasm observed in rats is unlikely to be relevant to humans.

Non-carcinogenic liver effects and kidney effects (including changes in kidney organ weights, urine chemistry and histopathology) observed in rodents from repeated DINP exposure were not replicated in primates.

4.8 Reproductive toxicity

Traditional hazard assessments consider effects on fertility separate from developmental toxicity. Fertility is tested by exposing sexually mature adults to a chemical and examining the effects on reproductive capacity. Developmental toxicity is studied by exposing pregnant dams and looking for effects in the foetuses. Chemicals that affect the developing reproductive system following prenatal exposure may also affect sexual maturation or functional reproductive disorders that are only apparent at maturity. Developmental toxicity can therefore lead to effects on fertility and the two endpoints cannot be clearly distinguished.

In this hazard assessment, data are presented on the basis of test procedure. Studies include repeat dose toxicity studies that dose adult animals for varying duration, two-generation studies, prenatal developmental toxicity studies (only the dam is dosed, study ends before parturition) and postnatal developmental toxicity studies (dam is dosed during gestation and allowed to litter, study ends during weaning). The effects on fertility (as adults) and development (as foetuses) are then discussed separately.

4.8.1 Human studies

Previous evaluations

Duty et al. (2003) examined whether phthalate monoesters present in urine in men attending an andrology clinic were associated with altered semen quality. Eight urinary phthalate monoesters including MINP were measured in a single spot urine sample collected on the same day as the semen sample. MINP levels were below the limit of detection.

The following information was summarised from ECB (2003). Major studies and results are summarised in Table 6.

4.8.2 Repeated dose toxicity studies

Previous evaluations

Minor effects on reproductive organs were noted in both the repeated dose and carcinogenicity studies discussed earlier (see Sections 3.5 & 3.7). In a 2-year study with Fischer 344 rats (Exxon Biochemical Sciences, 1986*), there was a slight increase in relative (statistically significant) and absolute (not statistically significant) testes weights at 0.6% (307 mg/kg bw/d). In sub-acute and sub-chronic studies in mice (Hazelton, 1991b*, 1992*), slight but significant decreases in absolute and/or relative testes weights were observed from 6000 ppm (1377 mg/kg bw/d) with some accompanying pathological changes. A NOAEL for reproductive effects of 275 mg/kg bw/d was derived in a 2-year study in mice, based on a decrease in testicular weight without histological changes at 742 mg/kg bw/d (Aristech Chemical Corporation, 1995*). Also observed were an increased incidence of testicular cell hyperplasia in high dose males (553 mg/kg bw/d) and endometrial hyperplasia in high dose females (672 mg/kg bw/d), in a 2-year study with Sprague Dawley rats (Bio/Dynamics, 1986*).

No changes in testes weight and histology were observed in a 13-week study in monkeys given 2500 mg/kg bw/d (Huntingdon Life Sciences, 1998*) (see section 3.5).

4.8.3 One/Two-generation reproductive toxicity studies

Previous evaluations

A one-generation reproductive toxicity study in rats (30/sex/dose) employed levels of 0, 0.5%, 1.0% and 1.5% DINP (CAS 68515-48-0) in the feed from 10 weeks prior to mating and through the mating period (Exxon Biomedical Sciences, 1996d*, Waterman et al., 2000). Dosing continued in dams through gestation to postnatal day 21.

Statistically significant decreases in food consumption and body weights were observed, particularly in the mid- and high-dose parental animals. There were statistically significant dose related increases in the absolute and/or relative liver and kidney weights of both male and female animals at all dose levels tested. There was a statistically significant increase in absolute and relative testes weight, right epididymis weights and relative left epididymis and seminal vesicle weights in the high dose males (1.5%) compared to controls. In females, there was a statistically significant decrease in absolute and relative right ovarian, and absolute left ovarian weights of the high dose females (1.5%) compared to controls. There was no gross post-mortem findings in the parental animals considered related to DINP treatment. No histopathological examinations were carried out.

