# Human Health Hazard Assessment


# Di-n-hexyl-phthalate (DnHP)
# (CAS No. 84-75-3)

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## INTRODUCTION

This review of di-n-hexyl phthalate (DnHP) is a health hazard assessment only. For this assessment, a key review on DnHP prepared by the Centre for the Evaluation of Risks to Human Reproduction (CERHR, 2003) was consulted. Information from this review was supplemented with relevant studies from more recent literature surveys conducted up to September 2006.
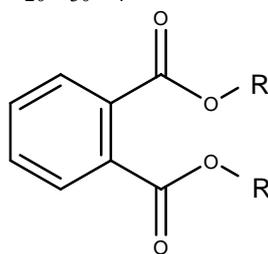
References not marked with an asterisk were examined for the purposes of this assessment. References not examined but quoted from the key review as secondary citations are also noted in this assessment and marked with an asterisk.

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:              84-75-3
Chemical Name:           1,2-Benzenedicarboxylic acid, dihexyl ester
Common Name              Di-n-hexyl phthalate (DnHP or DHP)
Molecular Formula:       $C_{20}H_{30}O_4$
Structural Formula:



Molecular Weight:        334.4
Synonyms:                Dihexyl phthalate; Dihexyl ester phthalic acid; Bis(n-hexyl) phthalate
Purity/Impurities/Additives: DnHP may be present either as a minor constituent (1%) of C6-10-phthalate mixtures (CAS No. 68515-51-5) or an isomer (≤25%) in mixtures of diisohexyl phthalates (DIHP, CAS no. 68515-50-4)

### 1.2 Physicochemical Properties

*Table 1: Summary of physicochemical properties*

| Property | Value |
| --- | --- |
| Physical state | Clear liquid, aromatic |
| Melting point | -27.4°C |
| Boiling point | 350°C |
| Density | 1011 kg/m$^3$ |

| | |
|---|---|
| Vapour pressure | $6.67 \times 10^{-7}$ kPa (25˚C) |
| Water solubility | $5.0 \times 10^{-5}$ g/L |
| Partition coefficient n-octanol/water (log Kow) | 6.30 |
| Henry's law constant | 4.458 Pa.m$^3$/mol# |
| Flash point | Not available |

# Henry's law constant value was derived from the experimental values for vapour pressure, $5 \times 10^{-6}$ mmHg, water solubility of 0.05 mg/L and a molecular weight of 334.4 for DnHP (isomer not clearly specified).
Source: CERHR (2003)

## 2. USES

DnHP is used in the making of plastisols that are subsequently used in the manufacture of automobile parts (air filters, battery covers) and dip-moulded products (tool handles, dishwasher baskets). Commercial phthalate substances containing DnHP may be added to the PVC utilised in the manufacture of flooring, canvas tarps, and notebook covers. Substances containing DnHP may also be used in traffic cones, toys, vinyl gloves, weather stripping, flea collars, shoes, and conveyor belts used in food packaging operations (CERHR, 2003).

Information on use of DnHP in Australia was not available.

## 3. HUMAN HEALTH HAZARD

### 3.1 Toxicokinetics

*Previous Evaluations*

Dermal absorption of DnHP was studied along with a series of phthalates using occluded application of $^{14}$C labelled phthalate diesters (157 μmol/kg) on the clipped back of male F344 rats (Elsisi et al., 1989*). Urine and faeces were collected every 24 hours for 7 days. The cumulative percentage dose excreted in 7 days was approximately 18% for DnHP. Urine was the major route of excretion. After 7 days, there was no specific tissue distribution with no tissue containing >0.6% of the applied DnHP dose. Most of the unexcreted dose remained in the area of application.

*Data not Reported in Previous Evaluations*

No accumulation of DnHP (isomer not specified, 25 or 250 ppm in the diet) occurred in starlings fed DnHP for 30 days (O'Shea & Stafford, 1980*).

**Conclusion**

Available toxicokinetic information indicates that DnHP is absorbed dermally in rats with approximately 18% of $^{14}$C excreted in urine within 7 days. There was no evidence of accumulation of DnHP in any tissue.

### 3.2 Acute Toxicity

*Previous Evaluations*

No data.

