Existing Chemical Hazard Assessment Report

Australian Government
Department of Health and Ageing
NICNAS

Diisohexyl Phthalate

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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME
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Preface

This report was compiled under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). This Scheme was established by the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act), which came into operation on 17 July 1990.

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Overview

This review of diisohexyl phthalate (DIHP) is a health hazard assessment only. For this assessment, no international assessment reports were available. The review was prepared using 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. DIHP possesses 2 branched and/or linear ester side chains each with a backbone of predominantly 5 carbons (C5). DIHP is considered to belong to a group of 'transitional' phthalates defined as those produced from alcohols with straight-chain carbon backbones of C4-6.

Limited use information is available for DIHP. Commercial blends of DIHP may contain up to 25% di-n-hexyl phthalate (DnHP).

In Australia, DIHP is imported for use in auto transmission lubricants.

There is very little toxicity information for DIHP. For individual health 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 DIHP.

No toxicokinetic data were available for DIHP. However, diisohexyl phthalate (DIHP) and di-n-hexyl phthalate (DnHP) are structurally similar, one with a branched (DIHP) and the other a linear (DnHP) backbone. Based on data for DnHP and other transitional phthalates, DIHP is likely to be rapidly absorbed as the monoester from the gut and excreted via the urine.

There was no information regarding the acute toxicity of DIHP, but it is expected to be similar to DnHP and other phthalates with a linear, similar sized backbone, which exhibit low acute oral and dermal toxicity. Similarly, DIHP is not expected to cause skin or eye irritation or skin sensitisation.

Based on negative in vitro tests for DIHP and DnHP, and the generally negative genotoxicity profile of phthalates of a similar molecular weight, DIHP is considered unlikely to be genotoxic.

There are no repeat dose or long-term studies (including carcinogenicity) available for DIHP. For those phthalates currently with no information such as DIHP, the severity of effects expected from repeat dose exposure is difficult to predict. However, liver and kidney effects from repeat doses would be expected, particularly at high doses. It is also not possible to extrapolate carcinogenic potential of DIHP due to insufficient testing of other phthalates.

There are no mammalian reproductive or developmental toxicity studies available for DIHP. In vitro studies have yielded conflicting results as to the antagonistic activity of DIHP to human androgen receptors. Other studies suggest that DIHP or an isomeric mixture of DIHP demonstrated human oestrogen receptor α-agonistic activity and androgen receptor-antagonistic activities in vitro but did not induce vaginal cornification response or an increase in uterine weight in vivo.

A closely related analogue, DnHP, causes fertility effects in both sexes of two rodent species and developmental toxicity. Other transitional phthalates such as butyl benzyl phthalate (BBP), dibutyl phthalate (DBP) and diethylhexyl phthalate (DEHP) have also been associated with male reproductive and developmental toxicity. Hence, DIHP is likely to cause similar adverse reproductive and developmental effects.

Table of Contents

Acronyms and Abbreviations

AR	androgen receptor
BBP	butylbenzyl phthalate
bw	body weight
C	Celsius
CAS	Chemical Abstracts Service
CERHR	Centre for the Evaluation of Risks to Human Reproduction
CHO	Chinese hampster ovary
DBP	dibutyl phthalate
DEHP	diethylhexyl phthalate
DIHP	diisohexyl phthalate
DNCB	1-chloro-2,4-dinitro-benzene
DnHP	Di-n-hexyl phthalate
ER	oestrogen receptor
f	female
g	gram
GLP	good laboratory practice
h	hour
IgE	immunoglobulin E
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
MCL	mononuclear cell leukaemia
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

TMA	trimellitic anhydride
w/w	weight per weight
μ	micro

1. Introduction

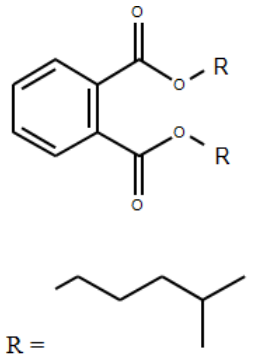
This review of diisohexyl phthalate (DIHP) is a health hazard assessment only. For this assessment, no international assessment report was available. The review was prepared using literature surveys conducted up to September 2006.

