**Existing Chemical
Hazard Assessment
Report**

Diisoheptyl Phthalate

June 2008

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.

The principal aim of NICNAS is to aid in the protection of people at work, the public and the environment from the harmful effects of industrial chemicals.

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.

For the purposes of Section 78(1) of the Act, copies of assessment reports for New and Existing Chemical assessments are freely available from the web (www.nicnas.gov.au). Summary Reports are published in the *Commonwealth Chemical Gazette* (http://www.nicnas.gov.au/publications/#gazette), and are available to the public on line at www.nicnas.gov.au.

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Overview

This review of diisoheptyl phthalate (DiHepP) is a health hazard assessment only. For this assessment, the Organisation for Economic Cooperation and Development, Screening Information Data Set (OECD SIDS) Initial Assessment Report on DiHepP was consulted. Information from this report was supplemented with literature surveys conducted up to September 2006.

DiHepP production volume is between 20000 and 200000 tonnes per annum world wide, with one production site in Europe and one in the USA. DiHepP is principally used with polymers as an additive to impart flexibility in polyvinylchloride (PVC) resins. PVC-containing phthalate ester applications include flooring and wall coverings. Polymers containing phthalate ester applications that are non-PVC based include cellulose plastics, rubbers and selected paints and adhesives.

In Australia, DiHepP is imported for use as a specialist PVC plasticiser and in screen printing inks.

Structurally, phthalate esters are characterized by a diester structure consisting of a benzenedicarboxylic acid head group linked to two ester side chains. DiHepP possesses 2 branched ester side chains each with a backbone of predominantly 6 carbons (C6). DiHepP is considered to belong to a group of 'transitional' phthalates defined as those produced from alcohols with straight-chain carbon backbones of C4-6.

Toxicity data for DiHepP were not available for all health endpoints. For endpoints with missing or incomplete data, information from structurally similar phthalates, where available, was used to extrapolate potential toxicity. Relevant read-across information was obtained from other NICNAS hazard assessment reports for phthalates and the NICNAS Phthalates Hazard Compendium, which contains a comparative analysis of toxicity endpoints across 24 *ortho*-phthalates, including DiHepP.

No toxicokinetic data were available for DiHepP. Based on the toxicokinetic profile of phthalates in general, DiHepP is likely to be rapidly absorbed as the monoester from the gut and excreted via the urine.

DiHepP has low acute oral and dermal toxicity. It caused minimal skin and eye irritant effects in rabbits, and did not induce any skin sensitisation in guinea pigs or humans.

No repeat dose toxicity studies were available for DiHepP. In reproductive toxicity studies, effects in the liver, kidney and pituitary were seen, with histopathology reported in all three organs. Liver effects, namely hepatocyte enlargement, were consistent with repeat dose effects seen with other transitional phthalates. A repeat dose oral NOAEL of approximately 50-168 mg/kg bw/d (m-f) was determined for rats in these studies, with a LOAEL of 222-750 mg/kg bw/d (m-f) based on liver and kidney changes.

DiHepP was not mutagenic in bacterial mutation and cytogenetic test. No in vitro mammalian mutation and in vivo genotoxicity data were available for DiHepP. Overall, results of in vitro tests indicate that DiHepP is non-genotoxic.

In vivo studies in rats and mice undertaken to investigate the effects of DiHepP on mechanisms associated with hepatic carcinogenicity found that DiHepP had no inhibitory

effect on gap junctional intercellular communication (GJIC). However significant elevations in hepatic DNA synthesis were seen in both species.

No adequate carcinogenicity data are available for DiHepP. Due to insufficient testing on other phthalates, it is not possible to extrapolate carcinogenic potential for DiHepP.

In a two-generation study, effects on fertility (decreased reproductive performance and fertility index) were seen in both sexes in the F1 generation at the highest dose level 8000 ppm (approximately 830 mg/kg bw/d prior to breeding and 540 mg/kg bw/d during gestation). The NOAEL for reproductive effects in males and females was 4500 ppm (227-750 mg/kg bw/d) and the LOAEL was 8000 ppm (419-1360 mg/kg bw/d) in the F1 generation based on decreased reproductive organ weight.

Developmental effects seen in the two-generation study occurred mainly with the 4500 and 8000 ppm groups in F1 and F2 generations, respectively. The maternal and developmental NOAEL was 1000 ppm (50-168 mg/kg bw/d), and the LOAEL was 4500 ppm (222-750 mg/kg bw/d) based on decreased anogenital distance (AGD) in the F2 male offspring.

In another developmental study, overt developmental effects were seen at 750 mg/kg bw/d, which included an increase in resorptions (per litter and per implantation site) and a related decrease in live foetuses and an increased incidence of foetuses with external, visceral and skeletal malformations compared to controls. The developmental NOAEL was established at 300 mg/kg bw/d, with a LOAEL of 750 mg/kg bw/d based on increased resorptions and malformations.