*Data not Reported in Previous Evaluations*

| Study | Species | Results (LD50/LC50) | References |
|-------|---------|---------------------|------------|
| Oral | Rat | 29600 mg/kg bw | RTECS, 2006 |
| Dermal | Rabbit | >20 mL/kg bw | RTECS, 2006 |

**Conclusion**

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

**3.3 Irritation**

Skin Irritation

*Previous Evaluations*

No data.

*Data not Reported in Previous Evaluations*

In a standard Draize test, rabbits exposed to a dermal application of 500 mg DnHP over 24 hours exhibited a mild irritation (RTECS, 2006).

**Conclusion**

DnHP causes minimal skin irritation in rabbits.

Eye Irritation

*Previous Evaluations*

No data.

*Data not Reported in Previous Evaluations*

No data.

**Conclusion**

No eye irritation studies were available for assessment.

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*

No data.

*Data not Reported in Previous Evaluations*

No data.

**Conclusion**

No sensitisation studies were available for assessment.

**3.5 Repeated Dose Toxicity**

*Previous Evaluations*

In a study, 4-week old male Wistar rats were exposed to a single high dose of DnHP (1824 mg/kg bw/d), DnOP or DEHP for 3, 10, or 21 days. Groups of 4 treated rats and 6 control rats were sacrificed and necropsied after 3, 10, or 21 days of treatment (Mann et al., 1985; Hilton et al., 1986*).

DnHP treatment did not cause a change in body weight gain, food intake levels, testes weight, or the gross appearance of testes, kidney or pancreas. The liver of treated rats was identified as the principal target organ for the effects of DnHP. Liver histopathology, enzyme activity, and peroxisome proliferation effects were examined alongside parameters of thyroid function (levels of thyroid hormones in serum and thyroid histopathology). Diets containing DnHP induced hepatic histology and liver chemistry changes at all three assessment times. At 3 days, effects of dietary DnHP on the liver included centrilobular necrosis, loss of glycogen, proliferation and dilation of smooth endoplasmic reticuli and shortening of the microvilli in bile canaliculi. By 10 days, accumulation of large lipid droplets in hepatocytes around central veins were observed, in addition to a slight increase in cyanide-insensitive palmitoyl CoA oxidation and of catalase in the peroxisomal fraction. A small, late-appearing, increase in liver weight and a significant decrease in glucose-6-phosphate activity were noted at 21 days. Apart from slight induction of one peroxisomal enzyme, DnHP did not induce other measures of peroxisome proliferation.

Hinton et al. (1986) reported that at 21 days, there was a significant decrease in serum thyroxine (T4) levels and increased serum triiodothyronine (T3) levels in rats. Electron microscopy analysis revealed changes indicative of thyroid hyperactivity such as increased lysosomal numbers and size, enlarged Golgi apparatus, and mitochondrial damage.

*Data not Reported in Previous Evaluations:*

A more recently published repeated-dose dietary study examined the effects of DnHP on the liver using lower doses over 14 days of exposure (Howarth et al., 2001; see Robust Summary in Section 7 for more details). Male Wistar albino rats weighing approximately 180 g were

administered diets of either a control powdered diet, DEHP (10000 ppm; ~1000 mg/kg bw/d), DnHP (10000 ppm; ~1000 mg/kg bw/d) or, a mixture of DEHP (~1000 mg/kg bw/d) and DnHP (~1000 mg/kg bw/d). DEHP caused peroxisomal fatty acid oxidation, induction of CYP4A1, liver enlargement, decreased serum cholesterol and triglyceride and, morphological evidence of thyroid hyperactivity. Dietary exposure of DnHP alone did not cause induction of peroxisomal fatty acid oxidation, CYP4A1 enzyme or changes in relative liver weight. However, there was a marked accumulation of fat in liver, a decrease in serum cholesterol, larger decreases in serum triglyceride than DEHP alone and morphological evidence of thyroid hyperactivity. In general, changes in rats treated with a mixture of DEHP and DnHP were very similar to those found in rats treated with DEHP alone except that the effect on serum cholesterol levels was additive and serum triglyceride levels were intermediate between effects of phthalates given alone.

**Conclusion**

There is sufficient evidence from rodent studies to show that repeated administration of high dietary doses of DnHP (>1000 mg/kg bw/d) can cause liver toxicity.