There were no statistically significant effects of DINP on male mating, female fecundity or female gestational indices. Mean days of gestation of the treated and control groups were essentially equivalent. There was a decrease in the live birth index and survival of offspring during lactation in the high dose animals (1.5%) compared to controls. The majority of offspring in all groups were free of external abnormalities from PND 0-21. Dose-related decreases in mean offspring body weight were observed during the postnatal period with all treatment doses at PND 0 and PND 14 to 21. Pup weights were below the historical range at the highest dose (1.5%). These findings were considered a result of decreased maternal food consumption and body weight and/or from direct effects of DINP on pup milk consumption via exposure through lactation. The NOAEL for fertility was 1.0% (622-1731 mg/kg bw/d) based on decrease in live birth and survival indices at the high dose group. The NOAEL for developmental toxicity could not be established due to decreased pup weight at the lowest dose tested (301-923 mg/kg bw/d).

A GLP compliant two-generation reproduction rat study was performed with DINP (CAS 68515-48-0) at 0, 0.2%, 0.4% and 0.8% in the diet for 10 weeks prior to mating and through the mating period (Exxon Biomedical Sciences, 1996e*, Waterman et al., 2000). F0 dams were dosed through pregnancy and lactation. F1 offspring were dosed from weaning, mating and pregnancy until PND 1.

Statistically significant increases in absolute kidney weights were observed from 0.2 % in F0 females, from 0.4% in F0 males and at 0.8% in F1 males. Statistically significant increases in absolute liver weights were observed from 0.4% in F0 females, at 0.8% in F0 males and at 0.8% in F1 females. Minimal to moderate increased hepatocytic cytoplasmic eosinophilia with occasional enlargement of affected hepatocytes was noted from 0.2% in males and females from all treatment

groups in both generations. There were no effects on reproductive organ weights. There were no changes in mating, male or female fertility, fecundity, gestational index, or length of gestation in the F0 or F1 generations.

DINP produced no changes in live birth index, sex ratio or offspring survival during lactation in the F1 or F2 generations. There was a dose-related decrease in offspring body weight during the postnatal period (PND 0-21) at PND0 for F1 high dose males only and at all doses at PND21. However, pup weights were within historical control range. The NOAEL for fertility effects was 0.8% (477-1541 mg/kg bw/d). The LOAEL for developmental effects was 0.2% (159-395 mg/kg bw/d) based on reduced pup weights in F1 and F2 pups during lactation.

4.8.4 Prenatal developmental toxicity studies

Previous evaluations

Exxon Biomedical Sciences (1994*, Waterman et al., 1999) conducted a developmental toxicity study using four groups of 25 rats administered DINP (CAS 68515-48-0) by gavage at doses of 0, 100, 500 or 1000 mg/kg bw/d between gestation days (GD) 6 and 15. The study was performed substantially in accordance with GLP procedures. There were no maternal effects except for statistically significant decreases in body weight gain and mean food consumption at 1000 mg/kg bw/d during the treatment period, leading to a NOAEL for maternal toxicity of 500 mg/kg bw/d. Note should be taken that normal weight and food consumption patterns were observed during the late gestation period, after exposure ceased, possibly indicating a recovery effect.

In terms of developmental effects, administration of DINP (CAS: 68515-48-0) to the dams resulted in a statistically significant increase in total foetuses with skeletal rudimentary lumbar ribs, dilated renal pelvis and visceral variations at 1000 mg/kg bw/d on a per litter basis. These variations are relatively common in rodents, however the induced frequencies (6/23 visceral (mainly dilated renal pelvis) variations at 1000 mg/kg vs. 0/23 in controls and 20/23 rudimentary lumbar ribs at 1000 mg/kg compared to 15/24 for controls) are outside historical control ranges. The NOAEL for developmental toxicity is therefore 500 mg/kg bw/d.

The ECB (2003) report discusses another rat prenatal screening toxicity study that used three variants of DINP (CAS 68515-48-0 and two other formulations that are both referred to as CAS 28553-12-0). In each case, gavage doses of 0, 40, 200 and 1000 mg/kg bw/d of DINP were administered to rats (8-10/group) on GD 6-15 (BASF 1995 a*,b*; Hellwig et al., 1997).

For CAS 68515-48-0, an increased occurrence of foetal skeletal variations at 1000 mg/kg bw/d, consisting mainly of rudimentary cervical and accessory 14^{th} ribs (no quantitative data was available), led to a NOAEL for developmental toxicity of 200 mg/kg bw/d. The NOAEL for maternal toxicity was 200 mg/kg bw/d. The LOAEL for maternal toxicity was 1000 mg/kg bw/d based on a slight decrease in food consumption and an increase in relative kidney weights.

