Hazard assessment of dicyclohexyl phthalate

[Dicyclohexyl phthalate, CAS No. 84-61-7]

Chemical name: Dicyclohexyl phthalate (DCHP)
Synonyms: Dicyclohexyl phthalate, 1,2-Benzenedicarboxylic acid dicyclohexylester, DCHP
Molecular formula: \( \text{C}_{20}\text{H}_{26}\text{O}_4 \)
Molecular weight: 330.42
Structural formula:

\[
\begin{array}{c}
\text{O} \\
\text{C-O} \\
\text{O} \\
\text{O} \\
\text{C-O} \\
\text{O} \\
\end{array}
\]

Appearance: A white powder\(^1\)
Melting point: 66°C\(^1\)
Boiling point: 222-228°C\(^1\)
Specific gravity: \( d_{20}^{\circ} = 1.383 \)\(^1\)
Vapor pressure: 0.093 Pa (115°C)\(^1\)
Partition coefficient: \( \text{Log Pow} = 6.2 \) (calculated value)\(^1\)
Degradability: Hydrolyzability: No report.
Biodegradability: Easily biodegradable (BOD=68.5%, 28 days)\(^2\).
Solubility: Water: 4 mg/l (24°C)\(^1\)
Organic solvents: Soluble in most organic solvents.\(^1\)
Amount of production/import: 1998: 276 t (Production 0 t, Import 276 t)\(^3\)
Usage: Plasticiser for nitrocellulose, ethylcellulose, vinyl acetate and vinyl chloride resins\(^1\).
Applied laws and regulations: No applied laws and regulations.

\(^{1}\text{HSDB, 2001; }^{2}\text{"Tsusansho Koho" (daily), 1975; }^{3}\text{Ministry of International Trade and Industry, 1999}\)
1. Toxicity Data

1) Information on adverse effects on human health

Dicyclohexyl phthalate (DCHP) is a principal ingredient (>60%) of hot melt adhesives and is reported to be present together with phthalic anhydride and 2,5-di-tert-amylquinone in steam generated during hot melting of adhesives (Vandervort and Brooks, 1977; Levy et al., 1978).

It is reported that a worker exposed to hot steam containing DCHP during hot melting of adhesives developed stridor when he was exposed again to similar steam containing DCHP (Andrasch et al., 1976; Levy et al., 1978). In the provocation test in asthmatic patients who were suspected to be exposed to DCHP-containing steam during hot melting operation of adhesives, bronchospasm was not induced upon 20 minutes of inhalation exposure to DCHP vapor at aerial concentration of 11.4 mg/m$^3$ (40 mg DCHP/3.5 m$^3$ chamber). The exposure concentration during operation is estimated to be 0.1-1 mg/m$^3$ (Pauli et al., 1979).

2) Information on endocrine system and reproductive system

(1) *in vitro* test result related to receptor binding (Attachment-1)

In receptor binding assay using human estrogen receptors expressed in Sf9/Baculovirus, the results suggested existence of two classes of binding sites for DCHP, i.e., low and high affinity sites, for which DCHP had the IC$_{50}$ values of 1.0 $\mu$M and 2,000 $\mu$M, respectively (the binding affinities for these sites were 1/480 and 1/960,000 those of 17$\beta$-estradiol (E$_2$), respectively) (Nakai et al., 1999). DCHP also binds to recombinant human estrogen receptors with affinity which is about 1/92,000 that of E$_2$ (CERI, 2001a).

In yeast estrogen screen (YES) assay, DCHP had estrogen-like activity (Nakama et al., 1999). In the yeast two-hybrid assay, however, DCHP did not activate the gene transcription (Nishihara et al., 2000). In reporter gene assay with HeLa cells transfected with human or rat estrogen receptor expression plasmid and ER responsive element, DCHP did not induce gene transcription activation (CERI, 2001a, Yamasaki et al., 2001).
(2) in vivo test result in mammals (Attachment-2)

In uterotrophic assay, a screening test for detection of estrogen or antiestrogen effects (in accordance with the OECD draft guidelines), DCHP was administered subcutaneously to juvenile female SD rats (aged 20 days) at doses of 0, 2, 20 and 200 mg/kg/day for 3 days, but uterine weight remained unaffected in all groups (Yamasaki et al., 2001).