Diets containing 1824 mg/kg bw/d DnHP induced accumulation of large droplets of fat around central veins leading by 10 days to mild centrilobular necrosis and an increase in liver weight at 21 days. Peroxisome proliferation was not observed as there was only a slight induction of one peroxisomal enzyme. A more recent study by Howarth et al. (2001) confirmed effects of prolonged administration of DnHP (~1000 mg/kg bw/d) on the rodent liver noting induction of a fatty liver and significant decreases in serum cholesterol and triglyceride levels. Evidence of thyroid hyperactivity was also apparent in both studies following DnHP administration.

No NOAEL can be established from these studies. A LOAEL of 1824 mg/kg bw/d can be derived from the 21-day study based on hepatocellular necrosis, fat accumulation, loss of glycogen and increases in liver enzymes at this dose.

**3.6 Genetic Toxicity**

*Previous Evaluations*

DnHP tested negative in *S. typhimurium* TA 98, 100, 1535, 1537 (with and without activation by rat and hamster S9 metabolic systems) and two other bacterial mutagenicity assays (not specified) (Zeiger et al., 1985*; CMA, 1999*).

*Data not Reported in Previous Evaluations*

No additional data were available.

**Conclusion**

DnHP tested negative in an array of bacterial mutagenicity assays (Zeiger et al., 1985*; CMA, 1999*). Equivocal results for C6-10 phthalate, that contains minor amounts of DnHP (1%), were obtained in a mouse lymphoma mutation assay due to a non-dose related increase in mutations, both in the presence and absence of S9 metabolic activation (Barber et al.,

2000*). DIHP, containing ≤25% DnHP, was reported to be inactive in a mouse micronucleus test that was conducted by Exxon Biomedical Sciences Inc. in 1996 (CERHR, 2003).

No *in vitro* cytogenetic, mammalian mutation or *in vivo* genotoxicity studies were conducted on DnHP.

## 3.7 Carcinogenicity

*Previous Evaluations*

No data.

*Data not Reported in Previous Evaluations*

No data.

**Conclusion**

No carcinogenicity studies were available for assessment.

## 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 repeat dose toxicity studies that dose adult animals for varying duration, prenatal developmental 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 Repeat Dose Toxicity Studies

*Previous Evaluations*

In a short-term study by Foster et al. (1980), a single dose of 2400 mg/kg bw/d DnHP was given by gavage in corn oil to a group of 12 pubertal Sprague-Dawley rats (4-week old) for 4 days. Marked effects on testis weight (65% of control value) were noted in the absence of body weight effects. Histologic examination revealed marked seminiferous tubular atrophy with the majority of tubules showing few spermatogonia and Sertoli cells, but normal Leydig cell morphology.

In the previously described rodent dietary studies (see Section 3.5), 4-week old male Wistar rats were exposed to 1824 mg/kg bw/d DnHP for 3, 10, or 21 days (Mann et al., 1985). DnHP treatment did not cause a change in testes weight or the gross appearance of testes despite inducing hepatic histological and liver chemistry changes at all three assessment times.

### 3.8.2 Continuous Breeding Reproductive Toxicity Studies

*Previous Evaluations*

One-generation mouse study was used by the NTP-CERHR Expert Panel for assessment of reproductive toxicity (Reel et al., 1985*; Lamb et al., 1987). In the study, twenty breeding pairs of CD-1 mice (40 pairs in control group) were dosed with DnHP (98% pure) for 7 days prior to and during a 98-day cohabitation period. The dietary doses were 0, 0.3, 0.6 or 1.2% w/w (0, 380, 800 or 1670 mg/kg bw/d). Dietary exposure to DnHP resulted in dose-related adverse effects on fertility during the continuous breeding phase including a reduction in the number of litters/pair, live pups/litter and proportion of pups born alive. Results were statistically significant for all parameters at and above 0.3%. No litters were produced at the high dose (1670 mg/kg bw/d), there was 1 litter in the mid-dose group (800 mg/kg bw/d) and 14 of 17 pairs had litters in the low-dose group (380 mg/kg bw/d), compared to all pairs with litters in the control group. Significant effects on these reproductive parameters occurred at the lowest dose level, with clear adverse effects seen in the absence of any maternal body weight effects.

