Existing Chemical Hazard Assessment Report

Australian Government
Department of Health and Ageing
NICNAS

Di-*n*-octyl Phthalate

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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*-octyl phthalate (DnOP) is a health hazard assessment only. For this assessment, two key reviews on DnOP prepared by the US Agency for Toxic Substances and Disease Registry (ATSDR) and the US Centre for the Evaluation of Risks to Human Reproduction (CERHR) were consulted. Information was also obtained from the Government of Canada and Ontario Ministry of the Environment. These reviews were supplemented with literature surveys conducted up to September 2006.

Structurally, phthalate esters are characterized by a diester structure consisting of a benzenedicarboxylic acid head group linked to two ester side chains. DnOP possesses 2 linear ester side chains each with a backbone of 8 carbons (C8). DnOP is considered a member of the High Molecular Weight Phthalate Esters (HMWPE) Category as defined by the American Chemistry Council Phthalate Esters Panel HPV Testing Group and OECD. The HMWPE group includes chemically similar substances produced from alcohols having backbone carbon lengths of ≥ C7.

DnOP is manufactured in the United States predominantly as a minor component of C6-10 phthalate (approximately 20%). DnOP is used in this form in polyvinyl chloride (PVC) for the manufacture of a variety of products including flooring and carpet tiles, canvas tarps, swimming pool liners, notebook covers, traffic cones, toys and dolls, vinyl furniture upholstery, shower curtains and gloves, garden hoses, weather stripping, flea collars and shoes. DnOP-containing PVC is also used in food applications. Other non-PVC uses of DnOP include as a dye carrier in plastics production, for manufacture of adhesives, plastisols and nitrocellulose lacquer coatings, as an active pesticide ingredient and in cosmetics and colourants. Current EU legislation restricts the use of DnOP in certain toys and childcare articles which can be placed in the mouth.

In Australia, DnOP is imported for use as a plasticiser in automotive and industrial hose and for the manufacture of PVC conveyer belts, insulation materials, polyurethane surface coatings, floor finishes and adhesives. Imported toys, play and exercise balls contain DnOP. DnOP is also distributed to various institutions for research purposes.

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

DnOP was rapidly absorbed from the gastrointestinal tract following oral administration and metabolised predominantly to mono-*n*-octylphthalate (MnOP). In rats after oral administration, peak blood and testes levels of MnOP were reached at 3 and 6 hours, respectively. Elimination occurs via the urine.

In animals, DnOP exhibited low acute oral toxicity. Information on dermal or inhalation toxicity was incomplete or unavailable. Data for other HMWPE suggests that dermal and inhalation toxicity would be expected to be low.

DnOP caused minimal skin and eye irritation in animals. Data were insufficient to determine the sensitisation potential of DnOP. However, it should be noted that phthalates, in general, have low skin sensitisation potential.

The liver appears to be the primary target organ from repeated exposure to DnOP. Liver toxicity was observed in several repeat dose studies. Overall, liver effects from DnOP did not appear to be associated with peroxisome proliferation. From a 13-week dietary study in rats, a LOAEL of 350 mg/kg bw/d was established based on histological changes in the liver and thyroid. The NOAEL was 37 mg/kg bw/d.

DnOP was negative in bacterial mutation and direct DNA damage assays. Mixtures containing DnOP tested negative in bacterial and mammalian mutation assays. Based on available in vitro data, DnOP was considered non-genotoxic.

Limited data on carcinogenic potential indicated that DnOP may act as a promoter of pre-neoplastic hepatic lesions in the rat via a non-peroxisome proliferative mechanism. Current literature indicates that rodents compared to humans are particularly susceptible to peroxisome proliferative effects and resultant carcinogenicity. However, peroxisome proliferation was not a notable effect in available repeated dose toxicity studies of DnOP. Overall, data were insufficient to determine the carcinogenic potential of DnOP.

DnOP did not appear to induce reproductive toxicity at the highest dose tested of 7500 mg/kg bw/d in a continuous breeding study in mice or 350 mg/kg bw/d in a 13-week subchronic study in rats. In vitro, a potential for testicular effects was suggested from findings of MnOP induced marked germ-cell detachment and disruption of Sertoli-cell monolayers in co-cultures of Sertoli-germ cells isolated from pubertal rats. However, testicular effects were not seen in subchronic studies in mice and rats. The NOAEL for fertility effects was 7500 mg/kg bw/d.

Studies in mice suggest a low potential for DnOP to induce developmental effects with a NOAEL for developmental effects of 7500 mg/kg bw/d.

In studies of potential oestrogenic mimicry, DnOP did not induce oestrogenic responses in vivo or in vitro and displayed no binding affinity for human oestrogen receptors.