DiHepP did not exhibit any oestrogenic activity when tested in most in vitro and in vivo assays with only an isomeric mixture demonstrating weak oestrogenic activities in a human oestrogen receptor a (ERa) (but not ERb) reporter gene assay.

Table of Contents

Acronyms and Abbreviations

AR	androgen receptor
bw	body weight
C	Celsius
CAS	Chemical Abstracts Service
CHO	Chinese hampster ovary
d	day
D711P	Di(heptyl, nonyl, undecyl) phthalate
DBP	dibutyl phthalate
DIDP	diisodecyl phthalate
DiHepP	diisoheptyl phthalate
DINP	diisononyl phthalate
DNA	deoxyribonucleic acid
DnOP	Di-n-octyl phthalate
ER	oestrogen receptor
f	female
F0	parental generation
F1	filial 1 (first generation)
F2	filial 2 (second generation)
g	gram
GJIC	gap junctional intercellular communication
GLP	good laboratory practice
h	hour
kg	kilogram
kPa	kilopascals
L	litre
LC50	median lethal concentration
LD50	median lethal dose
LOAEL	lowest-observed-adverse-effect level
m	male
mg	milligram
mL	millilitre
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOAEL	no-observed-adverse-effect level

OECD	Organisation for Economic Cooperation and Development
PBOX	peroxisomal beta-oxidation activity
ppm	parts per million
PVC	polyvinyl chloride
w/w	weight per weight
µ	micro

1. Introduction

This review of diisoheptyl phthalate (DiHepP) is a health hazard assessment only. For this assessment, an OECD SIDS Initial Assessment Report on Diisoheptyl Phthalate (OECD, 2005) was consulted. Information from this report was supplemented 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 report 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*-phthalates (NICNAS, 2008).

2. Identity

2.1 Identification of the substance

CAS Number(s): 71888-89-6

Note: DiHepP as the C7 isomer alone is known by CAS number 41451-28-9

Chemical Name: 1,2-Benzenedicarboxylic acid, di-C6-8-branched alkyl esters, C7 rich

Common Name: Diisoheptyl phthalate (DiHepP)

Molecular Formula: $C_{22}H_{34}O_4$

Structural Formula:
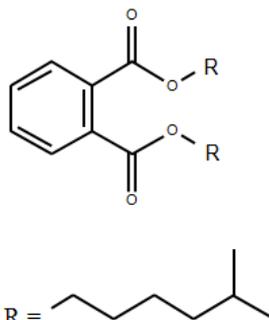
DiHepP consists of at least 80% of methylhexyl phthalate. Therefore the linear backbone is predominantly C6. The methyl group branching can be found on different C positions of the hexyl backbone chain.

Molecular Weight: 363 (based on di-C_7H_{15} alkyl ester)

Synonyms: DIHP; Diisoheptyl phthalate ester; 1,2-Benzenedicarboxylic acid, diisoheptyl ester

Purity/Impurities/Additives: Purity: >99.9% w/w

Impurities: ≤0.1% w/w, including isoheptyl alcohol (0.03%), diisoheptyl ether and isoheptyl benzoate (0.07%)

Additives: none

2.2 Physicochemical properties

Property	Value
Physical state	Liquid
Melting point	-45°C
Boiling point	398°C (101.3 kPa)
Density	994 kg/m^3 (20°C)
Vapour pressure	9.33 x 10^{-8} kPa (25°C)
Water solubility	1.7 x 10^{-5} g/L (22°C)
Partition coefficient n-octanol/water (log Kow)	6.87
Henry's law constant	1.99 Pa-m^3/mole (25°C)
Flash point	Not available

Source: OECD (2005). All values are calculated except relative density and water solubility.

3. Uses

DiHepP production volume is between 20000 and 200000 tonnes per annum world wide, with one production site in Europe and one in the USA. DiHepP is principally used with polymers as an additive to impart flexibility in polyvinylchloride (PVC) resins. PVC-containing phthalate ester applications include flooring and wall coverings. Polymers containing phthalate ester applications that are non-PVC based include cellulose plastics, rubbers and selected paints and adhesives (OECD, 2005).

In Australia, DiHepP is imported for use as a specialist PVC plasticiser and in screen printing inks.

4. Human Health Hazard

4.1   Toxicokinetics

Previous evaluations

No data.

Data not reported in previous evaluations

No data.

Conclusion

No toxicokinetic studies were available for assessment.

4.2   Acute toxicity

Previous evaluations

Study	Species	Results (LD50/LC50)	References	
Oral	Rats	>10000 mg/kg bw	MB Research	Laboratories, 1979*
Dermal	Rabbits	>3160 mg/kg bw	MB Research	Laboratories, 1979*

Data not reported in previous evaluations

No data.

Conclusion

DiHepP has low acute oral and dermal toxicity in laboratory animals. No acute toxicity data from inhalation exposure or human studies were available for DiHepP.