A crossover mating trial in the same study showed that both sexes were affected by exposure to DnHP. When high-dose males and control females were paired, there was a significant decrease in detected matings as evidenced by the number of copulatory plugs (56%) compared to controls (90%), and only 1 of 18 treated males sired a litter. When the high-dose females were mated with control males, there was no decrease in copulatory plugs, but none of the females became pregnant.

Only the control and high-dose DnHP groups were necropsied. Only 3 of 18 treated males had sufficient numbers of sperm to allow assessment of abnormal forms; sperm number in these 3 were diminished compared to control. Sperm assessment showed a significant decrease in sperm number (7% of control) and motility (22% of control) in high-dose DnHP treated parental mice. There were significant decreases in the relative weights of the epididymes, testes, and seminal vesicles. Extensive atrophy of the seminiferous epithelium was noted with mature sperm markedly diminished in the epididymis. No treatment-related microscopic lesions were detected in the ovaries, uterus, or vagina of the female mice. For females, liver to body weight ratio was significantly increased (31%) and uterine weight significantly decreased (31%). Body and relative kidney/adrenal weights were significantly decreased and liver to body weight ratio was significantly increased in both high-dose male and female treated groups, but histological changes were not noted. Reproductive function in $F_1$ offspring was not examined.

At the low dose (380 mg/kg bw/d), there was a significant reduction in the mean number of litters/pair, the number of live pups/litter and the proportion of pups born alive. Pup weight adjusted for litter size was unchanged. These developmental effects occurred in the absence of an effect on postpartum dam body weights. A NOAEL for fertility and developmental effects could not be established.

### 3.8.3 Developmental/Postnatal Toxicity Studies

*Previous Evaluations*

DnHP was evaluated in a Chernoff-Kavlock screening assay in which CD-1 mice (48-50 dams/group) were gavaged on GD 6-13 with a single dose level of 9900 mg/kg bw/d (undiluted chemical, 10 mL/kg/d) or corn oil (Hardin et al., 1987). Dams were allowed to litter and a postnatal evaluation was conducted. At that dose, no pregnant dams gave birth to a live litter and one exposed dam died.

*Data not Reported in Previous Evaluations*

No data.

### 3.8.4 Mode of Action

DnHP was negative for estrogenic activity in a yeast two-hybrid assay (Nishihara et al., 2000). DnHP did not increase proliferation of human breast cancer MCF-C7 cells (Okubo et al., 2003) and had no binding affinity (up to $10^{-5}$M) for the oestrogen receptor α or β *in vitro* (Toda et al., 2004). However, other studies have shown that DnHP exhibited weak competitive binding to the oestrogen receptor, ERα-agonistic and ERβ- and AR-antagonistic activities (Zacharewski et al., 1998, Takeuchi et al., 2005). DnHP also induced detachment of germ cells from a Sertoli cell monolayer *in vitro* (Gray & Gangolli, 1986).

**Conclusion**

*Effects on Fertility*

DnHP causes fertility effects in both sexes of two rodent species. The existing reproductive toxicity information for DnHP is derived from a one-generation rodent study that examined reproductive function in mice after dietary exposure (98 day continuous breeding protocol). Exposure to DnHP at doses at and above 380 mg/kg bw/d in mice reduced fertility in a dose-related manner. At the lowest dose, and in the absence of any effects on maternal body weight, fertility was reduced by about 18%. In cross-over studies, reproductive parameters in both sexes were affected. However, the mid- and low dose animals were not necroscopied. A NOAEL for fertility was not established.

Shorter durations of exposure yielded differing results. A sub-acute dose (4 day gavage) of 2400 mg/kg bw/day DnHP induced reduced testicular weight and testicular atrophy in the rat, whereas exposure to 1824 mg/kg bw/day DnHP for 3, 10 or 21 days had no effect on testes weight.

*Developmental Effects*

The available developmental toxicity data for DnHP do not provide adequate dose-response information for determination of LOAELs and NOAELs. Data from a Chernoff-Kavlock screening assay in mice gavaged with one dose level (9900 mg/kg bw/d) on GD 6-13 indicate that DnHP caused developmental effects (loss of all litters) at high doses in mice. Pup mortality in the same strain of mice at 380 mg/kg bw/d was also observed in a continuous 98-

day breeding study in the absence of signs of marked maternal toxicity. A NOAEL for developmental effects could not be established.