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Acronyms and Abbreviations

AGD	anogenital distance
AGI	anogenital index
ATSDR	Agency for Toxic Substances and Disease Registry (US)
bw	body weight
C	Celsius
CAS	Chemical Abstracts Service
CERHR	Centre for the Evaluation of Risks to Human Reproduction
CHO	Chinese hampster ovary
d	day
DNA	deoxyribonucleic acid
DnOP	dioctyl phthalate
ECB	European Chemicals Bureau
EU	European Union
F1	filial 1 (first generation)
g	gram
GD	gestation day
HMWPE	High Molecular Weight Phthalate Esters
HPV	High Production Volume
IUCLID	International Uniform ChemicaL Information Database
kg	kilogram
kPa	kilopascals
L	litre
LC50	median lethal concentration
LD50	median lethal dose
LOAEL	lowest-observed-adverse-effect level
M	moles
MCPP	mono-(3-carboxypropyl) phthalate
mg	milligram
mL	millilitre
MnOP	mono-*n*-octylphthalate
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program

OECD	Organisation for Economic Cooperation and Development
ppm	parts per million
PVC	polyvinyl chloride
µ	micro

1. Introduction

This review of di-*n*-octyl phthalate (DnOP) is a health hazard assessment only. For this assessment, two key reviews on DnOP prepared by the US Agency for Toxic Substances and Disease Registry (ATSDR, 1997) and the US Centre for the Evaluation of Risks to Human Reproduction (CERHR, 2003) were consulted. Information was also obtained from the Government of Canada (1993) and Ontario Ministry of the Environment (2005). These reviews were supplemented with literature surveys conducted up to September 2006.

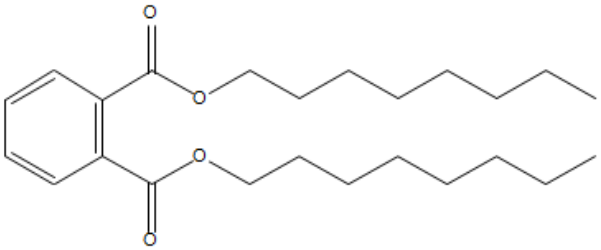
Information on Australian uses was compiled from data supplied by industry in 2004 and 2006.

References not marked with an asterisk were examined for the purposes of this assessment. References not examined but quoted from these two reports 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 phthalate 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:	117-84-0
Chemical Name:	1,2-Benzened icarboxylic acid, dioctyl ester
Common Name:	Di-*n*-octyl phthalate (DnOP or DOP)
Molecular Formula:	$C_{24}H_{38}O_4$
Structural Formula:	
Molecular Weight:	390.6
Synonyms:	Bis(*n*-octyl) phthalate; Di-*n*-octyl phthalate; Dioctyl-o-benzenedicarb late; *n*-Dioctyl phthalate; *n*-Octyl phthalate; Octyl phthalate; Phthalic acid, dioctyl ester
Purity/Impurities/ Additives	None identified

2.2 Physico-chemical properties

Table 1: Summary of physico-chemical properties

Property	Value
Physical state	Colourles s, odourless liquid
Melting point	-25°C
Boiling point	390°C (101.3 kPa)
Density	978 kg/m ^{3} (25°C)
Vapour pressure	1.92 x 10^{-5} kPa (25°C)
Water solubility	3.0 x 10^{-3} g/L (25°C)
Partition coefficient n-octanol/water (log Kow)	5.22
Henry's law constant	0.55, 6.6 8 x 10^{-3} atm-m^3/mole
Flash point	219°C

Source: ATSDR (1997)

3. Uses

DnOP is manufactured in the United States predominantly as a minor component of C6-10 phthalate (approximately 20%). DnOP is used in this form in PVC for the manufacture of a variety of products including flooring and carpet tiles, canvas tarps, swimming pool liners, notebook covers, traffic cones, toys and dolls, vinyl furniture upholstery, shower curtains and gloves, garden hoses, weather stripping, flea collars and shoes. DnOP-containing PVC is also used in food applications such as seam cements, bottle cap liners and conveyor belts. Other non-PVC uses of DnOP include as a dye carrier in plastics production, for manufacture of adhesives, plastisols and nitrocellulose lacquer coatings, as an active pesticide ingredient and in cosmetics and colorants (ATSDR, 1997; CERHR, 2003). Current EU legislation restricts the use of DnOP in certain toys and childcare articles which can be placed in the mouth.

In Australia, DnOP is imported for use as a plasticiser in automotive and industrial hose and for the manufacture of PVC conveyer belts, insulation materials, polyurethane surface coatings, floor finishes and adhesives. Imported toys, play and exercise balls contain DnOP. DnOP is also distributed to various institutions for research purposes.

4. Human Health Hazard

4.1 Toxicokinetics

Previous evaluations

Limited available data indicate that DnOP is readily absorbed following ingestion. Low levels of DnOP were reported in liver and adipose tissues of rats following administration in the diet over 13 weeks (Poon et al., 1995*, 1997*). No data are available on absorption following inhalation or dermal administration.