In another uterotrophic assay in ovariectomized female SD rats, oral administration of DCHP at 0, 10, 100 and 1,000 mg/kg/day for 7 days had no effect on uterine weight in any groups (CERI, 2001b). For the detection of antiestrogenic activity in 8-week-old ovariectomized female SD rats, concomitant oral gavage administration of DCHP at 0, 10, 100 and 1,000 mg/kg/day and 17-α ethinylestradiol at 30 µg/kg/day for 7 days had no effect on uterine weight in any groups, either (CERI, 2001b).

In Hershberger assay, a screening test for the detection of androgen and or antiandrogen effect (in accordance with the OECD draft guidelines), DCHP was administered by oral gavage to castrated male SD rats (aged 7 weeks) at doses of 0, 10, 100 and 1,000 mg/kg/day for 10 days, but male accessory reproductive organ weights remained unchanged in all groups (CERI, 2001b). In another Hershberger assay in castrated male rats (aged 7 weeks), DCHP was administered by oral gavage at doses of 0, 10, 100 and 1,000 mg/kg/day in combination with testosterone propionate at 0.4 mg/kg/day, s.c., for 10 days, but male accessory reproductive organ weights remained unchanged in all groups (CERI, 2001b).

In male SD rats (age, unspecified) given DCHP orally at 0, 500, 1,000, 1,500, 2,000 and 2,500 mg/kg/day for 7 days, bilateral testes showed atrophy of seminiferous tubules with 30-40% loss of germ cells (1/5 rats) in 2,500 mg/kg/day group (Lake et al., 1982).

In male rats (strain and age, unspecified) given 4,170 mg/kg of DCHP orally for 21 days, atrophy of seminiferous tubules with decreased spermatogenesis was observed (BIBRA, 1994).

In a feeding study, male and female Wistar rats (age, unspecified) were fed diet containing 100 ppm of DCHP (equivalent to 5 mg/kg) for 18 months, male reproductive organs remained unaffected (BIBRA, 1994). The dosage of this test, however, was quite low, and test conditions were unspecified and the results were not described in detail.

When female rats (strain and age, unspecified) were given 25% DCHP (in olive oil) by oral gavage at 2 ml/kg/day (equivalent to 600 mg/kg/day) for 6 weeks and then mated with untreated males, DCHP had no effects on reproductive performance and fetuses (BIBRA,
Dicyclohexyl phthalate

In a 4-generation reproductive toxicity study, male and female Wistar rats (age, unspecified) were fed diet containing DCHP at 100 ppm (equivalent to 5 mg/kg/day), but no toxic effects were observed in F₀ animals or succeeding generation offsprings (Lefaux, R., 1968; BIBRA, 1994).

3) Information on general toxicity

(1) Acute toxicity (Table 1) (Shibako and Blumenthal, 1973; BIBRA, 1994)

<table>
<thead>
<tr>
<th></th>
<th>Mouse</th>
<th>Rat</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral LD₅₀</td>
<td>&gt;3,200 mg/kg</td>
<td>&gt;3,200 mg/kg</td>
<td>-</td>
</tr>
<tr>
<td>Inhalation LD₅₀</td>
<td>-</td>
<td>&gt;3.2 mg/L (1h)</td>
<td>-</td>
</tr>
<tr>
<td>Percutaneous LD₅₀</td>
<td>-</td>
<td>-</td>
<td>&gt;300 mg/kg (24h)</td>
</tr>
<tr>
<td>Intraperitoneal LD₅₀</td>
<td>1,600 mg/kg</td>
<td>&gt;3,200 mg/kg</td>
<td>-</td>
</tr>
</tbody>
</table>

(2) Repeated-dose toxicity (Attachment-3)

In a 7-day repeated-dose toxicity study in which DCHP was administered by oral gavage to male SD rats (aged 30 days) at doses of 0, 500, 1,000, 1,500, 2,000 and 2,500 mg/kg/day for 7 days, 7-ethoxycoumarin O-de-ethylase and microsomal cytochrome P450 in liver increased at 500 mg/kg/day or higher groups, and histopathological examination of liver in 1,500, 2,000 and 2,500 mg/kg/day groups disclosed centrilobular swelling of hepatocytes in 1,500 mg/kg/day or higher groups and marked proliferation of smooth endoplasmic reticulum in hepatic lobules in 2,500 mg/kg/day (Lake et al., 1982).

