

# **Human Health Hazard Assessment**

**Diisoheptyl Phthalate (DiHepP)  
(CAS No. 71888-89-6)**

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## INTRODUCTION

This review of diisooheptyl phthalate (DiHepP) is a health hazard assessment only. For this assessment, an OECD SIDS Initial Assessment Report on Diisooheptyl Phthalate (OECD, 2005) was consulted. Information from this report was supplemented with relevant studies from more recent literature surveys conducted up to September 2006.

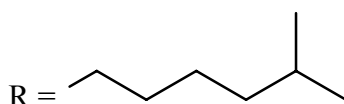
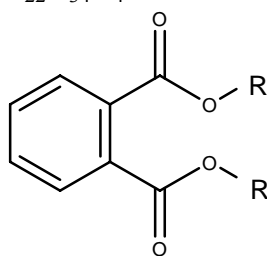
References not marked with an asterisk were examined for the purposes of this assessment. References not examined but quoted from the key report as secondary citations are also noted in this assessment and marked with an asterisk.

Hazard information from this assessment is published also in the form of a hazard compendium providing a comparative analysis of key toxicity endpoints for 25 phthalates (NICNAS, 2007).

## 1. IDENTITY

### 1.1 Identification of the Substance

CAS Number(s):	71888-89-6
Chemical Name:	1,2-Benzenedicarboxylic acid, di-C6-8-branched alkyl esters, C7 rich
Common Name:	Diisooheptyl phthalate (DiHepP)
Molecular Formula:	C <sub>22</sub> H <sub>34</sub> O <sub>4</sub>
Structural Formula:	



DiHepP consists of at least 80% of methyl hexyl 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 <sub>7</sub> H <sub>15</sub> alkyl ester)
Synonyms:	DIHP; Diisooheptyl phthalate ester; 1,2-Benzenedicarboxylic acid, diisooheptyl ester
Purity/Impurities/Additives:	Purity: >99.9% w/w Impurity: ≤0.1% w/w, including isooheptyl alcohol (0.03%), diisooheptyl ether and isooheptyl benzoate (0.07%) Additives: none

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

## 1.2 Physicochemical Properties

**Table 1: Summary of physicochemical properties**

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

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

## 2. 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.

## 3. HUMAN HEALTH HAZARD

### 3.1 Toxicokinetics

#### *Previous Evaluations*

No data.

#### *Data not Reported in Previous Evaluations*

No data.

### Conclusion

No toxicokinetic studies were available for assessment.

### 3.2 Acute Toxicity

#### *Previous Evaluations*

<b>Study</b>	<b>Species</b>	<b>Results (LD50/LC50)</b>	<b>References</b>
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.

**3.3 Irritation**Skin Irritation*Previous Evaluations*

A single 24-hour 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.

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.

Respiratory Irritation

*Previous Evaluations*

No data.

*Data not Reported in Previous Evaluations*

No data.

**Conclusion**

No respiratory irritation studies were available for assessment.

**3.4 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.

**3.5 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 3.8.

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 F<sub>0</sub> and F<sub>1</sub> animals at 4500 ppm (222-750 mg/kg/d) and 8000 ppm (404-1360 mg/kg/d) and increased pituitary weights (F<sub>1</sub> males at 8000 ppm). F<sub>0</sub> 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 F<sub>1</sub> animals, hypertrophy of the pars distalis of the pituitary gland. The NOAEL for systemic toxicity in F<sub>0</sub> and F<sub>1</sub> 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.

### **3.6 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.

### 3.7 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.

### 3.8 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.

#### **3.8.1 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). F<sub>0</sub> and F<sub>1</sub> 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 F<sub>0</sub> and F<sub>1</sub> parental

generations). The F<sub>1</sub> and F<sub>2</sub> offspring (pups) were evaluated for neonatal survival, growth and development.

Parental toxicity (both F<sub>0</sub> and F<sub>1</sub> animals) was seen at 4500 and 8000 ppm, with dose-related increases in liver and kidney weights and increased pituitary weights (in F<sub>1</sub> males at 8000 ppm). There was no difference in F<sub>0</sub> body weight during treatment and no difference in sperm parameters between control and treated rats. The F<sub>1</sub> 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 F<sub>1</sub> generation at 8000 ppm. However, the mean F<sub>2</sub> 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 F<sub>1</sub> males at all dose levels but there were no differences in F<sub>1</sub> 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 F<sub>1</sub> 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 F<sub>1</sub> pups at 8000 ppm and F<sub>2</sub> pups at 4500 and 8000 ppm. Other developmental effects reported in this study were decreased gonad, kidney, and pituitary weights in the F<sub>1</sub> generation (both sexes) and decreased secondary sex organ weights for F<sub>1</sub> and F<sub>2</sub> offspring (males) at 8000 ppm; reduced anogenital distance (absolute and relative) and delays in balanopreputial separation in F<sub>1</sub> pups at 8000 ppm; reduced anogenital distance (absolute and relative) at 4500 ppm and above in the F<sub>2</sub> generation; hypospadias, swelling of the prepuce, undescended testes and retention of thoracic nipples in F<sub>1</sub> 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 F<sub>0</sub> and F<sub>1</sub> 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/day) and the LOAEL was 8000 ppm (419-1360 mg/kg bw/day) in the F<sub>1</sub> 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 F<sub>2</sub> male offspring.