Following oral administration of DnOP (2000 mg/kg) in rats, peak blood and testes levels of mono-*n*-octylphthalate (MnOP) were reached at 3 and 6 hours after dosing, respectively. Half-life of this metabolite in blood was 3.3 hours (Oishi, 1990*). DnOP in addition to other dialkyl phthalates was reported to be metabolised to their monoesters and alcohol by enzymes present in gut tissues (Rowland et al., 1977*). Following administration of 0.2 mL of DnOP to rats by gavage, 31% of the administered dose was recovered in urine within 48 hours as both MnOP and free phthalic acid (Albro and Moore, 1974*).

Data not reported in previous evaluations

In a more recent study of DnOP metabolism in rats, the monoester MnOP, mono-(3-carboxypropyl) phthalate (MCPP), phthalic acid and 5 additional oxidative products were detected in urine 24 hours after oral administration (300 mg/kg). MCPP was the major component with MnOP being the minor (Silva et al., 2005). In a similar study, MCPP levels were found to be 560-fold higher than MnOP levels in 24-hour urinary samples (Calafat et al., 2006). An in vitro study of the metabolism of DnOP by rat liver microsomes showed rapid production of MnOP with production also of mono-hydroxy-*n*-octyl phthalate and phthalic acid (Silva et al., 2005).

Conclusion

Following ingestion, DnOP is rapidly metabolised and absorbed from the gastrointestinal tract as the monoester mono-*n*-octylphthalate. Half-life of the monoester in the blood is approximately 3 hours. The liver is capable of metabolising DnOP. Elimination occurs via the urine with levels of MnOP exceeded after 24 hours by the other oxidative metabolite MCPP.

4.2 Acute toxicity

Previous evaluations

Results for acute toxicity are summarised in Table 2.

Data not reported in previous evaluations

No data.

Table 2: Acute animal toxicity studies

Study	Species	Result (LD50)	Reference
Oral	Rat	53700 mg/kg bw	Dogra et al. (1987)*
Oral	Mouse	13000 mg/kg bw	Dogra et al. (1989)*
Oral	Mouse	>12800 mg/kg bw	Eastman Kodak Company (1978)*
Dermal	Guinea pig	75 mL/kg bw	Bisesi (1994); CMA (1999)*

Source: ATSDR, 1997; CERHR, 2003

Conclusion

DnOP has oral LD50 values of 53700 mg/kg bw in rats and >12800 mg/kg bw in mice. A dermal LD50 value of 75 mL/kg bw in guinea pigs was reported, however, the concentration of the applied substances was not available. No data were available regarding inhalation toxicity.

4.3 Irritation

4.3.1 Skin irritation

Previous evaluations

DnOP was reported to be a slight skin irritant when applied to the depilated skin of guinea pigs (Eastman Kodak Company, 1978*). According to information from RTECS (2004), DnOP caused mild skin irritation when applied to rabbit skin (Marhold, 1986*).

Data on effects in humans are restricted to poorly documented clinical studies of skin irritation and sensitisation in volunteers upon dermal contact with dioctyl phthalate (isomers unspecified) (Government of Canada, 1993).

Data not reported in previous evaluations

No data.

Conclusion

DnOP causes minimal skin irritation in guinea pigs. Limited data suggest similar effects in humans.

4.3.2 Eye irritation

Previous evaluations

Ocular administration of DnOP to guinea pigs resulted in slight conjunctival irritation. No further details were provided (Eastman Kodak Company, 1978*).

According to information from RTECS (2004), application of 500 mg into the rabbit eye resulted in only mild eye irritation (Marhold, 1986*). Another poorly reported

study in RTECS (authorship unknown) noted that application of DnOP (20 mg) into the rabbit eye caused severe eye irritation (Anon., 1946*).

Data on effects in humans are restricted to a case report of irritation of the eye of workers exposed to phthalates including dioctyl phthalate (isomer unspecified) (Government of Canada, 1993).

Data not reported in previous evaluations

No data.

Conclusion

DnOP causes minimal eye irritation in guinea pigs. Limited data suggest similar effects in humans.

4.3.3 Respiratory irritation

Previous evaluations

Data are restricted to a case report of irritation of the upper respiratory tract of workers exposed to phthalates including dioctyl phthalate (isomer unspecified) (Government of Canada, 1993).

Data not reported in previous evaluations

No data.

Conclusion

Data are insufficient to determine the respiratory irritation potential of DnOP.

4.4 Sensitisation

Previous evaluations

DnOP was not a skin sensitiser in guinea pigs (Eastman Kodak Company, 1978*). Data on effects in humans are restricted to poorly documented clinical studies of skin irritation and sensitisation in volunteers upon dermal contact with dioctyl phthalate (isomers unspecified).

Data not reported in previous evaluations

A European Chemicals Bureau (ECB) IUCLID dataset for a related phthalate mixture likely containing DnOP (1,2-benzenedicarboxylic acid, di-C8-10-alkyl esters, CAS no. 71662-46-9) noted negative results from a guinea pig maximisation test conducted to OECD Test Guidelines and Good Laboratory Practice guidelines.