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

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:	68515-50-4
Chemical Name:	1,2-Benzenedicarboxylic acid, dihexyl ester, branched and linear
Common Name	Diisohexyl phthalate (DIHP) DIHP is also referred to by CAS number 146-50-9 or 71850-09-4 representing DIHP specific isomers.
Molecular Formula:	$C_{20}H_{30}O_4$
Structural Formula:	
Molecular Weight:	334
Synonyms:	Dihexyl phthalate, branched and linear; DHP; 1,2-Benzenedicarboxylic acid, bis(4-methyl-2-pentyl) ester; Phthalic acid, diisohexyl ester
Purity/Impurities/Additives:	Commercial blends may contain isomeric mixtures of Di-n-hexyl phthalate (DnHP – up to 25%) and DIHP

2.2 Physicochemical properties

Table 1: Summary of physicochemical properties

Property	Value
Physical state	Not available
Melting point	Not available
Boiling point	Not available
Density	Not available
Vapour pressure	Not available
Water solubility	Not available
Partition coefficient n-octanol/water (log Kow)	Not available
Henry's law constant	Not available
Flash point	Not available

3. Uses

Less than 2000 tons of DIHP is used in Europe (CERHR, 2003). Commercial blends of DIHP may contain up to 25% DnHP.

In Australia, DIHP is imported for use in auto transmission lubricants.

4. Human Health Hazard

4.1 Toxicokinetics

No data.

4.2 Acute toxicity

No data.

4.3 Irritation

4.3.1 Skin irritation

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

Conclusion

DIHP did not cause skin irritation in human patch tests.

4.3.2 Eye irritation

No data.

4.3.3 Respiratory irritation

No data

4.4 Sensitisation

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

To examine the effect of phthalate exposure on IgE levels, undiluted DIHP was applied with semi-occlusive wraps to each flank region of B6C3F1 mice 5 times per week for 2 weeks during the induction phase. Seven days later animals were challenged, and 7 days after that, all animals were sacrificed for IgE determinations. DIHP had no significant effect on levels of serum IgE, IL-4 or IL-13 proteins (Butala et al., 2004).

Conclusion

DIHP did not induce dermal sensitisation in humans.

4.5 Repeated dose toxicity

No data.

4.6 Genetic toxicity

CERHR (2003) reported that DIHP (which may contain up to 25% DnHP) was inactive in a mouse micronucleus test conducted by Exxon Biomedical Sciences in 1996; no other information on this study was supplied.

Conclusion

DIHP tested negative in a mouse micronucleus test. No in vitro bacterial and mammalian mutation and in vivo genotoxicity studies are available for DIHP.

4.7 Carcinogenicity

No data.

4.8 Reproductive toxicity

No data.

4.8.1 Mode of action

The oestrogenic activity of DIHP has been examined using a battery of short-term in vitro and in vivo assays. DIHP was negative for oestrogenic activity in a recombinant yeast assay (Harris et al., 1997). DIHP (mixture of isomeric isomers) demonstrated oestrogenic activities in a human oestrogen receptor (ER) α (but not β) reporter gene assay in CHO-K1 cells transfected with expression vectors for human oestrogen receptor ERα, ERβ and androgen receptor (AR) (Takeuchi et al., 2005). DIHP demonstrated anti-oestrogenic activity via ERβ in the presence of 17β-oestradiol and anti-androgenic activity in the hAR-transactivation assay. DIHP was a weak competitive agonist at the oestrogen receptor in an in vitro competitive ligand-binding assay and weakly induced oestrogen receptor-mediated gene expression in MCF-7 cells (Zacharewski et al., 1998). DIHP did not induce oestrogenic responses in vivo in an uterotrophic and vaginal cornification assays using immature and mature ovariectomised rats at any of the concentrations tested (20, 200, and 2000 mg/kg) over the course of a 5-day experiment (Zacharewski et al., 1998).

5. Hazard Characterisation

There is very little toxicity information for DIHP. For individual health 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, 2008a), which contains a comparative analysis of toxicity endpoints across 24 *ortho*-phthalate esters, including DIHP.

DIHP has an alkyl carbon backbone of C5 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, 2008a). The transitional phthalates are considered to produce similar reproductive and developmental effects.

No toxicokinetic data are available for DIHP. However, DIHP and DnHP are structurally similar, one with a branched (DIHP) and the other a linear (DnHP) backbone. Based on data for DnHP (NICNAS, 2008b) and other transitional phthalates, DIHP is likely to be rapidly absorbed as the monoester from the gut and excreted via the urine.

There is no information regarding the acute toxicity of DIHP, but it is expected to be similar to DnHP and other phthalates with a linear backbone, which exhibit low acute oral and dermal toxicity. Similarly, DIHP is not expected to cause skin or eye irritation or skin sensitisation.

DIHP and DnHP both have only one in vitro genotoxicity study available. DIHP was negative in a mouse micronuclei assay whereas DnHP was negative in bacterial mutagenicity tests. When assessed together, and noting the generally negative genotoxicity profile of phthalates of a similar molecular weight, DIHP is considered unlikely to be genotoxic.