## 4. HAZARD CHARACTERISATION

Toxicity data for DnHP were not available for the majority of 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 DnHP.

DnHP has an alkyl carbon backbone of C6 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 oral or inhalation toxicokinetic data are available for DnHP. Based on the toxicokinetic profile of phthalates in general, DnHP is likely to be rapidly absorbed as the monoester from the gut and excreted via the urine. DnHP is dermally absorbed in rats, with approximately 18% of $^{14}$C excreted in urine within 7 days. There is no systemic accumulation of DnHP in any tissue 7 days post application or longer from percutaneous or dietary exposure respectively.

DnHP has low acute oral and dermal toxicity. Mild skin irritation was observed in rabbits following exposure. No eye irritation or sensitisation studies are available, however, DnHP is not expected to cause eye irritation or skin sensitisation based on data obtained from other transitional phthalates.

DnHP is negative in the bacterial mutagenicity. No *in vitro* cytogenetic, mammalian mutation and *in vivo* genotoxicity studies were conducted on DnHP. Its analogue, DIHP, containing ≤25% DnHP, is negative in a mouse micronucleus assay. When assessed together, and noting the generally negative genotoxicity profile of phthalates of a similar molecular weight, DnHP is considered unlikely to be genotoxic.

Biological effects of DnHP in experimental rodent studies include reduced fertility, developmental toxicity, liver damage, reduced serum cholesterol and triglycerides, and thyroid hyperactivity. In a comparative study, the magnitude of these effects was similar to another linear chain phthalate, DnOP, but not as great as that evoked by the branched chain phthalate, DEHP (Mann et al., 1985; Hinton et al., 1986)

Limited repeated-dose oral toxicity data in rodents indicate that the liver is the target organ following exposure to high doses of DnHP. No NOAEL can be established from these studies. A LOAEL of 1824 mg/kg bw/d can be derived from the 21-day study based on hepatocellular necrosis, fat accumulation, loss of glycogen and increases in liver enzymes at this dose.

Peroxisome proliferation is a process characterized by the ability of subcellular organelles called peroxisomes to increase in size and number. This process is accompanied by the induction of peroxisomal fatty acid β-oxidation enzyme expression and activity. Based upon only the late induction of the commonly used marker of peroxisomal proliferation, cyanide-insensitive palmitoyl-CoA oxidation and the absence of other indicators such as changes in peroxisome morphology and numbers, limited repeat-dose studies suggest that DnHP induces

only weak peroxisome proliferative changes compared to the known peroxisome proliferator DEHP (CERHR, 2003).

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

Experimental data in rodents clearly demonstrated that DnHP causes reproductive effects, with a dose-related manner (380-1670 mg/kg bw/d) on mice fertility during a continuous 98 day breeding study (Lamb et al., 1987). Crossover mating studies also demonstrated that reproductive parameters were affected in both male (including reduced testes weights) and females (Lamb et al., 1987). The NOAEL could not be established. The LOAEL is 380 mg/kg bw/day based on decreased male and female fertility. A toxic effect on Sertoli cells, demonstrated *in vitro*, is one proposed mechanism for DnHP-induced testicular toxicity and subsequent reductions in male fertility (Gray & Gangolli, 1986). There is conflicting evidence that DnHP has estrogenic activity.

In a screening protocol design in mice, complete litter loss was observed at a high oral dose (9900 mg/kg bw/d) (Hardin et al., 1987). Pup mortality in the same strain of mice at 380 mg/kg bw/d was also observed in a continuous 98-day breeding study in the absence of signs of marked maternal toxicity (Lamb et al., 1987). A NOAEL for developmental effects could not be established.

Overall, the reproductive effects of DnHP are similar to other transitional phthalates (NICNAS, 2007). Transitional phthalates which have been tested all demonstrated effects on male reproductive organs, most notably decreased testes weight. DnHP has not been adequately tested for developmental effects. However, transitional phthalates have been shown to induce 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. Therefore, it is likely that DnHp will induce similar developmental effects.