The ECB IUCLID dataset above also contains short summaries of human sensitisation data for DnOP. Sixty workers chosen from 400 workers in a plastic shoe factor were patch tested with a standard battery of substances including 4 phthalates. Of 11 workers with contact dermatitis, 6 workers showed sensitisation reactions. Reactions to DnOP were observed. In another summary of a case report of occupational asthma, DnOP was reported to evoke an asthmatic reaction in a single

patient following exposure to DnOP vapours. The reaction was inhibited by prior administration of sodium cromoglycate.

No other details were provided for either of these summaries and the data have not undergone any evaluation by the European Commission (ECB, 2000).

Conclusion

A single animal study indicated that DnOP is not a skin sensitiser. Negative animal results were also noted above for a di-C8-10-alkyl ester mixture likely containing DnOP. In contrast, poorly documented human patch test studies and a case report from occupational exposure suggest the potential for skin and respiratory sensitisation in humans.

Overall, data are insufficient to determine the sensitisation potential of DnOP.

4.5 Repeated dose toxicity

Previous evaluations

Administration of DnOP (2% in diet) to male JCL:Wistar rats for 1 week produced statistically significant increases in absolute and relative liver weights but not kidney weights (Oishi and Hiraga, 1980). Administration of dietary mono-octyl phthalate also induced liver enlargement but also altered serum lipid composition (Oishi and Hiraga 1982*).

Wistar rats received DnOP orally at doses of 2266 mg/kg bw/d for 3 days, 2078 mg/kg bw/d for 10 days, and 1906 mg/kg bw/d for 21 days. The livers of treated rats had a pale greasy appearance. Relative liver weights were statistically significantly increased after 10 and 21 days of treatment, but not after 3 days of treatment. After 3 days, loss of centrilobular glycogen, proliferation and dilation of smooth endoplasmic reticulum, loss of rough endoplasmic reticulum, and shortened microvilli in some bile canaliculi were observed. No significant changes were noted in enzyme parameters associated with peroxisome proliferation. After 10- or 21-day treatment, centrilobular glycogen reduction became more severe and was associated with fat accumulation and some necrosis. Small but statistically significant increases were also noted for hepatic cyanide-insensitive palmitoyl CoA oxidase and peroxisomal catalase activities for these prolonged treatments (Mann et al., 1985*).

Thyroid effects were re-examined in the above study. Serum from 4 Wistar rats administered DnOP for 21 days in the above study were reassayed (Hinton et al., 1986*). Statistically significant decreases in serum thyroxine (T4) levels, but not triiodothyronine (T3) levels, were observed. In addition, in the thyroid, ultrastructural changes consisting of increases in the numbers and sizes of lysosomes, enlargement of the Golgi apparatus and damage to mitochondria were also detected.

To examine whether liver metabolism and biochemical changes are associated with peroxisome proliferation, 6 Sprague-Dawley rats/dose were treated by gavage with 1000 or 2000 mg/kg bw/d DnOP for 14 days. Relative liver weights were increased in both groups. There were no increases in peroxisomal enzyme activities (Lake et al., 1984*; 1986*).

In an incomplete report of a study in which male rats were exposed to 0, 300 or 600 mg/kg bw/d of DnOP in the diet for 11 weeks, marked liver damage (characterized by cellular enlargement and proliferation, vacuolization, chronic inflammation, and necrosis) was observed at 300 mg/kg bw/d and above. Alterations in serum enzyme levels were consistent with the observed liver damage (DeAngelo et al., 1988*).

Rats received DnOP intraperitoneally at doses of 100, 300, or 600 mg/kg bw/d for 90 days. Half of the animals from each dose group were killed at the end of the study. Microscopic examination of the kidneys revealed dose related changes mainly to glomeruli, proximal and distal tubules, the loop of Henle and collecting tubules in the medulla. Effects persisted 45 days after cessation of treatment (Khanna et al., 1990*).

Systemic effects of DnOP were studied in Sprague-Dawley rats (10/sex) fed DnOP at dietary concentrations of 0, 5, 50, 500, or 5000 ppm (0, 0.4-0.4, 3.5-4.1, 36.8-40.8, or 350-403 mg/kg bw/d males-females respectively) for 13 weeks. At 5000 ppm, liver and thyroid effects were observed. Hepatic effects including anisokaryosis, nuclear hyperchromicity, vesiculation, cytoplasmic vacuolation, nuclear endothelial prominence, and accentuation of zonation were observed. Statistically significant increases in hepatic ethoxyresorufin-o-demethylase activity were also seen at this dose level. Thyroid effects included decreases in follicle size and colloid density. There were no morphological effects on reproductive organs at the highest dose level. The NOAEL was 500 ppm (37 mg/kg bw/d), based on liver and thyroid effects at 5000 ppm (Poon et al., 1995*; 1997*).