### **3.8.2 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 fetuses 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.

### **3.8.3 Mode of action**

DiHepP (up to 2000 mg/kg) did not induce estrogenic responses *in vivo* in a uterotrophic and vaginal cornification assays using immature and mature ovariectomised rats (Zacharewski et al., 1998). DiHepP (unknown isomer) was negative for estrogenic 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 estrogenic activities in a human estrogen receptor (ER)  $\alpha$  (but not  $\beta$ ) reporter gene assay in CHO-K1 cells transfected with expression vectors for human estrogen receptor ER $\alpha$ , ER $\beta$  and androgen receptor (AR) (Takeuchi et al., 2005). However, DiHepP (up to  $10^{-5}$ M) had no binding affinity for the oestrogen receptor  $\alpha$  or  $\beta$  *in vitro* (Toda et al., 2004). DiHepP also demonstrated anti-estrogenic activity via ER  $\beta$  in the presence of 17 $\beta$ -estradiol and antiandrogenic activity in the hAR-transactivation assay. (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 F<sub>1</sub> males) was considered to be an experimental artefact rather than a treatment-related effect, as no differences were seen in F<sub>1</sub> 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 F<sub>0</sub> and F<sub>1</sub> generations was 1000 ppm in the diet (50-168 mg/kg bw/day, 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/day) and the LOAEL was 8000 ppm (419-1360 mg/kg bw/day) in the F<sub>1</sub> 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 F<sub>2</sub> 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.

#### 4. 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 (2007) which contains a comparative analysis of toxicity endpoints across 25 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, 2007).

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 F<sub>1</sub> 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 F<sub>1</sub> 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 F<sub>1</sub> 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/day) and the LOAEL was 8000 ppm (419-1360 mg/kg bw/day) in the F<sub>1</sub> 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 F<sub>1</sub> and F<sub>2</sub> 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 F<sub>2</sub> 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 estrogenic activity when tested in most *in vitro* and *in vivo* assays with only an isomeric mixture demonstrating weak estrogenic activities in a human estrogen receptor (ER)  $\alpha$  (but not  $\beta$ ) reporter gene assay.

Overall, the reproductive and developmental effects of DiHepP are similar to other transitional phthalates (NICNAS, 2007). 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.

## 5. HUMAN HEALTH HAZARD SUMMARY TABLE

<b><i>Phthalate</i></b>	<b><i>Acute Toxicity</i></b>	<b><i>Irritation &amp; Sensitisation</i></b>	<b><i>Repeated Dose Toxicity</i></b>	<b><i>Genetic Toxicity</i></b>	<b><i>Carcinogenicity</i></b>	<b><i>Fertility</i></b>	<b><i>Developmental Toxicity</i></b>
Diisooheptyl phthalate (DiHepP)	Oral Rat: LD50 >10000 mg/kg bw  Dermal Rabbit: LD50 >3160 mg/kg bw	Skin irritation: Minimal effects  Eye irritation: Minimal effects  Respiratory irritation: No data  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 and kidney weights with associated histopathology.  PP noted.	<i>In vitro</i> Negative in bacterial mutation and chromosomal aberrations assays  <i>In vivo</i> 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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## 7. ROBUST STUDY SUMMARIES

### *Repeated Dose Toxicity (mechanistic study)*

<b>Test Substance</b>	Di-isoheptyl phthalate (DiHepP), purity >98%, CAS 71888-89-6 Di-n-octyl phthalate (DnOP), purity >99%, CAS 117-84-0 Two isomeric forms of di-isononyl phthalate designated as DINP-1, CAS 6815-48-0 and DINP-A, CAS 71549-78-5, purity >98% Di-isodecyl phthalate (DIDP), purity >98%, CAS 68515-49-1 Di-(heptyl, nonyl, undecyl) phthalate (D711P), purity >98%
<b>Type of Test</b>	Monoester forms of all phthalates listed above were also tested. Determination of gap junctional intercellular communication (GJIC). Replicative DNA synthesis. Peroxisomal beta-oxidation activity (PBOX).
<b>Species</b>	Rats, Fischer 344, and Mice, B6C3F1, 5 males/dose, 6-8 weeks old from Harlan Sprague-Dawley, Indianapolis, IN.
<b>Route of admin.</b>	Oral (in the diet)
<b>Study Duration</b>	2-4 weeks
<b>Frequency of treatment</b>	Daily
<b>Post exposure period</b>	None
<b>Doses</b>	<i>Rats:</i> 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 <i>Mice:</i> 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
<b>Control group</b>	All groups were treated for 2 and 4 weeks. Untreated
<b>NOAEL / NOEL</b>	Insufficient data.
<b>LOAEL / LOEL</b>	Insufficient data.
<b>GLP &amp; QA</b>	No information provided in study report.
<b>Guidelines</b>	No information provided in study.
<b>Method</b>	Male rats and mice were fed NIH-07 diets containing individual di-alkyl 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).
<b>Result</b>	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, mono-isononyl 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 di-isononyl 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.