## 5. HUMAN HEALTH HAZARD SUMMARY TABLE

| Phthalate | Acute Toxicity | Irritation & Sensitisation | Repeated Dose Toxicity | Genetic Toxicity | Carcinogenicity | Fertility | Developmental Toxicity |
|---|---|---|---|---|---|---|---|
| Di-n-hexyl phthalate (DnHP) | Oral Rat: LD50 = 29600 mg/kg bw<br><br>Dermal Rabbit: LD50 > 20 mL/kg bw | Skin irritation: minimal effect<br><br>Eye irritation: no data<br><br>Skin sensitisation: no data | Rat: NOAEL = not established. LOAEL = 1824 mg/kg bw/d, hepatocellular necrosis, fat accumulation, loss of glycogen; ↑ liver enzymes. | *In vitro* Negative in bacterial mutation assays.<br><br>*In vivo* No data. | No data. | Rat: NOAEL = not established. LOAEL = 380 mg/kg bw/d, dose-related ↓ number of litters/pair, live pups/litter and proportion of pups born alive. | Rat: NOAEL = not established. LOAEL = 380 mg/kg bw/day; ↑ pup mortality. |

↑: increase; ↓: decrease

## 6. REFERENCES

Barber ED, Cifone M, Rundell J, Przygoda R, Astill BD, Moran E, Mulholland A, Robinson E, & Schneider B (2000) Results of the L5178Y mouse lymphoma assay and the Balb/3t3 cell in vitro transformation assay for eight phthalate esters. J Appl Toxicol, 20:69-80.

CERHR (2003) NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-n-Hexyl Phthalate (DnHP). NIH Publication No. 03-4489. National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction, U.S. Department of Health and Human Services.

CMA (1999) Comments of the Chemical Manufacturers Association phthalate esters panel in response to request for public input on seven phthalate esters. FR Doc. 99-9484. Washington, DC, Chemical Manufacturers Association.

Elsisi AE, Carter DE, & Sipes IG (1989) Dermal absorption of phthalate diesters in rats. Fundam Appl Toxicol, 12:70-77.

Foster PMD, Thomas LV, Cook MW, & Gangolli SD (1980) Study of the testicular effects and changes in zinc excretion produced by some n-alkyl phthalates in the rat. Toxicol Appl Pharmacol, 54:392-398.

Gray TJ & Gangolli SD (1986) Aspects of the testicular toxicity of phthalate esters. Environ Health Perspect, 65:229-235.

Hardin BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, MacKenzie KM, Piccirillo VJ, & Smith KN (1987) Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratog Carcinog Mutagen, 7:29-48.

Hinton RH, Mitchell FE, Mann A, Chescoe D, Price SC, Nunn A, Grasso P, & Bridges JW (1986) Effects of phthalic acid esters on the liver and thyroid. Environ Health Perspect, 70:195-210.

Howarth JA, Price SC, Dobrota M, Kentish PA, & Hinton RH (2001) Effects on male rats of di-(2-ethylhexyl) phthalate and di-n-hexylphthalate administered alone or in combination. Toxicol Lett, 121:35-43.

Lamb JC 4th, Chapin RE, Teague J, Lawton AD, & Reel JR (1987) Reproductive effects of four phthalic acid esters in the mouse. Toxicol Appl Pharmacol, 88:255-269.

Mann AH, Price SC, Mitchell FE, Grasso P, Hinton RH, & Bridges JW (1985) Comparison of the short-term effects of di(2-ethylhexyl) phthalate, di(n-hexyl) phthalate, and di(n-octyl) phthalate in rats. Toxicol Appl Pharmacol, 77:116-132.

RTECS (2006) Registry of Toxic Effects of Chemical Substances. Compiled by the National Institute for Occupational Safety and Health of the U.S. Department of Health and Human Services. Included in TOMES Plus(R) System published by Thomson Micromedex. Greenwood Village, Colorado (Accessed 2006).

NICNAS (2007) Phthalate Hazard Compendium: A summary of physicochemical and human health hazard data for 25 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.

O'Shea TJ & Stafford CJ (1980) Phthalate plasticizers: accumulation and effects on weight and food consumption in captive starlings. Bull Environ Contam Toxicol, 25:345-352.