DnOP was also studied in mice. Following a continuous breeding protocol, offspring CD-1 mice taken from the last litter of treated parental mice exposed to approximately 7500 mg/kg bw/d DnOP for 105 days, including mating, were exposed to DnOP for 85 to 105 days, first via lactation, then through diet. The average DnOP dose in these offspring over this period was calculated by the study authors as 8640 mg/kg bw/d. Statistically significant increases in absolute liver weights were observed in both sexes. Absolute kidney weights were also statistically significantly increased in females but not males. However, no gross morphological or histopathological lesions were noted in these two organs (Heindel et al., 1989, Morrissey et al., 1989*, NTP 1985*).

CD-1 mice receiving DnOP in the diet at concentrations of 1800, 3600, 7500, and 15000 mg/kg bw/d for 14 days showed no clinical symptoms of toxicity except for rough hair coats at the highest dose (Heindel et al., 1989*).

Information is limited on chronic toxicity of DnOP in experimental animals. In an incomplete account, a single dose (175 mg/kg bw/d) of an unspecified isomer of dioctyl phthalate was administered to an unspecified species and strain of animal for 12 months. The reported effects were restricted to a decrease in body weights and increases in liver and kidney weights and activities of serum enzymes (Pieckacz, 1971*). In another chronic toxicity study, "numerous" liver nodules were reported in male rats given 1% DnOP (600 mg/kg bw/d) in the diet for 15 months. The activity of a number of lysosomal enzymes was also increased in these rats (Carter et al., 1989*).

There are few human data for repeat dose exposure to DnOP. Government of Canada (1993) and Ontario Ministry of the Environment (2005) contain the following

references to poorly documented studies of occupational exposure to phthalates including DnOP.

Gilioli et al. (1978*) reported that 12 of 23 workers at a plasticizer manufacturing facility who had been exposed to phthalate esters (< 1 to 60 mg/m^3, specific isomers not identified) for an average of 4.5 years had a mild to moderate sensory-motor and motor polyneuropathy. Another study investigated Russian workers (87 females and 60 males) in the artificial leather industry in which several phthalate plasticisers including DnOP were used (Milkov et al., 1973*). Duration of employment ranged from 0.5 to 19 years. Phthalates were found to be the principal air contaminants in the work areas with ambient air concentrations of the phthalate plasticisers ranging from 1.7 to 66 mg/m^3. Neurological testing revealed that 32% of the employees had polyneuritis, the incidence of which correlated positively with length of service. A subset (81 workers) was examined for vestibular function effects, with 78% of subjects showing a depression of vestibular receptors. Some workers also displayed a lowering of the excitability threshold level for olfactory receptors.

Data not reported in previous evaluations

In a US New Jersey Department of Health and Senior Services Hazardous Substances Fact Sheet, repeated exposure of humans to DnOP is claimed to cause liver damage, while repeated skin contact can cause dryness, cracking and rashes (NJDHSS, 2002).

Conclusion

The liver appears to be the primary target organ of DnOP. Liver toxicity (weight, histological or clinical chemistry changes) caused by DnOP was observed in several repeat dose studies. In a 21-day oral study, DnOP caused dose-related statistically significant increases in relative liver weights, loss of centrilobular glycogen and changes in liver microarchitecture including fat accumulation and necrosis. In other studies, DnOP also induced ultrastructural changes in the thyroid and kidney.

Overall, liver effects from DnOP did not appear to be associated with peroxisome proliferation. Increases in peroxisomal enzyme activities generally were not observed.

From a 13-week rat dietary study of 4 dose levels, a LOAEL of 350 mg/kg bw/d was established, based on histological changes in the liver (anisokaryosis, nuclear hyperchromicity, vesiculation, cytoplasmic vacuolation, nuclear endothelial prominence, accentuation of zonation) and thyroid (decreases in follicle size and colloid density). The NOAEL was 37 mg/kg bw/d.

4.6 Genetic toxicity

Previous evaluations

ATSDR (1997) notes a sizable database of in vitro microbial assays for DnOP. DnOP ranging from 100 to 10000 µg/plate in either the presence or absence of exogenous metabolic activation was not mutagenic in Ames tests using *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100) (Zeiger et al., 1982*; 1985*).

DnOP at 2000 µg/mL with metabolic activation and ranging from 100 to 2000 µg/mL without metabolic activation did not induce DNA damage in *E-coli* (Goodyear Tire & Rubber Company, 1981*). Similar results are reported by Seed (1982) and

Shibamoto and Wei (1986*). DnOP also showed a negative response in a prokaryotic SOS chromotest assay to detect DNA damage in *E-coli* (Sato et al., 1994*).

Data on mixtures containing DnOP were noted in CERHR (2003). Results from testing a C6-10 phthalate mixture (containing 20% DnOP) in a mouse lymphoma mutation assay were considered equivocal due to a non-dose related increase in mutations in the presence and absence of metabolic activation (Barber et al., 2000). According to CMA (1999*), di(*n*-octyl, *n*-decyl) phthalate containing DnOP as a component was reported to be negative in an Ames test and the Chinese hamster ovary/HPRT locus assay.

Data not reported in previous evaluations

No data.

Conclusion

DnOP is negative in bacterial mutation and DNA damage assays. No information is available regarding genotoxic potential in vivo.