4.3   Irritation

4.3.1 Skin irritation

Previous evaluations

A single 24-h application of DiHepP to abraded rabbit skin, under occluded conditions, produced very slight erythema in 3 of 4 animals. No signs of oedema were observed (MB Research Laboratories, 1979*).

In preparation for skin sensitisation testing in a Human Repeated Insult Patch Test (HRIPT), 15 subjects were exposed to a group of C6 to C13 phthalates, including DiHepP. Undiluted test substances were applied to the skin under an occluded patch for 24 hours and readings were taken at 30 min and 24 h after patch removal. No significant irritation was noted from DiHepP (Medeiros et al., 1999).

Data not reported in previous evaluations

No data.

Conclusion

DiHepP caused minimal skin irritation in rabbits and humans.

4.3.2 Eye irritation

Previous evaluations

In a modified Draize test, DiHepP was a mild irritant, with a maximum total score of 10 observed at 1 h (Draize scale of 0-110). Mean scores at 24, 48, and 72 hours for the various indices were 0.50, 0.50, and 0.17, respectively for conjunctival redness; 0.33, 0.00, and 0.00, respectively for chemosis; 0.67, 0.50, and 0.00, respectively for discharge; no iridial or corneal effects were noted (MB Research Laboratories, 1979*).

Data not reported in previous evaluations

No data.

Conclusion

DiHepP caused minimal eye irritation in rabbits.

4.4 Sensitisation

Previous evaluations

Skin sensitisation studies have been conducted in guinea pigs using the Magnusson-Kligman and Buehler test methods. In the Magnusson-Kligman test, a weak sensitisation response was observed for DiHepP in guinea pigs during the re-challenge phase, but not in the challenge phase (Exxon Biomedical Sciences, 1991*). In the Buehler test, no indication of a sensitisation response was seen in guinea pigs (Huntingdon Research Centre, 1994*).

A Human Repeated Insult Patch Test (HRIPT) was conducted in a 104 people exposed to a group of C6 to C13 phthalates using the modified Draize procedure. Undiluted test substances including DiHepP were individually applied to the skin 3 times per week for 3 successive weeks during the induction and challenge phases. No evidence of skin sensitisation was noted from exposure to DiHepP (Medeiros et al., 1999).

Data not reported in previous evaluations

No data.

Conclusion

DiHepP did not induce skin sensitisation in guinea pigs or in humans.

4.5 Repeated dose toxicity

Previous evaluations

No repeat dose (oral) toxicity studies have been conducted on DiHepP, apart from reproductive toxicity studies, as reported below and in detail in Section 4.8.

In a developmental toxicity study (14 days gavage dosing at doses of 0, 100, 300 and 750 mg/kg bw/d) using Sprague-Dawley dams, the only data reported for non-reproductive effects were dose-related increases in mean absolute and relative maternal liver weights, which were statistically significant in the 750 mg/kg bw/d groups. The LOAEL for maternal effects in this study was determined as 750 mg/kg bw/d DiHepP (Exxon Biomedical Sciences Inc., 1997*; McKee et al., 2006).

In a two-generation reproduction toxicity (dietary) study in Sprague-Dawley rats (30/sex/dose, doses of 0, 1000, 4500 and 8000 ppm), dose-related increases in liver and kidney weights were seen in both sexes of parental F0 and F1 animals at 4500 ppm (222-750 mg/kg/d) and 8000 ppm (404-1360 mg/kg/d) and increased pituitary weights (F1 males at 8000 ppm). F0 animals were dosed for approximately 90 days (ie 70 days prior to mating). Histopathological findings in liver, kidney and pituitary included centrilobular hepatocellular hypertrophy and vacuolation, dilated renal pelves/hydronephrosis and for F1 animals, hypertrophy of the pars distalis of the pituitary gland. The NOAEL for systemic toxicity in F0 and F1 animals was determined as 1000 ppm in the diet (approximately 50-168 mg/kg/d, m-f), with a LOAEL of 4500 ppm (222-750 mg/kg/d, m-f) based on liver and kidney effects (Wil Research Laboratories Inc., 2003*; McKee et al., 2006).

Smith et al. (2000) reported that rats and mice fed diets containing DiHepP (0, 1000, 12000 mg/kg in rats; 0, 500, 6000 mg/kg in mice, for 2 and 4 weeks) produced effects indicative of peroxisome proliferation. This included increased periportal DNA synthesis and elevated peroxisomal beta-oxidation (PBOX) in the liver of both F344 rats and B6C3F1 mice, along with increased liver weights (F344 species only).

Data not reported in previous evaluations

No data.

Conclusion

In rats, the liver and kidney were the primary target organs, with increased organ weights and histological effects observed in these organs and to a lesser extent in the pituitary. Hepatocellular hypertrophy and vacuolation in the liver were likely associated with peroxisome proliferation. The repeat dose subchronic oral NOAEL in rats was 50-168 mg/kg bw/d (m-f) with a LOAEL of 222-750 mg/kg bw/d (m-f) based on liver and kidney effects.