For CAS 28553-12-0 ("DINP2"), a NOAEL for developmental toxicity was established at 200 mg/kg bw/d based on a statistically significant increased incidence of a skeletal variation namely accessory 14^{th} ribs (5/10 vs. 0/10 in controls on a per litter basis) at 1000 mg/kg bw/d. In addition, multiple malformations were seen in one foetus among 67 foetuses, examined at 1000 mg/kg bw/d, namely globular-

shaped heart, unilobular lung, hydrocephaly, dilation of the aortic arch and anasarca. At 200 mg/kg bw/d, transposition of great vessels was observed in one foetus among 65 foetuses examined. The NOAEL for maternal toxicity was stated to be 200 mg/kg bw/d, although this was apparently based on the occurrence of vaginal haemorrhage in one dam at 1000 mg/kg bw/d. A study was also performed on CAS 28553-12-0 ("DINP3"), but as this material has reportedly not been manufactured since 1995, it will not be considered here.

Another developmental toxicity study was performed using doses of 0, 10, 500 and 1000 mg/kg bw/d (CAS not specified) administered by gavage to rats on GD 6-15 (Hazleton, 1981*). In this study, no significant maternal or foetal effects were noted and the relevant NOAELs were 1000 mg/kg bw/d, the highest dose tested.

Data not reported in previous evaluations

Pregnant Wistar rats were gavaged during GD 7-21 with DINP (CAS 28553-12-0) at 750 mg/kg bw/d (Borch et al., 2004). Administration of DINP resulted in significant reduction in testicular testosterone content and production at GD 21 in male foetuses. The authors stated that the doses of DINP used were not expected to cause maternal toxicity, but no supportive data were provided. Note should be taken, however, that the same dose (750 mg/kg bw/d) was able to produce parental toxicity in the one and two-generation reproductive studies discussed above (see Robust Study Summary at Section 7).

4.8.5 Developmental/postnatal toxicity studies

Previous evaluations

In a study on rats using a range of phthalates (Gray et al., 2000), DINP was administered by gavage to dams at 750 mg/kg bw/d from GD 14 to PND 3. There was no overt maternal toxicity or reduced litter sizes, although DINP reduced pregnancy weight gain to GD 21. As infants, male offspring displayed female-like areolas/nipples in 22% of cases (PND 13) compared with 0% in the controls. There was no effect on male reproductive organ weight, but DINP induced reproductive malformations in 7.7% of male offspring ($p<0.05$). Preputial separation was not delayed by DINP (see Robust Study Summary at Section 6). The authors suggested that the weak antiandrogenic activity of DINP may be due to the presence of some phthalates with a 6-7 ester group in the mixture.

Data not reported in previous evaluations

In a follow up study by the Gray group (Ostby et al., 2001), DINP was also administered by gavage in Sprague-Dawley rats from GD 14 to PND 3 using higher dosage levels of 0, 1000, and 1500 mg/kg bw/d to confirm its antiandrogenic action in utero. At PND 2, males exposed to 1500 mg DINP displayed reduced anogenital distance while female anogenital distance was unaffected by treatment. DINP also increased the percentage of males with areolas on PND 13 in a dose related fashion from 14% in control males to 55% and 75% in the 1000 mg and 1500 mg DINP groups, respectively.

The potential impact of dietary exposure to DINP (400, 4000 and 20000 ppm) from GD 15 – PND 10 was examined in Sprague-Dawley rats (5/sex/dose) (Masutomi et al., 2003). A decrease in body weight gain in dams, as well as reduction in their food

consumption were observed at 20000 ppm. A slight, but not significant decrease in litter size was also noted at this dose.

Neonatal body weights in 4000 and 20000 ppm DINP groups, measured on PND 2, showed a non-significant tendency to decrease. Reduction in offspring body weight gain was noted in both sexes during PND 2-10, and in males during PND 21-42 at 20000 ppm (maternal intake of 1164-2656 mg/kg bw/d). Recovery was noted after cessation of exposure. There were also reductions in absolute and relative testes weights and increases in relative adrenal weight in PNW 3. No developmental alterations were observed, other than slight degeneration of meiotic spermatocytes at stage XIV and vacuolar degeneration of Sertoli cells at 20000 ppm. In female offspring, there was a decrease of corpora lutea in the ovary observed on PNW 11. Anogenital distances were not significantly changed in either sex in any treatment group but vaginal opening and preputial separation were decreased in the high dose group.