Based on negative results from in vitro bacterial mutation and DNA damage assays, DnOP is considered non-genotoxic.

4.7 Carcinogenicity

Previous evaluations

In two studies, male Sprague-Dawley rats were given a partial hepatectomy and then injected intraperitoneally with diethylnitrosamine, then exposed to 0.5 or 1% DnOP via the diet (approximately 250 mg/kg bw/d or 500 mg/kg bw/d) for up to 26 weeks. Increases in numbers of gamma-glutamyl transpeptidase-positive foci and levels of glutathione-S-transferase staining in the livers compared to controls were observed, suggesting pre-tumour activity. Only a 3-fold increase was observed for carnitine acetyltransferase activity, a marker for peroxisome proliferation (DeAngelo et al., 1986* and 1989*; Carter et al., 1992). These results suggest that DnOP may promote preneoplastic lesions in rat livers via a mechanism that does not rely solely on peroxisome proliferation.

In a poorly documented chronic toxicity study, "numerous" liver nodules were reported in male rats given 1% DnOP (600 mg/kg bw/d) in the diet for 15 months. The activity of a number of lysosomal enzymes was also increased in these rats (Carter et al., 1989*).

Data from an in vitro test where a C6-10 phthalate mixture (containing 20% DnOP) was examined in the Balb/3T3 mammalian cell transformation assay showed a negative result (Barber et al., 2000).

Data not reported in previous evaluations

No data.

Conclusion

Limited data suggest that dietary exposure to DnOP might act as a promoter of pre-neoplastic lesions in the rat liver. Overall, data are insufficient to determine the carcinogenic potential of DnOP.

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. The effects on fertility and development are then discussed separately.

4.8.1 Human studies

Previous evaluations

Human sperm suspensions incubated with DnOP (0, 64, 640 µM) for up to 18 h showed dose-dependent decreases in motility (80% motility at 64 µM) (Fredricsson et al., 1993).

Data not reported in previous evaluations

A group of 45 women with endometriosis showed significantly higher plasma concentrations of DnOP as well as DBP, BBP, DEHP and polychlorinated biphenyls compared to a group of 135 fertile women (Reddy et al., 2006a; b).

Association between 11 maternal urinary phthalate monoester concentrations and genital parameters such as anogenital index (AGI) [i.e. anogenital distance (AGD) normalised for body weight] and testicular descent in children was investigated in 85 mother-son pairs (Swan et al., 2005). There was no significant association between maternal urinary MCPP (a DnOP metabolite) concentration and infant AGI.

4.8.2 Repeat dose toxicity studies

Previous evaluations

Male Sprague-Dawley rats receiving DnOP by gavage at 2800 mg/kg bw/d for 4 or 10 days showed no testicular atrophy or histological lesions, testicular zinc loss or prostate or seminal vehicle weight loss (Foster et al., 1980; Gray and Butterworth 1980*).

Similarly, no effects on testes weights or testicular concentration of testosterone or dihydrotestosterone were observed in male rats fed a diet containing 2% DnOP for one week. However, a statistically significant 15% reduction in testicular zinc was observed (Oishi & Hiraga, 1980). No effect on testis weight, gross morphology, or

histopathology was found in male rats receiving dietary exposure to approximately 2000 mg/kg/d for 10 or 21 days (Mann et al., 1985).

In a subchronic study, Sprague-Dawley rats received DnOP in diet at doses as high as 5000 ppm (350-403 mg/kg bw/d males-females respectively) for 13 weeks. Testes weights were unaffected and testes histology was normal. No reproductive effects were seen (Poon et al., 1995* and 1997*).

Data not reported in previous evaluations

No data.

4.8.3 Continuous breeding reproductive toxicity studies

Previous evaluations

In a continuous breeding protocol oral reproductive/developmental study, CD-1 Swiss mice (20 pairs/dose) were fed DnOP in the diet at 0, 1.25, 2.5, or 5% (0, 1800, 3600, and 7500 mg/kg bw/d) for 14 weeks (Gulati et al., 1985*; Heindel et al., 1989). Litters born during the 14 weeks period were evaluated and removed so that the adults could continue breeding. Over 5 successive litters, there was no effect on the number of litters, litter size, sex ratios, pup weight or viability.

In sexually mature F1 adults mated within dose groups, DnOP similarly had no effects on fertility indices, litter size, pup weight or viability. In control and high-dose (7500 mg/kg bw/d) F1 adults, increased absolute and relative liver weights and decreased relative seminal vesicles weights were observed in males. However, there was no evidence of morphological or histopathological changes in these organs. No changes were observed in testis, cauda epididymis or prostate weights, sperm concentrations, percent mobile sperm or percent abnormal sperm. Females at this high dose showed increased relative liver and kidney weights. However, no changes were observed in oestrous cycle length or in reproductive organ weights or histopathology. Over 5 litters, there was no effect on the number of litters, litter size, sex ratios, pup weight or viability. Overall, DnOP did not show significant reproductive effects in this study even at hepatotoxic doses. The NOAEL for reproductive and developmental effects was 7500 mg/kg bw/d, the highest dose tested.