4.6 Genetic toxicity

Previous evaluations

DiHepP was not mutagenic in *S. typhimurium* (TA 98, 100, 1535, 1537, 1538) reverse mutation assays, at concentrations up to 5000 mg/mL, with and without S9 metabolic activation (Exxon Biomedical Sciences Inc., 1995*).

DiHepP did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cells at concentrations up to 4990 mg/mL (Hazleton Laboratories America Inc., 1991*).

Data not reported in previous evaluations

No data.

Conclusion

DiHepP was negative in bacterial mutation and in vitro chromosomal aberration tests. No in vitro mammalian mutation and in vivo genotoxicity data were available for DiHepP.

4.7   Carcinogenicity

Previous evaluations

No in vivo carcinogenicity studies were available for assessment. Smith et al. (2000) investigated the effects of DiHepP on a number of mechanisms associated with hepatocarcinogenicity in male F344 rats and male B6C3F1 mice. DiHepP at dietary doses up to 12000 ppm in rats and up to 6000 ppm in mice (for up to 4 weeks) had no inhibitory effect on gap junctional intercellular communication (GJIC) in either species. However significant elevation of periportal DNA synthesis was seen in both species, after 2 weeks dosing of 1000 ppm (rats) and 500 ppm (mice).

Data not reported in previous evaluations

No data.

Conclusion

No in vivo carcinogenicity data were available for DiHepP.

4.8   Reproductive toxicity

Traditional hazard assessments consider reproductive toxicity separate from developmental toxicity. Reproductive toxicity is tested by exposing sexually mature adults to a chemical and examining the effects on the animal capacity to reproduce. Developmental toxicity is studied by exposing pregnant dams and looking for effects in the foetuses. However, these tests generally do not detect chemicals that induce effects that only appear postnatally. Thus, 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 reproductive toxicity and the two endpoints cannot be clearly distinguished.

In this hazard assessment, data will be presented on the basis of test procedure, including two-generation studies, developmental/prenatal toxicity studies (only the dam is dosed, study ends before parturition) and developmental/postnatal studies (dam is dosed during gestation and allowed to litter, study ends during weaning). The effects on fertility and development will then be discussed separately in the conclusion.

4.8.1 One/two-generation reproduction toxicity studies

Previous evaluations

In a dietary two-generation reproductive toxicity study in Sprague-Dawley rats (30/sex/dose), DiHepP was administered (to both sexes) at concentrations of 0, 1000, 4500, or 8000 ppm (in the diet) (Wil Research Laboratories Inc., 2003*; McKee et al., 2006). F0 and F1 animals received the diet for 70 days prior to mating, through the mating period and until the scheduled termination period for adults. Due to DiHepP being administered in the diet, the daily doses were significantly different at different life stages. The effects of DiHepP on all reproductive capabilities were evaluated (including gonadal function, oestrous cyclicity, mating behaviour, conception, gestation, parturition, and lactation in the F0 and F1 parental generations). The F1 and F2 offspring (pups) were evaluated for neonatal survival, growth and development.

Parental toxicity (both F0 and F1 animals) was seen at 4500 and 8000 ppm, with dose-related increases in liver and kidney weights and increased pituitary weights (in F1 males at 8000 ppm). There was no difference in F0 body weight during treatment and no difference in sperm parameters between control and treated rats. The F1 litter size was similar in control and treated animals.

Reproductive effects included decreased male and female reproductive performance (mating) and fertility for both sexes in the F1 generation at 8000 ppm. However, the mean F2 litter size was no different than controls. This dose equated to approximately 830 mg/kg bw/d prior to breeding and 540 mg/kg bw/d during gestation. Decreased sperm production rates and reduced testicular sperm concentrations were seen in F1 males at all dose levels but there were no differences in F1 testicular weights and no pathological evidence of aspermia or testicular atrophy was seen in either the low or mid dose groups. Testes and ovary weight as well as male accessory organ weights were reduced in high dose F1 offspring. Seminiferous tubule degeneration was prevalent in high dose group as well as epididymal hypospermia. There were no treatment-related effects on the percentages of motile and progressively motile sperm or absolute number and percentages of morphologically normal sperm at any dose level.