4.8.6 Mode of action

Studies were conducted to examine the in vitro (Nishihara et al., 2000, Harris et al, 1997; Takeuchi et al., 2005) and in vivo (Zacharewski et al., 1998) oestrogenic activities of DINP. DINP did not induce oestrogenic responses in vivo in uterotrophic and vaginal cornification assays using immature and mature ovariectomised rats (Zacharewski et al., 1998). DINP was negative for oestrogenic activity in a yeast two-hybrid assay (Nishihara et al., 2000), and showed extremely weak oestrogenic activity in a recombinant yeast assay (Harris et al., 1997). DINP did not demonstrate oestrogenic activities in a human oestrogen receptor (ER) α and β reporter gene assay in CHO-K1 cells transfected with expression vectors for ERα, ERβ and androgen receptor (AR) (Takeuchi et al., 2005). DINP was not a competitive agonist at the oestrogen receptor in an in vitro competitive ligand-binding assay and did not induce oestrogen receptor-mediated gene expression in MCF-7 cells (Zacharewski et al., 1998).

The results indicate that DINP is at best weakly oestrogenic in vitro. Current data indicate that DINP is not oestrogenic in vivo.

Conclusion

Effects on fertility

In general, DINP had no effect on male mating, female fecundity, and gestational index or length of gestation up to 1% in the diet (622-1731mg/kg bw/d) in rats. From a one-generation reproductive toxicity study in rats, the NOAEL for fertility was 622 mg/kg bw/d based on decrease in live birth and survival indices.

With regards to effects on reproductive organs, effects of DINP on testes weight were inconsistent. Increased testes weight was induced following exposure of rats to 1.5 % (966–2246 mg/kg bw/d) DINP in the diet from 10 weeks prior to mating to birth of litter without accompanying histopathological changes. Also, in a chronic toxicity study, 0.6% DINP in the diet (307-375 mg/kg bw/d) was associated with a small (statistically insignificant) increase in testes weight. However, decreased testes weight was noted at weaning in rats exposed prenatally and lactationally to 20000 ppm DINP in the diet (1164-2656 mg/kg bw/d). In repeat dose studies in mice, DINP in the diet at 4000 ppm (742-910 mg/kg/d) for 2 years, 6000 ppm (1377-1671

mg/kg/d) for 4 weeks or 10000 ppm (2600 mg/kg/d) for 13 weeks were associated with decreased testes weight. In the majority of studies, histopathological changes in the testes were not observed.

Overall, no overt toxicity was observed in reproductive organs in rats. From a 2-year carcinogenicity study in mice, a NOAEL for reproductive effects in males was established at 275 mg/kg bw/d with a LOAEL of 742 mg/kg bw/d based on decreased testes weight.

Developmental effects

In general, developmental toxicity studies suggested that exposure to DINP during early gestation induced skeletal and visceral variations but only at materno-toxic doses. However, as these variations were induced in a dose-responsive manner, the induction of variations were considered to be significant, leading to a NOAEL in a prenatal developmental toxicity study in rats of 500 mg/kg bw/d. The LOAEL was 1000 mg/kg bw/d.

Later gestational exposure was associated with retention of nipples in males and increases in malformations of the male reproductive tract. For these effects the LOAEL was 750 mg/kg bw/d. No NOAEL was derived.

A well-conducted two-generation study in rats noted decreased pup weight at PND 21 establishing a developmental LOAEL of 159–395 mg/kg bw/d.