Data not reported in previous evaluations

No data.

4.8.4 Prenatal developmental toxicity studies

Previous evaluations

Singh et al. (1972) administered DnOP at 0, 4890, 9780 mg/kg bw/d to Sprague-Dawley rats (15/group) prenatally on gestation days 5, 10 and 15 via intraperitoneal injection. A small but significant decrease in average foetal weight and a significant increase in the incidence in gross foetal malformations were observed in offspring. Possible confounding maternal effects were not reported.

Administration of n-octanol, a primary metabolite of DnOP (~130, 650, 945, and 1300 mg/kg bw/d) by gavage to pregnant Wistar rats on days 6−15 of gestation

induced dose-related symptoms of clinical intoxication of the nervous system with maternal death seen in the three highest dose levels. However, no effects on foetal weight, viability, or incidence of malformations were seen (Hellwig and Jack, 1997*)

Data not reported in previous evaluations

No data.

4.8.5 Postnatal developmental toxicity studies

Previous evaluations

Hardin et al. (1987) administered DnOP via oral gavage to female CD-1 mice at 9780 mg/kg bw/d during GD 6-13. There was no effect on maternal weight. The test group varied from the concurrent control (vehicle treated) group only in an 11% reduction in numbers of liveborn pups per litter and a 14% reduction in average pup weight gains on postnatal days 1-3 (both statistically significant). The authors reported that concurrent control values for these parameters were higher than other control groups from the same study, thus casting uncertainty on the biological significance of these changes.

Data not reported in previous evaluations

No data.

4.8.6 Mode of action

Previous evaluations

DnOP did not induce oestrogenic responses in vivo in a uterotrophic and vaginal cornification assay using immature and mature ovariectomised rats (Zacharewski et al., 1998). DnOP was negative for oestrogenic activity in recombinant yeast assay (Harris et al., 1997) and 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).

Phthalate-induced germ cell detachment was examined in vitro in co-cultures of Sertoli and germ cells isolated from pubertal rats (Gray and Beamand, 1984). Mono-*n*-octyl phthalate (MnOP) induced marked germ-cell detachment and disruption of the Sertoli-cell monolayer at 10^{-4} M, the magnitude of which exceeded that of the other 8 monoesters tested.

Data not reported in previous evaluations

DnOP (up to 10^{-3} M) showed no detectable binding to oestrogen receptor α or β in vitro (Toda et al., 2004) and did not demonstrate oestrogenic activities in in vitro reporter gene assays with CHO-K1 cells transfected with expression vectors for human oestrogen receptor α, β or androgen receptor (Takeuchi et al., 2005).

Conclusion

Effects on fertility

Data were insufficient to determine fertility effects of DnOP in humans.

In animals, no effects on number of litters, litter size, sex ratios, pup weight or viability were observed for DnOP in an oral continuous breeding reproductive/developmental study in mice. The NOAEL for fertility effects was 7500 mg/kg bw/d.

In a 13-week subchronic study in rats, no effects on testes weight or morphology were observed at the highest dose level (350 mg/kg bw/d). No testicular atrophy or histological lesions were observed in male rats gavaged with DnOP at 2800 mg/kg bw/d for 4 or 10 days. Decreased testicular zinc was the only effect observed in a one-week rat study in which rats were fed a diet containing 2% DnOP. No effects on testes weights or testicular concentration of testosterone or dihydrotestosterone were observed.

Developmental effects

Decreased foetal weight and increased visceral malformations were noted in the offspring of rats given high doses of DnOP (up to 9780 mg/kg bw/d) by intraperitoneal injection. However, potentially confounding maternal effects were not reported. In a second short-term oral (gavage) study in mice, statistically significant decreases were observed in viability and pup weight gains. However, concurrent control values for these parameters were not comparable with those of other control groups in the study casting uncertainties over the biological significance of these findings. In contrast, no developmental toxicity was observed in a continuous breeding multigenerational reproductive/developmental study in mice at concentrations up to 7500 mg/kg bw/d.

In view of the magnitude of doses used, the results of two oral studies in mice suggest a low potential for DnOP to induce developmental effects. The NOAEL for developmental effects was 7500 mg/kg bw/d.

5. Hazard Characterisation

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

DnOP is a linear C8 phthalate and a member of the High Molecular Weight Phthalate Esters (HMWPE) Category as defined by the American Chemistry Council Phthalate Esters Panel HPV Testing Group (2001) and OECD (2004). The HMWPE group includes chemically similar substances produced from alcohols having backbone carbon lengths of ≥ C7. Due to their similar chemical structure, category members are generally similar with respect to physicochemical, biological and toxicological properties or display an expected trend. Thus, read-across for toxicity endpoints is an appropriate approach to characterise selected endpoints for members of this category.

DnOP is rapidly absorbed from the GIT system following oral administration and metabolised predominantly to mono-*n*-octylphthalate. In rats after oral administration, peak blood and testes levels of mono-*n*-octylphthalate are reached at 3 and 6 hours respectively. Elimination occurs via the urine, with levels of MnOP exceeded after 24 hours by MCPP.