There are no repeat dose or long-term studies (including carcinogenicity) available for DIHP. For those phthalates currently with no information such as DIHP, the severity of effects expected from repeat dose exposure is difficult to predict. However, liver and kidney effects from repeat doses would be expected, particularly at high doses. It is also not possible to extrapolate carcinogenic potential of DIHP due to insufficient testing of other phthalates.

There are no mammalian reproductive or developmental toxicity studies available for DIHP. In vitro studies have yielded conflicting results as to the antagonistic activity of DIHP to human androgen receptors. Other studies suggest that DIHP or an isomeric mixture of DIHP, demonstrated human oestrogen receptor α-agonistic activity and androgen receptor-antagonistic activities in vitro but did not induce vaginal cornification response or an increase in uterine weight in vivo.

A closely related analogue, DnHP, causes fertility effects in both sexes of two rodent species and developmental toxicity. Other transitional phthalates such as BBP, DBP and DEHP have also been associated with male reproductive and developmental toxicity (NICNAS, 2008a). Hence, DIHP is likely to cause adverse reproductive and developmental effects.

6. Human Health Hazard Summary Table

Phthalate	**Acute Toxicity**	**Irritation & Sensitisation**	**Repeated Dose Toxicity**	**Genetic Toxicity**	**Carcinogenicity**	**Fertility**	**Developmental Toxicity**
Diisohexyl phthalate (DIHP)	No data	Skin irritation: Negative Eye irritation: No data Respiratory irritation: No data Skin sensitisation: Negative	No data	In vitro: Negative in mouse micronucleus test In vivo: No data	No data	No data	No data

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References

Butala JH, David RM, Gans G, McKee RH, Guo TL, Peachee VL, & White KL Jr. (2004) Phthalate treatment does not influence levels of IgE or Th2 cytokines in B6C3F1 mice. Toxicology, 201: 77-85.

CERHR (2003) NTP-CERHR Monograph on the potential human reproductive and developmental effects of di-n-hexyl phthalate (DnHP). NIH Publication No. 03-4489. Research Triangle Park, National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction, U.S. Department of Health and Human Services.

Harris CA, Henttu P, Parker MG, & Sumpter JP (1997) The oestrogenic activity of phthalate esters in vitro. Environ Health Perspect, 105(8): 802-811

Medeiros AM, Devlin DJ, & Keller LH (1999) Evaluation of skin sensitisation response of dialkyl (C6-C13) phthalate esters. Contact Dermatitis, 41:287-289.

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

NICNAS (2008b) Human health hazard assessment: Di-n-hexyl-phthalate (DnHP) (CAS No. 84-75-3). Sydney, National Industrial Chemicals Notification and Assessment Scheme.

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

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

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Appendix - Robust Study Summaries

Test Substance	Diisohexyl phthalate (DIHP) + other 6 phthalates
Method	Human repeated insult patch test (HRIPT)
GLP	Yes
Year	1999
Test Conditions	
No. of Volunteers	104 male and females (ages 21-55 years)
Rout of Administration	Dermal, occlusive
Doses	Undiluted
Method Remarks	24 h, 3 times/week for 3 weeks, challenge on the 6th week
Results	There was no evidence of dermal irritation or sensitisation for any of the seven phthalates tested, including DIHP
Reference	Medeiros AM, Devlin DJ, & Keller LH (1999) Evaluation of skin sensitisation response of dialkyl (C6-C13) phthalate esters. Contact Dermatitis, 41:287-289.

Test Substance	Diisohexyl phthalate (DIHP)
Method	Measurement of the elevations of IgE, IL-4 and IL-13 in serum
GLP	Yes
Year	2004
Test Conditions	
Species/Strain/Sex	Mouse, B6C3F1, female
No. of Animals/sex/dose	10/dose
Rout of Administration	Dermal, semi-occlusive
Concentrations	Undiluted
Method Remarks	6 h, 5 times/week for 2 weeks, challenge 1 week later
Results	DIHP did not result in significant elevations in total serum IgE, IL-4, or IL-13 protein, or IL-4 or IL-13 mRNA. The positive control (TMA, CAS no. 552- 30-7) and the selectivity control (DNCB, CAS no. 97-00-7) responded appropriately confirming the validity of the assay system.
Reference	Butala JH, David RM, Gans C , McKee RH, Guo TL, Peachee VL, & White KL Jr. (2004) Phthalate treatment does not influence levels of IgE or Th2 cytokines in B6C3F1 mice. Toxicology, 201: 77-85.