Table 6: Summary of reproductive and developmental studies on DINP in rats

Species/study duration	Route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & endpoint	Test Substance	Reference
One generation studies						
Rat Crl: CDBR	Diet	0, 0.5, 1, 1.5% (0, 301-923, 622-1731, 966-2246; m-f); premating-postpartum	Mat: NE Repro: 622–1731 (m-f) Devp: NE	Mat: 301-923 (m-f) ↑ liver & kidney wt Repro: 966-2246 (m-f) ↓ live birth index, ↓ pup survival Devp: 301-923 (m-f) ↓ pup wt in F1;	68515-48-0	Exxon Biomedical Sciences (1996d*) Waterman et al. (2000)
Two-generation studies						
Rat Crl: CDBR	Diet	0,0.2,0.4,0.8% (0, 118-395, 236-758, 477-1541 mg/kg/d; m-f); premating-postpartum	Mat: NE Repro: 477-1541 (m-f) Devp: NE	Mat: 114-395 (m-f) ↑ kidney, liver wt & histologic changes Repro: NE Devp: 159-395 (m-f) ↓ pup wt in F1 at PND21	68515-48-0	Exxon Biomedical Sciences (1996e*) Waterman et al. (2000)
Developmental toxicity studies						
Rat Sprague Dawley GD6-15	Gavage	0, 100, 500, 1000	Mat: 500 Devp: 500	Mat: 1000 Devp: 1000 ↑ dilated renal pelvis	68515-48-0	Exxon Biomedical Sciences (1994*) Waterman et al. (1999)
Rat Wistar GD6-15	Gavage	0, 40, 200, 1000	Mat: 200 Devp: 200	Mat: 1000 Devp: 1000 ↑ cerv & 14th rib	68515-48-0	BASF (1995a*) Hellwig et al. (1997)
Rat Wistar GD6-15	Gavage	0, 40, 200, 1000	Mat: 200 Devp: 200	Mat: 1000 Devp: 1000 ↑ 14th rib	28553-12-0	Hellwig et al. (1997)
Rat Sprague Dawley GD6-15	Gavage	0, 10, 500, 1000	Mat: 1000 Devp:1000	Mat: NE Devp: NE	CAS not specified	Hazleton (1981*)
Rat Sprague Dawley	Diet	0, 400, 4000, 20000 ppm (0, no	Mat: 306-656	Mat: 1164-2656	28553-12-0	Masutomi et al.

Species/study duration	Route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & endpoint	Test Substance	Reference
GD15-PND10		data, 306-656, 1164-2656)	Devp: 306-656	Devp: 1164-2656 ↓pup wt, ↓ testes wt		(2003)
Rat Wistar GD7-21	Gavage	0, 750	Devp: NE	Devp: 750 ↓ testicular testosterone content & production at GD21	28553-12-0	Borch et al., 2004
Rats Sprague Dawley GD14 - PND3	Gavage	0, 750	Mat: 750 Devp: NE	Mat: NE Devp: 750 ↑ nipples/nipples in males; ↑ male reproductive malformations		Gray et al., 2000
Repeat dose studies						
13-week study Marmoset monkeys (16-25-month old)		0, 100, 500, 2500	500	2500; ↓ body weight; ↓ body weight gain	CAS not specified	Huntington Life Sciences (1998*)
Rat Fischer 344 2-year	diet	0,0.03,0.3,0.6% (0, 15-18, 152-184, 307-375; m-f)	15-18	307-375 ↑ testes wt	68515-48-0	Exxon Biomedical Sciences (1986*)
Mouse B6C3F1 2-year	diet	0,500,1500,4000, 8000 ppm (0, 90-112, 275-335, 742-910, 1562-1887; m-f)	275-335	742-910 ↓ abs & rel testes wt	68515-48-0	Aristech Chemical Corporation (1995*) Moore (1998*)
Mouse B6C3F1 4-week	diet	0, 3000, 6000, 12000, 25000 ppm (0, 635-780, 1377-1671, 2689-3287, 6518-6920; m-f)	635-780	1377-1671 ↓ abs/rel testes wt	28553-12-0	Hazleton (1991b*)
Mouse B6C3F1 13-week	diet	0, 1500, 4000, 10000, 20000 ppm (0, 365, 972, 2600, 5700)	972	2600 ↓ abs epididymis & testes wt	28553-12-0	Hazleton (1992*)

Based on ECB (2003)
Mat: maternal; Repro: reproductive; Devp: developmental; Fert: Fertility; wt: weight; rel: relative; abs: absolute
NE = not established; ↓ = decreased; ↑ = increased