In animals, DnOP exhibits low acute oral toxicity. Information on dermal or inhalation toxicity is incomplete or unavailable. Data for other high molecular weight phthalates (NICNAS, 2008) suggests that dermal and inhalation toxicity would be expected to be low.

DnOP caused minimal skin and eye irritation in animals. Data are insufficient to determine the sensitisation potential of DnOP. However, it should be noted that phthalates, in general, have low skin sensitisation potential (NICNAS, 2008).

The liver appears to be the primary target organ from repeated exposure to DnOP. Liver toxicity (weight, histological or clinical chemistry changes) caused by DnOP was observed in several repeat dose studies. DnOP also induced ultrastructural changes in the thyroid and kidney. Overall, liver effects from DnOP did not appear to be associated with peroxisome proliferation. From a 13-week dietary study in rats, a LOAEL of 350 mg/kg bw/d was established based on histological changes in the liver and thyroid. The NOAEL was 37 mg/kg bw/d.

DnOP was negative in bacterial mutation and direct DNA damage assays. Mixtures containing DnOP tested negative in bacterial and mammalian mutation assays. Based on available in vitro data, DnOP is considered non-genotoxic.

Limited data on carcinogenic potential indicated that DnOP may act as a promoter of pre-neoplastic hepatic lesions in the rat via a non-peroxisome proliferative mechanism. A large increase in numbers of gamma-glutamyltransferase-positive foci was observed in the livers of rats following 26 weeks dietary administration. Gamma-glutamyltransferase is often significantly increased in human tumours and its role in

tumour progression and invasion has been suggested. In another study, liver nodules were reported in rats after dietary administration of DnOP for 15 months. Current literature indicates that rodents compared to humans are particularly susceptible to peroxisome proliferative effects and resultant carcinogenicity (NICNAS, 2008). However, peroxisome proliferation was not a notable effect in available repeated dose toxicity studies of DnOP. Overall, data are insufficient to determine the carcinogenic potential of DnOP.

DnOP did not appear to induce reproductive toxicity at the highest dose tested of 7500 mg/kg bw/d in a continuous breeding study in mice or 350 mg/kg bw/d in a 13-week subchronic study in rats. In vitro, a potential for testicular effects is suggested from findings of mono-*n*-octyl phthalate induced marked germ-cell detachment and disruption of Sertoli-cell monolayers in co-cultures of Sertoli-germ cells isolated from pubertal rats. However, testicular effects were not seen in subchronic studies in mice and rats. The NOAEL for fertility effects was 7500 mg/kg bw/d.

Decreased foetal weight and increased visceral malformations were noted in the offspring of rats given high doses of DnOP post-gestation by intraperitoneal injection. However, the extent to which these might be attributable to maternal toxicity is not known. In a second short-term developmental study in mice, statistically significant decreases were observed in viability and pup weight gains with high doses of DnOP given via oral gavage. However, there are uncertainties over the biological significance of these findings given the magnitude of concurrent control values. In contrast, no developmental toxicity was observed in a continuous breeding multigenerational reproductive/developmental study in mice at DnOP concentrations up to 7500 mg/kg bw/d. In studies of potential oestrogenic mimicry, DnOP did not induce oestrogenic responses in vivo or in vitro and displayed no binding affinity for human oestrogen receptors.

Studies in mice suggest a low potential for DnOP to induce developmental effects with a NOAEL for developmental effects of 7500 mg/kg bw/d.

6. Human Health Hazard Summary Table

Phthalate	**Acute Toxicity**	**Irritation & Sensitisation**	**Repeated Dose Toxicity**	**Genetic Toxicity**	**Carcinogenicity**	**Fertility**	**Developmental Toxicity**
Di-*n*-octyl phthalate (DnOP)	Oral Rat: LD50 = 53700 mg/kg bw Dermal Guinea pig: LD50 = 75 mL/kg bw Inhalation No data	Skin Irritation: ME Eye Irritation: ME Respiratory Irritation: Insufficient data Skin Sensitisation: Negative	Oral Rat: NOAEL = 37 mg/kg bw/d LOAEL = 350 mg/kg bw/d Ultrastructural changes in liver and thyroid. High doses: ↑ liver weight, vacuolisation and necrosis; ↓ centrilobular glycogen. PP not generally noted.	In vitro: Bacterial mutation assays and direct DNA damage assays: Negative In vivo: No data	In vitro: No data In vivo: Rat: Liver nodules following 15 month dietary administration Rat: 26 week dietary ↑ gamma-glutamyltransferase-positive foci in liver	Continuous breeding study Mouse: NOAEL = 7500 mg/kg bw/d (highest dose tested) LOAEL: NE	Continuous breeding study Mouse: NOAEL = 7500 mg/kg bw/d (highest dose tested) LOAEL: NE

ME – minimal effects; NE – not established; PP – peroxisome proliferation

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