In this study, significantly reduced offspring body weights (and weight gains) were noted in F1 pups at 8000 ppm and F2 pups at 4500 and 8000 ppm. Other developmental effects reported in this study were decreased gonad, kidney, and pituitary weights in the F1 generation (both sexes) and decreased secondary sex organ weights for F1 and F2 offspring (males) at 8000 ppm; reduced anogenital distance (absolute and relative) and delays in balanopreputial separation in F1 pups at 8000 ppm; reduced anogenital distance (absolute and relative) at 4500 ppm and above in the F2 generation; hypospadia, swelling of the prepuce, undescended testes and retention of thoracic nipples in F1 males at 8000 ppm. The high dose (8000 ppm in diet) equated to approximately 540 mg/kg bw/d during gestation and 1360 mg/kg bw/d during lactation. The NOAEL for systemic toxicity in the F0 and F1 generations was 1000 ppm (approximately 50-168 mg/kg bw/d for males and females respectively), with a LOAEL of 4500 ppm (222-750 mg/kg bw/d), based on histopathological findings in liver and kidney. The NOAEL for effects on fertility was 4500 ppm (227-750 mg/kg bw/d) and the LOAEL was 8000 ppm (419-1360 mg/kg bw/d) in the F1 generation based on decreased reproductive organ weight. The NOAEL for developmental effects and for parental systemic toxicity was 1000 ppm

(approximately 50-168 mg/kg bw/d for males and females respectively). The LOAEL was 4500 ppm (222-750 mg/kg bw/d) based on decreased anogenital distance in the F2 male offspring.

4.8.2 Prenatal developmental toxicity studies

Previous evaluations

A developmental toxicity study was conducted on DiHepP in Sprague-Dawley female rats using oral gavage at doses of 0, 100, 300, and 750 mg/kg bw/d on gestation days 6-20 (Exxon Biomedical Sciences Inc., 1997*; McKee et al., 2006). Overt maternal toxicity was not evident, although there was an increase in liver weights at and above 300 mg/kg bw/d. Developmental effects were seen only in the high dose group, which included an increase in resorptions (per litter and per implantation site), decrease in live foetuses (increased embryo/foetal death) and decreased pup weight. In addition, there was an increased incidence of foetal malformations and variations, including anophthalmia, microphthalmia, ectopic testis/ ovaries, abnormal origin or agenesis of the blood vessels, and agenesis, misshapen, fused or malformed bones of the skull sternebrae, ribs or vertebrae, with stunted foetuses in approximately half of the litters, compared to controls. In this study, the developmental NOAEL was established at 300 mg/kg bw/d, with a LOAEL of 750 mg/kg bw/d based on increased resorptions and malformations.

Data not reported in previous evaluations

No data.

4.8.3 Mode of action

DiHepP (up to 2000 mg/kg) did not induce oestrogenic responses in vivo in uterotrophic and vaginal cornification assays using immature and mature ovariectomised rats (Zacharewski et al., 1998). DiHepP (unknown isomer) was negative for oestrogenic activity in a yeast two-hybrid assay (Nishihara et al., 2000). DBP 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). DiHepP (isomeric mixture) demonstrated weak oestrogenic activities in a human oestrogen receptor (ER) α (but not β) reporter gene assay in CHO-K1 cells transfected with expression vectors for ERα, ERβ and androgen receptor (AR) (Takeuchi et al., 2005). However, DiHepP (up to 10^{-5}M) had no binding affinity for the ERα or ERβ in vitro (Toda et al., 2004). DiHepP demonstrated anti-oestrogenic and anti-androgenic activity in the hERβ- and hAR-transactivation assays, respectively (Takeuchi et al., 2005).

Conclusion

Effects on fertility

Reproductive effects reported in a two-generation reproductive toxicity study were mainly at the high dose (8000 ppm) and at or above the systemic toxic dose (4500 ppm). Decreased sperm production rates and reduced testicular sperm concentrations seen at and above 1000 ppm (in F1 males) was considered to be an experimental artefact rather than a treatment-related effect, as no differences were seen in F1 testicular weights and no pathological evidence of aspermia or testicular atrophy was

seen in either the low or mid dose groups (OECD, 2005). Also there were no treatment-related effects on sperm motility or percentages of morphologically normal sperm at any dose level. The NOAEL for parental systemic toxicity in the F0 and F1 generations was 1000 ppm in the diet (50-168 mg/kg bw/d, m-f), with a LOAEL of 4500 ppm (222-750 mg/kg bw/d, m-f), based on liver and kidney effects. The NOAEL for reproductive effects in males and females was 4500 ppm (227-750 mg/kg bw/d) and the LOAEL was 8000 ppm (419-1360 mg/kg bw/d) in the F1 generation based on decreased reproductive organ weight.

Developmental effects

Developmental effects seen in the two-generation study occurred either at or above maternally toxic dose levels. The maternal and developmental NOAEL was 1000 ppm (50-168 mg/kg bw/d), and the LOAEL was 4500 ppm (222-750 mg/kg bw/d) based on decreased anogenital distance in the F2 male offspring. In a developmental study, overt developmental effects were seen at 750 mg/kg bw/d. Increased maternal liver weight was observed at and above 300 mg/kg bw/d. The developmental NOAEL was established at 300 mg/kg bw/d, with a LOAEL of 750 mg/kg bw/d based on increased resorptions and malformations.