In another study, 7-day repeated oral administration of DCHP at 1,500 mg/kg/day or its major metabolites, monocyclohexyl phthalate or cyclohexanol, at 1,130 and 455 mg/kg/day, respectively, caused increase in relative liver weight and induction of hepatic enzymes such as biphenyl 4-hydroxylase, 7-ethoxycoumarin- O-de-ethylase, aniline 4-hydroxylase and microsomal P450s in treated groups (Lake et al., 1982).

In a 21-day repeated administration study in which DCHP was administered by oral gavage to rats (strain, age and gender, unspecified) at 4,170 mg/kg/day, hepatomegaly, squamous cell proliferation in forestomach and alopecia were observed in the treated group (BIBRA, 1994).

In a 90-day repeated administration study in which DCHP was administered by oral...
gavage to albino rats (age and sex, unspecified) at doses of 25-200 mg/kg/day, liver weight increased in 25 mg/kg/day and higher groups, but liver was histologically intact (BIBRA, 1994).

In a 90-day feeding study in which male and female rats were fed diet mixed with 8.8% DCHP-containing plastic at concentration of 10% (800 mg/kg/day as DCHP), rats showed suppression of body weight gain, decrease in adrenal and spleen weights and slight histopathological changes in various organs except for testis and liver. However, the causal relationship with DCHP is not documented (BIBRA, 1994).

In another repeated-dose toxicity study in which 25% DCHP was administered by oral gavage to male and female rats (strain and age, unspecified) at the volumes of 1 and 2 ml (equivalent to 100 and 200 mg/kg/day) twice daily for 6 weeks or 1 year, no abnormal changes were observed in body weight or hematological and histopathological examinations (BIBRA, 1994).

4) Information on mutagenicity/genotoxicity and carcinogenicity

(1) Mutagenicity/genotoxicity (Table 2)

In *in vitro* assays, DCHP is reported to be negative for mutagenicity in reverse mutation test using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 regardless of the presence or absence of S9mix from rat or hamster liver. Except for the above results, no *in vitro* nor *in vivo* data are available for the mutagenicity/genotoxicity assessment of DCHP.

<table>
<thead>
<tr>
<th>Test method</th>
<th>Test conditions</th>
<th>Results*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vitro</em></td>
<td>Reverse mutation test</td>
<td></td>
<td>Zeiger, et al., 1985</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella typhimurium</em> strains TA98, TA100, TA1535, TA1537, 10,000 µg/plate</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hamster S9(+/-), or Rat S9(+/-)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* - : Negative
(2) Carcinogenicity (Table 3)

No data are found on carcinogenicity in humans and laboratory animals.

<table>
<thead>
<tr>
<th>Organization</th>
<th>Category</th>
<th>Significance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA</td>
<td>-</td>
<td>No evaluation.</td>
<td>IRIS, 2000</td>
</tr>
<tr>
<td>EU</td>
<td>-</td>
<td>No evaluation.</td>
<td>ECB, 2000</td>
</tr>
<tr>
<td>NTP</td>
<td>-</td>
<td>No evaluation.</td>
<td>NTP, 2000</td>
</tr>
<tr>
<td>IARC</td>
<td>-</td>
<td>No evaluation.</td>
<td>IARC, 2001</td>
</tr>
<tr>
<td>ACGIH</td>
<td>-</td>
<td>No evaluation.</td>
<td>ACGIH, 2001</td>
</tr>
</tbody>
</table>

5) Information on immune system

At present, no data are found on immunological effects of DCHP.

6) Fate and Metabolism

In liver and small intestinal homogenates from rat, ferret and baboon, and human small intestinal homogenates, DCHP was slowly hydrolyzed to give major metabolites such as mono-cyclohexyl phthalate and cyclohexanol (Lake et al., 1977; 1982) (Fig. 1). It is also reported that rat gastric, small intestinal and cecal homogenates and human feces have similar hydrolytic activity for DCHP (Rowland et al., 1977).
2. Hazard assessment at present

No data are found on the effects on endocrine and reproductive systems in humans.

In *in vitro* assays to detect endocrine effect of DCHP, weak binding affinity for estrogen receptors (1/480 - 1/960,000 of that of E2) and estrogen-like activity were observed in binding assay with human ER and human ER-dependent yeast growth assay. But no estrogen-like activity was observed in yeast two-hybrid assay and reporter gene assay. In uterotrophic and Hershberger assays, DCHP had no estrogen-like or androgen-like activities, suggesting the low possibility for endocrine-disrupting effect of DCHP mediated by these sex hormone receptors.