5. Hazard Characterisation

DINP is not a single compound, but a complex mixture containing mainly C8, C9-branched isomers. The composition of CAS 68515-48-0 is represented as mixed phthalates with side chains made up of 5-10% methyl ethyl hexanol, 45-55% dimethyl heptanol, 5-20% methyl octanol, 0-1% n-nonanol, and 15-25% isodecanol; and the composition of CAS 28553-12-0 is represented as mixed phthalates with side chains made up of 5-10% methyl ethyl hexanol, 40-45% dimethyl heptanol, 35-40% methyl octanol, and 0-10% n-nonanol. Thus, diisononyl phthalate [side chains of dimethyl heptanol (i.e. isononanol)] makes up about 50% of the two 'DINP' mixtures which appear to be available on the market. The above structural formulas are those associated with the CAS numbers given but they do not reflect the complexity of the commercially available phthalate mixtures.

When orally administered to rats, a large proportion of DINP is rapidly metabolised in the GIT to the corresponding monoester (MINP) and absorbed and excreted, primarily in the urine as oxidation products and phthalic acid. Generally, at least 50% of an oral dose is absorbed and excreted in the urine. DINP is excreted in the faeces as the parent compound as well as MINP and oxidation products. DINP and its metabolites do not accumulate in the body. There is limited absorption by the dermal route (<4% in 7 days).

In animal studies, DINP has a low order of acute toxicity by the oral (LD50 >10000 mg/kg bw), dermal (LD50 >3160 mg/kg bw) and inhalation toxicity (LC50 >4.4 mg/L). DINP caused minimal skin and eye irritation and is unlikely to cause skin sensitisation.

The target organs for chronic toxicity are the liver and kidney. A NOAEL of 88-108 mg/kg bw/d was derived from a two-year well-conducted study in rats (Aristech Chemical Corporation, 1994*). This NOAEL was based on liver and kidney toxicity at higher doses indicated by biochemical parameter changes (increased serum ALT, AST), liver weight changes (increased absolute and relative liver weights) and concurrent histopathological alterations, as well as kidney changes (increased absolute/relative kidney weights) in both sexes. In monkeys, oral administration of DINP for 13 weeks with doses up to 2500 mg/kg bw/d produced no treatment-related changes in liver weights, biochemical parameters or histological findings. Slight increases in liver enzyme activities were not regarded as treatment-related due to lack of concomitant histological or organ weight changes (Huntington Life Sciences, 1998).

No studies involving the inhalation route have been conducted. Based on a 6-week dermal study in rabbits, a NOAEL of 0.5 mL/kg (approx 500 mg/kg bw/d) was derived for dermal application, with gross skin alterations seen at a higher dose (2500 mg/kg bw/d).

Based on the available data, DINP is considered not to be genotoxic.

Increased incidences of MCL, kidney and liver neoplasia were observed in animals. Liver carcinogenicity observed in both sexes was linked to peroxisome proliferative effects. On this basis, it is considered that DINP-induced hepatocarcinogenicity in animals is not a toxicological effect that is relevant for humans (NICNAS, 2008).

Kidney tumours in male rats are consistent with a specific male rat alpha 2µ-globulin accumulation mechanism. Retrospective histological studies of kidneys in male rats exposed to DINP noted accumulation of alpha 2µ-globulin in areas of cellular proliferation accompanying renal tubular nephropathy. Alpha 2µ-globulin nephropathy is regarded as a species-specific effect in male rats (Caldwell, 1999a).

MCL was observed in Fischer 344 rat studies. However, as MCL is a common neoplasm in Fischer 344 rats and its increased incidence after chronic exposure to some substances is considered to be a strain specific effect. This neoplasm observed in rats has no human correlate and so is unlikely to be relevant to humans (Caldwell 1999b; ECB, 2003).

Non-carcinogenic liver and kidney effects (including changes in kidney organ weights, urine chemistry and histopathology) observed in rodents from repeated DINP exposure were not replicated in primates.

In general, DINP had no effect on male mating, female fecundity or female gestational indices. A NOAEL for fertility was established at 622 mg/kg bw/d based on decrease in live birth and survival indices at the high dose group.

With regards to effects on reproductive organs, effects of DINP on testes weight were inconsistent, with increased testes weight noted in a two-generation reproduction study and chronic toxicity study in rats (doses up to 2246 mg/kg bw/d) but decreasing testes weight in rats exposed prenatally and lactationally to 20000 ppm DINP in the diet (1164-2656 mg/kg bw/d) and in a number of chronic repeat dose toxicity studies in mice. Histopathological changes in the testes were not observed in most of these studies. The NOAEL for reproductive effects is 275 mg/kg bw/d from a 2 year carcinogenicity study in mice, with decreased testes weight observed at 742 mg/kg bw/d.