5. Hazard Characterisation

Toxicity data for DiHepP were not available for all health endpoints. For endpoints with missing or incomplete data, information from structurally similar phthalates, where available, was used to extrapolate potential toxicity. Relevant read-across information was obtained from other NICNAS assessment reports for relevant phthalates and the NICNAS Phthalates Hazard Compendium (NICNAS, 2008) which contains a comparative analysis of toxicity endpoints across 24 *ortho*-phthalates, including DiHepP.

DiHepP has an alkyl carbon backbone of C6-8 and is considered to belong to a group of "transitional" phthalates defined as those produced from alcohols with straight-chain carbon backbones of C4-6 (NICNAS, 2008).

No toxicokinetic data are available for DiHepP. Based on the toxicokinetic profile of phthalates in general, DiHepP is likely to be rapidly absorbed as the monoester from the gut and excreted via the urine.

DiHepP has low acute oral and dermal toxicity. It causes minimal skin and eye irritant effects in rabbits, and did not induce any skin sensitisation in guinea pigs or humans.

No repeat dose toxicity data are available for DiHepP, apart from reproductive toxicity studies. Effects were seen in these studies on liver, kidney and pituitary, with histopathology reported in all three organs. Effects on the liver, namely hepatocyte enlargement, were consistent with repeat dose studies with other transitional phthalates. A repeat dose oral NOAEL of approximately 50-168 mg/kg bw/d (m-f) was determined for rats in these studies, with a LOAEL of 222-750 mg/kg bw/d (m-f) based on liver and kidney effects.

DiHepP was not mutagenic when tested in different strains of *S. typhimurium* with and without metabolic activation and did not induce structural chromosome aberrations in CHO cells, without metabolic activation. No in vitro mammalian mutation and in vivo genotoxicity data are available for DiHepP. Overall, results of in vitro (bacterial mutation and cytogenetic) tests indicate that DiHepP is non-genotoxic.

In vivo studies in rats and mice undertaken to investigate the effects of DiHepP on mechanisms associated with hepatic carcinogenicity found that DiHepP had no inhibitory effect on gap junctional intercellular communication (GJIC). However significant elevations in hepatic DNA synthesis were seen in both species.

No adequate carcinogenicity data are available for DiHepP. Due to insufficient testing on other phthalates, it is not possible to extrapolate carcinogenic potential for DiHepP.

In a two-generation study, effects on fertility (decreased reproductive performance and fertility index) were seen in both sexes in the F1 generation at the highest dose level 8000 ppm (approximately 830 mg/kg bw/d prior to breeding and 540 mg/kg bw/d during gestation). Decreased mean sperm production rates and decreased testicular sperm concentrations were observed in F1 males at all doses, but this finding may have been an experimental artefact rather than a treatment related effect

as no differences were seen in F1 testicular weights and no pathological evidence of aspermia or testicular atrophy was seen in either the low or mid dose groups. The NOAEL for reproductive effects in males and females was 4500 ppm (227-750 mg/kg bw/d) and the LOAEL was 8000 ppm (419-1360 mg/kg bw/d) in the F1 generation based on decreased reproductive organ weight.

Developmental effects seen in the two-generation study occurred mainly with the 4500 and 8000 ppm groups in F1 and F2 generations, including reduced anogenital distance, delays in balanopreputial separation, testicular abnormalities, changes in external genitalia, and retention of thoracic nipples. The maternal and developmental NOAEL was 1000 ppm (50-168 mg/kg bw/d), and the LOAEL was 4500 ppm (222-750 mg/kg bw/d) based on decreased anogenital distance in the F2 male offspring. In a developmental study, overt developmental effects were seen at 750 mg/kg bw/d, which included an increase in resorptions (per litter and per implantation site) and a related decrease in live foetuses and an increased incidence of foetuses with external, visceral and skeletal malformations compared to controls. Overt maternal toxicity was not evident in this study, although there was an increase in liver weights at and above 300 mg/kg bw/d. The developmental NOAEL was established at 300 mg/kg bw/d, with a LOAEL of 750 mg/kg bw/d based on increased resorptions and malformations.

DiHepP did not exhibit any oestrogenic activity when tested in most in vitro and in vivo assays with only an isomeric mixture demonstrating weak oestrogenic activities in a human oestrogen receptor (ER) α (but not β) reporter gene assay.

Overall, the reproductive and developmental effects of DiHepP are similar to other transitional phthalates (NICNAS, 2008). Transitional phthalates which have been tested all demonstrated effects on male reproductive organs, and induced a recognisable pattern of malformations in offspring including decreased anogenital distance, delayed preputial separation and retained thoracic nipples in male pups. At high doses, hypospadias and cryptorchidism are induced, as well as increased frequency of supernumerary ribs.