Okubo T, Suzuki T, Yokoyama Y, Kano K, & Kano I (2003) Estimation of estrogenic and anti-estrogenic activities of some phthalate diesters and monoesters by MCF-7 cell proliferation assay *in vitro*. Biol Pharm Bull, 26(8): 1219-1224.

Reel JR, Lawton AD, & Myers CB (1985) Di-n-hexyl phthalate: Reproduction and fertility assessment in CD-1 mice when administered in the feed. NTP-85-187. NTIS#PB85-249332. National Toxicology Program, National Institute of Environmental Health Sciences.

Takeuchi S, Iida M, Kobayashi S, Jin K, Matsuda T and Kojima H (2005) Differential effects phthalate ester human estrogen receptors. Toxicology, 210, 223-233.

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

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

Zeiger E, Haworth S, Mortelmans K, & Speck W (1985) Mutagenicity testing of di(2-ethylhexyl)phthalate and related chemicals in Salmonella. Environ Mutagen, 7:213-232.

## 7. ROBUST STUDY SUMMARIES

### *Repeated Dose Toxicity*

| | |
|---|---|
| Test Substance | DEHP and DnHP |
| Type of Test | Subacute Oral Toxicity – 14 Days Study |
| Species | Rats, Wistar albino, age unknown, 180g, University of Surrey Experimental Biology Unit. |
| Route of admin. | Oral |
| Study Duration | 14 days |
| Frequency of treatm. | Daily |
| Post exposure period | None |
| Doses | |
| Control group | 5 male rats fed a control powdered diet (B and K Universal Ltd.) |
| NOAEL / NOEL | Insufficient data; one dose only |
| LOAEL / LOEL | Insufficient data; one dose only |
| GLP& QA | No information provided in study report |
| Guidelines | No information provided in study |
| Method | Male Wistar albino rats weighing approximately 180 g were fed diets of either: (a) control powdered diet, (b) 10000 ppm of DEHP (1%; ~1000 mg/kg bw/d), (c) 10000 ppm of DnHP (1%; ~1000 mg/kg bw/d) or (d) 10000 ppm of DEHP (1%; ~1000 mg/kg bw/d) and 10000 ppm of DnHP (1%; ~1000 mg/kg bw/ d). After 14-d of daily dietary exposure rats were sacrificed. The liver, kidneys and spleen were removed and weighed. Liver homogenates were prepared for determination of glucose-6-phosphatase activity, cyanide insensitive palmitoyl CoA oxidation, lauric acid oxidation and catalase activity. Haematocrit, serum triglyceride, cholesterol, total red and white cell number were measured. Blood films were prepared and stained (May-Grunwald/Giemsa procedure) and a differential white-cell count was made. |
| Result | There was no significant difference in food consumption. Treatments caused a slight reduction in weight gain compared with controls although there were no clinical or behavioural signs of systemic toxicity. Livers of rats receiving 1000 mg/kg bw/d DEHP treatments showed markedly increased relative weights. There was a slight but significant increase in liver weight for rats receiving 1000 mg/kg bw/d DnHP alone. The livers of animals receiving DnHP alone were fatty. No treatment related changes were noted in the weight of spleen and kidneys or in any haematological parameter. A marked treatment related decrease in serum triglyceride was observed: DnHP alone caused the largest effect, DEHP the smallest effect and a combination of the two resulting in intermediate level of effect (Table I). DEHP and DnHP given alone caused equivalent decreases in serum cholesterol. The effect of giving DEHP and DnHP in combination caused an additive decrease in cholesterol. Histological examination showed no treatment-related changes in any tissue other than the liver and the thyroid. Large numbers of small vacuoles identified as lipid droplets were present in the livers of rats treated with DnHP alone. |