The developmental toxicity studies in rats indicate that exposure to DINP during early gestation induces skeletal and visceral variations but only at materno-toxic doses (LOAEL was 1000 mg/kg bw/d). A NOAEL of 500 mg/kg bw/d was derived. Later gestational exposure was associated with retention of nipples in males and increases in malformation of the male reproductive tract. The LOAEL was 750 mg/kg bw/d. No NOAEL was derived. Decreased pup weight was noted on PND 21 in a two-generation study in rats with a LOAEL of 159-395 mg/kg bw/d. No NOAEL was derived.

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6. Human Health Hazard Summary Table

Phthalate	***Acute***	***Irritation & Sensitisation***	***Repeated Dose Toxicity***	***Genetic Toxicity***	***Carcinogenicity***	***Fertility***	***Developmental Toxicity***
Diisononyl phthalate (DINP)	Oral Rat: LD50 >10000 mg/kg bw (CAS 68515-48-0), LD50 >40000 mg/kg bw (CAS 28553-12-0) Dermal Rabbit: LD50 >3160 mg/kg bw (CAS 68515-48-0) Inhalation Rat: LC50 >4.4 mg/L/4h	Skin Irritation: minimal effects Eye Irritation: minimal effects Skin Sensitisation: negative	Rat: NOAEL = 88-108 (m-f) mg/kg bw/d LOAEL = 358-442 (m-f) mg/kg bw/d ↑ kidney and liver weight, liver histopathological changes, serum enzyme changes. PP noted High doses: liver enlargement and necrosis, renal tubular necrosis	In vitro: Negative in bacterial mutation assays Negative in chromosomal aberrations assay Negative in mouse lymphoma assay Negative in unscheduled DNA synthesis assay In vivo: Negative in cytogenetic assay	In vitro Positive in 1 of 7 cell transformation assays In vivo: *Two year dietary study* F-344 rat: NOAEL = 88-108 (m-f) mg/kg bw/d LOAEL = 358-442 (m-f) mg/kg bw/d ↑ MCL *Two year dietary study* B6C3F1 mouse: NOAEL = 112 (f) & 275 (m) mg/kg bw/d LOAEL = 335 (f) & 742 (m) mg/kg bw/d ↑ total hepatocellular neoplasms (adenomas and carcinomas combined)	*One-generation study* Rat: NOAEL = 622 mg/kg bw/d LOAEL = 966 mg/kg bw/d ↓ live birth and survival indices *Two-repeat dose study* Mouse: NOAEL = 275 (m) mg/kg bw/d LOAEL = 742 (m) mg/kg bw/d ↓ testes wt	*Two-generation study* Rat: NOAEL = NE LOAEL = 159-395 (m-f) mg/kg bw/d ↓ pup wt at weaning *Developmental studies:* Rat: NOAEL = NE LOAEL = 750 mg/kg bw/d ↑ areolas/nipples in males; ↑ male reproductive malformations Rat: NOAEL = 500 mg/kg bw/d LOAEL = 1000 mg/kg bw/d ↑ skeletal & visceral variations

M: male; f: female; ↑: increased; ↓: decreased; PP: peroxisome proliferation.

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Appendix - Robust Study Summaries

Reproductive toxicity

Test substance	:	DINP (CAS no. unstated)
Type	:	Study on effect of phthalates administered to dams on foetal male rat steroidogenesis.
Species	:	Pregnant Wistar rats
Route of admin.	:	Oral
Exposure period	:	GD 7 to GD 21
Study Duration	:	Not specified
Frequency of treatment.	:	Daily
Duration of test	:	21 days
Doses	:	750 mg/kg bw/d
Control group	:	Dosed with vehicle only (peanut oil)
NOAEL parental	:	Not determined
NOAEL F1 offspring	:	N/A
Other: systemic effects	:	Reduced testicular testosterone content and production in male foetuses at GD21.
Guidelines	:	Not stated
GLP	:	Not stated
Method	:	Control group of 8 dams administered vehicle only. Test group of 8 dams dosed with 750 mg/kg bw/d of DINP from GD7 to GD21.
Result	:	Testicular testosterone content and production significantly reduced in male foetuses at gestation day 21.
Conclusion	:	Testicular testosterone reduction in male foetuses during gestation following DINP maternal exposure.
Reference	:	Borch J, Ladefoged O, Haas U & Vinggaard AM (2004). Steroidogenesis in foetal male rats is reduced by DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in foetal, prepubertal and adult male rats. Reproductive Toxicology, 18: 53-61.