6. Human Health Hazard Summary Table

Phthalate	Acute Toxicity	Irritation & Sensitisation	Repeated Dose Toxicity	Genetic Toxicity	Carcinogenicity	Fertility	Developmental Toxicity
Diisoheptyl phthalate (DiHepP)	Oral Rat: LD50 >10000 mg/kg bw Dermal Rabbit: LD50 >3160 mg/kg bw	Skin irritation: Minimal effects Eye irritation: Minimal effects Skin sensitisation: Negative	Oral Rat (2-gen. repro study): NOAEL = 50-168 mg/kg bw/d (m-f) LOAEL = 222-750 mg/kg bw/d (m-f), ↑ liver, kidney and pituitary weights with associated histopathology. PP noted.	In vitro: Negative in bacterial mutation and chromosomal aberrations assays In vivo: No data	Rat, Mouse: No effect on gap junctional intercellular communication, ↑ hepatic DNA synthesis and peroxisomal beta-oxidation	Rat: NOAEL = 227-750 mg/kg bw/d (m-f) LOAEL = 419-1360 mg/kg bw/d (m-f), ↓ reproductive organ weight	Two generation study Rat: NOAEL = 50-168 mg/kg bw/d (m-f) LOAEL = 222-750 mg/kg bw/d (m-f), ↓ anogenital distance in F2 Developmental study Rat: NOAEL = 300 mg/kg bw/d LOAEL = 750 mg/kg bw/d, ↑ resorptions and malformations

PP: peroxisome proliferation; m-f: male-female; ↑: increase; ↓: decrease.

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References

Exxon Biomedical Sciences (1991) Dermal sensitisation test in the guinea pig. Study no.181221. Exxon Biomedical Sciences Inc. (Unpublished report).

Exxon Biomedical Sciences (1995) Microbial mutagenesis in salmonella mammalian microsome plate incorporation assay. Study no. 195725. Exxon Biomedical Sciences Inc. (Unpublished report).

Exxon Biomedical Sciences (1997) Developmental toxicity study in rats with diisoheptyl phthalate. Study no. 167634. Exxon Biomedical Sciences Inc. (Unpublished report).

Hazleton Laboratories America, Inc. (1991) Mutagenicity test in an in vitro cytogenetic assay. Study No. 181232. Unpublished report.

Huntingdon Research Center (1994) Skin sensitization in the guinea pig (Buehler method). Study no. Exx 12e/940627/SS. Huntingdon Research Center (Unpublished report).

IARC (2000) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Industrial Chemicals Vol 77. Lyon, France, International Agency for Research on Cancer.

MB Research Laboratories (1979) Test for oral and dermal toxicity in rats, and eye irritation in rabbits. Study no. MB 79-3967. MB Research Laboratories Inc. (Unpublished report).

McKeeRH, Pavkov KL, Trimmer GW, Keller LH, & Stump DG (2006) An assessment of the potential developmental and reproductive toxicity of diisoheptyl phthalate in rodents. Reprod Toxicol, 21:241-252.

Medeiros AM, Devlin DJ, & Keller LH (1999) Evaluation of skin sensitisation response of dialkyl (C_6-C_{13}) phthalate esters. Contact Dermatitis, 41:287-289.

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

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

OECD (2005) SIDS Initial Assessment Report for Diisoheptyl Phthalate for SIAM 20 (Draft). Organization for Economic Cooperation and Development, Paris, France, 19-22 April 2005.

Smith JH, Isenberg JS, Pugh G Jr, Kamendulis LM, Ackley D, Lington AW, & Klaunig JE (2000) Comparative in vivo hepatic effects of Diisononyl phthalate (DINP) and related C7-C11 dialkyl phthalates on gap junctional intercellular communication (GJIC), peroxisomal beta-oxidation (PBOX), and DNA synthesis in rat and mouse liver. Toxicol Sci, 54: 312-321.

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

Toda C, Okamoto Y, Ueda K, Hashizume K, Itoh K, & Kojima N (2004) Unequivocal oestrogen receptor-binding affinity of phthalate esters feaured with ring hydroxylation and proper alkyl chain size. Arch Biochem Biophys, 431: 16-21

Wil Research Laboratories Inc. (2003) A dietary two-generation reproductive toxicity study of diisoheptyl phthalate in rats. Study No. Wil-438002. Unpublished report.

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

Appendix - Robust Study Summaries

Repeated Dose Toxicity (mechanistic study)