Table I: Body and liver weights, serum triglyceride and cholesterol in groups of five male rats either receiving control diet or diet containing 1% DEHP, 1% DnHP or 1% DEHP and 1% DnHP

| Treatment | Initial body | Final body | Liver/body weight ratio | Levels of serum | Levels of serum triglyceride |
|---|---|---|---|---|---|

| Treatment | weight (g) | | (%) | cholesterol (mmol/L) | (mmol/L) |
|---|---|---|---|---|---|
| Control | 221 ± 8 | 312 ± 6 | 4.10 ± 0.04 | 1.49 ± 0.08 | 1.27 ± 0.17 |
| DEHP | 227 ± 5 | 299 ± 9 | 6.09 ± 0.06*** | 0.78 ± 0.02*** | 0.76 ± 0.09*** |
| DnHP | 226 ± 5 | 287 ± 10 | 4.78 ± 0.17** | 0.79 ± 0.08*** | 0.39 ± 0.04*** |
| DEHP+DnHP | 231 ± 5 | 290 ± 8 | 6.54 ± 0.16*** | 0.61 ± 0.07*** | 0.58 ± 0.11*** |

The results are presented as mean ± S.E. Asterisks indicate results significantly different from controls using Student's t-test (**P<0.01, ***P<0.001)

Fewer, scattered lipid droplets were noted in the centrilobular zone of livers from rats given a combination of DEHP and DnHP. Evidence of thyroid hyperactivity was apparent for all treatments either alone or in combination as indicated by a reduction in follicular size and increased numbers of follicular cells with a columnar appearance. DEHP alone significantly increased cyanide-insensitive palmitoyl CoA oxidation and ω-oxidation of lauric acid (Table II). Equivalent increases were found for DEHP and DnHP in combination. However, there was no increase in either enzyme activity for DnHP alone. There was a slight but insignificant increase in catalase due to DEHP alone and DEHP in combination with DnHP but not for DnHP alone. Glucose-6-phosphate was slightly lowered in rats receiving DEHP alone, but this did not reach statistical significance.

Table II: Enzyme levels in the livers of groups of 5 male rats receiving either control diet or diet containing 1% DEHP, 1% DnHP or a mixture of DEHP and DnHP

| Treatment | Lauric acid ω-oxidation (nmol/min/mg protein) | Lauric acid ω-1 oxidation (nmol/min/mg protein) | Palmitoyl Co-A oxidation (nmol/min/mg protein) | Glucose-6-phosphatase (μmol/min/mg protein) | Catalase (units/mg protein) |
|---|---|---|---|---|---|
| Control | 0.20 ± 0.03 | 0.130 ± 0.012 | 3.5 ± 0.3 | 0.080 ± 0.004 | 1.86 ± 0.38 |
| DEHP | 0.44 ± 0.02*** | 0.036 ± 0.003*** | 29.2 ± 1.2*** | 0.068 ± 0.003* | 2.38 ± 0.24 |
| DnHP | 0.26 ± 0.02 | 0.107 ± 0.008 | 3.4 ± 0.3 | 0.077 ± 0.008 | 1.92 ± 0.12 |
| DEHP+DnHP | 0.45 ± 0.05*** | 0.037 ± 0.005** | 29.0 ± 4.3*** | 0.067 ± 0.007 | 2.35 ± 0.11 |

The results are presented as mean ± SE Asterisks indicate results significantly different from controls using Student's t-test (*P<0.05, **P<0.01, ***P<0.001).

Conclusion   Induction of a fatty liver is induced by dietary exposure to both DnHP and DEHP. Induction of peroxisomal proliferation, indicated by specific alterations in hepatic histology and peroxisomal enzyme activities, are found only after administration of DEHP.
DEHP caused peroxisomal fatty acid oxidation, induction of CYP4A1, liver enlargement, decreased serum cholesterol and triglyceride and, morphological evidence of thyroid hyperactivity. Dietary exposure of DnHP alone did not cause induction of peroxisomal fatty acid oxidation, CYP4A1 or changes in relative liver weight. However, there was a marked accumulation of fat in liver, a decrease in serum cholesterol, larger decreases in serum triglyceride than DEHP alone and morphological evidence of thyroid hyperactivity. In general, changes in rats treated with a mixture of DEHP and DnHP were very similar to those found in rats treated with DEHP alone except that the effect on serum cholesterol levels was additive and serum triglyceride levels were intermediate between effects of phthalates given alone.

Reference   Howarth JA, Price SC, Dobrota M, Kentish PA, & Hinton RH (2001) Effects on male rats of di-(2-ethylhexyl) phthalate and di-n-hexylphthalate administered alone or in combination. Toxicol Lett, 121:35-43.