Existing Chemical Hazard Assessment Report

Australian Government
Department of Health and Ageing
NICNAS

Di-*n*-hexyl Phthalate

June 2008

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME
GPO Box 58, Sydney NSW 2001, Australia **www.nicnas.gov.au**

Preface

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Overview

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 US Centre for the Evaluation of Risks to Human Reproduction (CERHR) was consulted. This was supplemented with literature surveys up to September 2006.

DnHP is used in making plastisols that are subsequently used in the manufacture of automobile parts and dip-moulded products. Commercial phthalate substances containing DnHP may be added to the polyvinyl chloride (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.

A survey of Australian industry in 2004 and 2006 provided no information on the use of this phthalate in Australia.

Structurally, phthalate esters are characterized by a diester structure consisting of a benzenedicarboxylic acid head group linked to two ester side chains. DnHP possesses 2 linear ester side chains each with a backbone of 6 carbons (C6). DnHP 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 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. Such 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 DnHP.

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 tissues.

DnHP has low acute oral and dermal toxicity. Mild skin irritation was observed in rabbits following exposure. No eye irritation or sensitisation studies were available, however, DnHP was not expected to cause eye irritation or skin sensitization.

DnHP was negative in bacterial mutagenicity tests. No in vitro cytogenetic, mammalian mutation and in vivo genotoxicity studies were conducted. An analogue, DIHP, containing $\leq$ 25% DnHP, was negative in a mouse micronucleus assay. When assessed together, and noting the generally negative genotoxicity profile of phthalates of a similar molecular weight, DnHP was 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.

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 could be established from these

studies. A LOAEL of 1824 mg/kg bw/d was derived from the 21-day study based on hepatocellular necrosis, fat accumulation, loss of glycogen and increases in liver enzymes at this dose. DnHP induced only weak peroxisome proliferative changes compared to known peroxisome proliferators such as diethylhexyl phthalate (DEHP).

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

Experimental data in mice demonstrated that DnHP causes dose-related fertility effects (380-1670 mg/kg bw/d) during a continuous 98 day breeding study. Crossover mating studies also demonstrated that reproductive parameters were affected in both male (including reduced testes weights) and females. The LOAEL was 380 mg/kg bw/d based on decreased male and female fertility. A NOAEL could not be established. There was conflicting evidence that DnHP possesses oestrogenic activity.

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

Overall, 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.

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Acronyms and Abbreviations

AGD	anogenital distance
AR	androgen receptor
bw	body weight
C	Celsius
CAS	Chemical Abstracts Service
CERHR	Centre for the Evaluation of Risks to Human Reproduction
DEHP	diethylhexyl phthalate
d	day
DNA	deoxyribonucleic acid
DnOP	di-*n*-octyl Phthalate
F1	filial 1 (first generation)
g	gram
GD	gestation day
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
MCF-7	human breast adenocarcinoma cell line
mg	milligram
mL	millilitre
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
ppm	parts per million
PVC	polyvinyl chloride
w/w	weight per weight
µ	micro

1. 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.

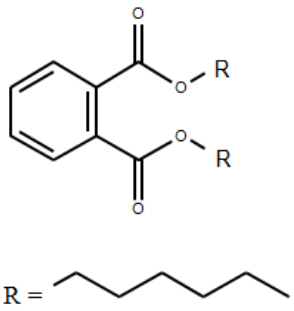
Information on Australian uses was compiled from data supplied by industry in 2004 and 2006.

References not marked with an asterisk were examined for the purposes of this assessment. References not examined but quoted from the key 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 24 *ortho*-phthalate esters (NICNAS, 2008).

2. Identity

2.1 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)

2.2 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 x 10^{-7} kPa (25˚C)
Water solubility	5.0 x 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 x 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)

3. 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).

No information on use in Australia was available. A survey of Australian industry in 2004 and 2006 provided no information on this phthalate.

4. Human Health Hazard

4.1 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.

4.2 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.

4.3 Irritation

4.3.1 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.

4.3.2 Eye irritation

Previous evaluations

No data.

Data not reported in previous evaluations

No data.

Conclusion

No eye irritation studies were available for assessment.

4.3.3 Respiratory irritation

Previous evaluations

No data.

Data not reported in previous evaluations

No data.

Conclusion

No respiratory irritation studies were available for assessment.

4.4 Sensitisation

Previous evaluations

No data.

Data not reported in previous evaluations

No data.

Conclusion

No sensitisation studies were available for assessment.

4.5 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 Appendix 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.

4.6 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.

4.7 Carcinogenicity

Previous evaluations

No data.

Data not reported in previous evaluations

No data.

Conclusion

No carcinogenicity studies were available for assessment.

4.8 Reproductive toxicity

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

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

4.8.1 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 4.5), 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.

4.8.2 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 F1 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.

4.8.3 Postnatal developmental 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.

4.8.4 Mode of action

DnHP was negative for oestrogenic activity in a yeast two-hybrid assay (Nishihara et al., 2000). DnHP did not increase proliferation of human breast cancer MCF-7 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).

4.8.5 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/d DnHP induced reduced testicular weight and testicular atrophy in the rat, whereas exposure to 1824 mg/kg bw/d 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.

5. 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 (NICNAS, 2008) which contains a comparative analysis of toxicity endpoints across 24 *ortho*- 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, 2008).

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 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/d 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 oestrogenic 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, 2008). 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 AGD, 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.

6. 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 Dermal Rabbit: LD50 > 20 mL/kg bw	Skin irritation: minimal effect Eye irritation: no data 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 In vivo: No data	No data	Mice: NOAEL = not established. LOAEL = 380 mg/kg bw/d, dose-related ↓ number of litters/pair, live pups/litter and proportion of pups born alive	Mice: NOAEL = not established LOAEL = 380 mg/kg bw/day; ↑ pup mortality

↑: increase; ↓: decrease

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References

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Appendix - 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 group	Initial body weight (g)	Final body weight (g)	Liver/body weight ratio (%)	Levels of serum cholesterol (mmol/L)	Levels of serum triglyceride (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

Treat-ment	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.

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