Test Substance	Diisoheptyl phthalate (DiHepP), purity >98%, CAS 71888-89-6
	Di-n-octyl phthalate (DnOP), purity >99%, CAS 117-84-0
	Two isomeric forms of diisononyl phthalate designated as DINP-1, CAS 6815-48-0 and DINP-A, CAS 71549-78-5, purity >98%
	Diisodecyl phthalate (DIDP), purity>98%, CAS 68515-49-1
	Di(heptyl, nonyl, undecyl) phthalate (D711P), purity >98%
	Monoester forms of all phthalates listed above were also tested.
Type of Test	Determination of gap junctional intercellular communication (GJIC).
	Replicative DNA synthesis.
	Peroxisomal beta-oxidation activity (PBOX).
Species	Rats, Fischer 344, and Mice, B6C3F1, 5 males/dose, 6-8 weeks old from Harlan Sprague-Dawley, Indianapolis, IN
Route of admin.	Oral (in the diet)
Study Duration	2-4 weeks
Frequency of treatment	Daily
Post exposure period	None
Doses	*Rats:*
	0, 1000 and 12000 mg/kg bw/d of DINP-1, DINP-A, DIDP, DIHepP and D711P in the diet;
	0, 1000 and 10000 mg/kg bw/d of DnOP in the diet
	Mice:
	0, 500 and 6000 mg/kg bw/d of DINP-1, DINP-A, DIDP, DIHepP and D711P in the diet;
	0, 500 and 10000 mg/kg bw/d of DnOP in the diet
Control group	Untreated
NOAEL / NOEL	Insufficient data.
LOAEL / LOEL	Insufficient data.
GLP& QA	No information provided in study report.
Guidelines	No information provided in study.
Method	Male rats and mice were fed NIH-07 diets containing individual dialkyl phthalates at 500, 1000, 6000, 10000 and 12000 mg/kg bw/d for 2 and 4 weeks. Osmotic mini-pumps, containing 5-bromo-2'-deoxyuridine, were surgically implanted subcutaneously in animals a week prior to sacrifice in order to assess the hepatic effects of the treatments. Animals were sacrificed, weighed and necropsied. The livers were extracted and processed to determine gap junctional intercellular

	communication (GJIC), replicative DNA synthesis and peroxisomal beta-oxidation activity (PBOX).
Result	Relative liver weights were significantly elevated in rats at high doses (6000 mg/kg bw/d) of all phthalates after 2 and 4 weeks, except DINP-1 at 2 weeks and DnOP at 4 weeks. No significant increases were seen at the low dose (1000 mg/kg bw/d) except DiHepP at 2 weeks ($p < 0.05$). In mice, relative liver weights were significantly increased at high doses (6000 mg/kg bw/d) of DINP-1, DINP-A and D711P at 2 and 4 weeks and DIDP at 2 weeks. Low doses (500 mg/kg bw/d) of both isomers of DINP induced significant increases in relative liver weight after 2 weeks only ($p < 0.05$).
	PBOX activity was significantly increased at high doses in rats (12000 mg/kg bw/d) of all phthalates (10000 mg/kg bw/d for DnOP) after 2 weeks. Only high doses of DINP-1 and DINP-A, DIDP and DIHepP caused significant increases in PBOX activity after 4 weeks ($p < 0.05$). In mice, PBOX activity was significantly elevated at high doses (6000 mg/kg bw/d) of all phthalates after 2 and 4 weeks. Low doses (500 mg/kg bw/d) of DNOP also caused a significant increase in PBOX activity ($p < 0.05$).
	GJIC was significantly inhibited in rats (indicated by a decreased transfer of lucifer yellow dye through adjacent hepatocytes) at high doses (12000 mg/kg bw/d) of both isomers of DINP after 2 weeks. High doses of DINP-A and D711P caused significant GJIC inhibition after 4 weeks ($p < 0.05$). In mice, high doses (6000 mg/kg bw/d) of DINP-A and DINP-1 caused significant inhibition after 2 and 4 weeks, respectively ($p < 0.05$).
	Periportal hepatocellular replicative DNA synthesis was significantly elevated in rats at high doses (12000 mg/kg bw/d) of phthalates after 2 and 4 weeks (except DINP-1 and D711P after 4 weeks), and low doses (1000 mg/kg bw/d) of DiHepP and DIDP after 2 and 4 weeks ($p < 0.05$). In mice, DNA synthesis was significantly increased at high doses (6000 mg/kg bw/d) of all DINP-1, DIDP and DiHepP and low doses (500 mg/kg bw/d) of DIDP, DiHepP and D711P after 2 weeks. Both doses of DIDP and high doses of DiHepP maintained a significant increase in DNA synthesis after 4 weeks ($p < 0.05$).
	Liver concentrations of DNIP-1 and its primary metabolites, monoisononyl phthalate-1 (MINP-1) and phthalic acid (PA), were significantly higher than controls, in both rats and mice, at all treated doses after 2 and 4 weeks. The levels of both metabolites were significantly higher than controls in the serum (intact phthalate was not detectable in the serum) in both species.
Conclusion	DINP and other C7-C11 phthalates caused changes in GJIC, DNA synthesis, PBOX and liver weight after 2-4 weeks of treatment in the liver of rats and mice.
Reference	Smith JH, Isenberg JS, Pugh G Jr, Kamendulis LM, Ackley D, Lington AW, & Klaunig JE (2000) Comparative in vivo hepatic effects of diisononyl phthalate (DINP) and related C7-C11 dialkyl phthalates on gap junctional intercellular communication

(GJIC), peroxisomal beta-oxidation (PBOX), and DNA synthesis in rat and mouse liver. Toxicol Sci, 54: 312-321.