Reproductive Toxicity

Test substance	:	DINP (CAS no. unstated)
Type	:	Study on effect on perinatal exposure of phthalates on sexual differentiation of the male rat
Species	:	Pregnant Sprague-Dawley rats
Route of admin.	:	Oral
Exposure period	:	GD 14 to PND 3
Study Duration	:	Not specified
Frequency of treatment.	:	Daily
Duration of test	:	Not specified, but examination up to the onset of puberty was noted
Doses	:	750 mg/kg bw/d
Control group	:	Dosed with vehicle only (laboratory grade corn oil)
NOAEL parental	:	Not determined
NOAEL F1 offspring	:	N/A
Other: systemic effects	:	Nipple retention and testes atrophy after perinatal exposure to 750 mg/kg bw/d in male rats
Guidelines	:	Not stated
GLP	:	Not stated
Method	:	Control group of 19 dams administered vehicle only. Test group of 14 dams dosed with 750 mg/kg bw/d of DINP from GD14 to PND3.
Result	:	Male offspring displayed female like areolas/nipples in 22% of cases compared with 0% in the controls. DINP also induced reproductive malformations in 7.7% of male offspring ($p<0.05$) compared with controls.
Conclusion	:	Maternal administration of DINP induced areolas/nipples development and malformations in male offspring.
Reference	:	Gray Jr LE, Ostby J, Furr J, Price M, Veeramachaneni DNR & Parks L (2000) Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicological Sciences **58**, 350-365.

Reproductive Toxicity

Test substance		DINP (CAS no. unstated)
Type	:	Study on the potential impact of dietary exposure to DINP during the sensitive period of brain sexual differentiation of foetuses
Species	:	Pregnant Sprague-Dawley rats (Charles River Japan, Inc)
Route of admin.	:	Oral
Exposure period	:	GD 15 to PND 10
Study Duration	:	Postnatal Week 11
Frequency of treatment.	:	Daily
Duration of test	:	Not specified, but prepubertal necropsy was conducted on PND 27
Doses	:	400, 4000, 20000 ppm
Control group	:	Powdered soy-free diet without DINP
NOAEL parental	:	Not determined
NOAEL F1 offspring	:	N/A
Other: systemic effects	:	There were signs of maternal toxicity at 20000 ppm (1164-2656 mg/kg bw/d: decreased food intake and body weight) with slight decrease (but not significantly) in litter size as compared to corresponding controls. Decreased body weight gain was observed in dams from GD15 – PND 10 with reduction in food consumption noted at the same period
Guidelines	:	Not stated
GLP	:	Not compliant
Method	:	Group of 5 dams were provided with powdered soy-free diet with 0, 400, 4000, 20000 ppm of DINP from GD7 to PND 10. Prepubertal necropsy was conducted on PND 27.
Result	:	Neonatal body weights of 4000 and 20000 ppm (306-656 and 1164-2656 mg/kg/d) of DINP, measured on PND 2, showed non-significant decrease. At 20000 ppm (1164-2656 mg/kg/d), reduced offspring body weight gain of both sexes during PND 2-10 and male only during PND 21-42 were observed (recovery was noted after cessation of exposure). There was also reduction in absolute and relative weights of the testes in PNW 3. Histopathological alterations in the gonadal organs, including degeneration of meiotic spermatocytes at stage XIV, vacuolar degeneration of Sertoli in the testes and decrease of corpora lutea in the ovary was observed in PNW 11.
Conclusion	:	Maternal exposure to DINP at 20000 ppm induced effects on the testes of male offspring.
Reference	:	Masutomi N, Shibutani M, Takagi H, Uneyama C, Takahashi N, Hirose M (2003) Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life. Toxicology 192, 149-170.