As the effects on endocrine and reproductive systems in laboratory animals, atrophy of seminiferous tubule and reduced spermatogenesis were observed in rats in repeated-dose toxicity test at higher doses (2,500 or 4,170 mg/kg/day). As the toxic effects on other organs, increase in liver weight and induction of hepatic enzymes are reported. As for reproductive & developmental toxicology test, 4-generation test was conducted and no adverse effects are reported at the lower dose (5 mg/kg/day).

As the information on DCHP-related hazards, DCHP was negative in reverse mutation.
tests in assessment of its mutagenicity/genotoxicity, but no data are found on *in vivo* assays. No reports are available about carcinogenicity of this compound in humans or experimental animals.

3. Risk assessment and other necessary future measures

2-generation reproductive toxicity study of DCHP is currently being performed. Although possibility that DCHP has endocrine disruptive activity mediated by sex hormone receptors, findings on the effects on fertility and development of offspring in multi-generations are not necessarily sufficient, though effects were not observed in some reproductive and developmental toxicology studies. Thus, the endocrine disrupting effect and related toxicities of DCHP will be comprehensively assessed by incorporating the results of the above 2-generation reproductive toxicity test.
References


NTP (2000) U.S. Department of Health and Human Services Public Health Service,
National Toxicology Program, 9th Report on Carcinogens.


"Tsusansho Koho" (daily) (1975)


Japan Society for Occupational Health (2001): Advice on the tolerance limit. Journal of
Dicyclohexyl phthalate

Japan Society for Occupational Health, 43: 95-119.
## Attachment-1 Results of in vitro studies on receptor binding

<table>
<thead>
<tr>
<th>Item</th>
<th>Test methods and conditions</th>
<th>Results</th>
<th>Conclusion</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER binding assay</td>
<td>Method: Competitive binding assay using [³H]-E2 as a ligand. Receptor: Human ER expressed in Sf9/Baculovirus. Temperature: 25°C pH: 7.4</td>
<td>IC50: 10⁻⁶ M (High affinity) 2×10⁻³ M (Low affinity) (E2: 2.09×10⁻³ M)</td>
<td>Binds to ER (The presence of high and low affinity binding sites has been suggested. The binding affinities of DCHP for ER are 1/480 (high affinity site) and 1/960,000 (low affinity site) of E2.</td>
<td>Nakai et al., 1999</td>
</tr>
<tr>
<td></td>
<td>Method: Human ER binding assay (recombinant ERα ligand domain)</td>
<td>IC50: 1.2×10⁻⁴ M (E2: 1.3×10⁻⁸ M) RBA: 0.0011%</td>
<td>Binds to ER (The binding affinity of DCHP for ER is 1/92,000 that of E2.)</td>
<td>CERI, 2001a</td>
</tr>
<tr>
<td>Human ER-dependent yeast growth assay</td>
<td>Cells: <em>S. cerevisiae</em> transfected with human ER Concentration: 10⁻⁵ M (DCHP) 10⁻⁹ M (E2) Test period: 2, 5 days</td>
<td>Weak yeast growth-promoting activity was detected on 5th day (S9+). No significant activity on 2nd day (S9+-) and 5th day (S9-). EC50: 7.1×10⁻⁴ M (S9+) (E2: 6.9×10⁻¹¹ M (S9-))</td>
<td>ER-mediated cell growth-promoting activity only on 5th day (S9+).</td>
<td>Nakama et al., 1999</td>
</tr>
<tr>
<td>Reporter gene assay using recombinant cultured cells</td>
<td>Cells: the HeLa cells transfected with human ER gene expression plasmids and ER response element. Test concentration: 10⁻¹¹ - 10⁻⁵ M (DCHP)</td>
<td>Negative for agonist activity within a range of 10⁻¹¹-10⁻⁸ M. (E2: PC50: &lt;10⁻¹¹ M)</td>
<td>Does not induce ER-mediated gene transcription activation.</td>
<td>CERI, 2001a</td>
</tr>
</tbody>
</table>

ER: Estrogen receptor  E2: 17β-estradiol  REC10: Concentration corresponding to 10% of the activity of 10⁻⁷ M E2  PC50: Concentration corresponding to 50% of the maximal activity of E2  IC50: Concentration corresponding to 50% inhibition concentration by E2  RBA: Relative binding potency (%)
## Attachment-2  Results of studies on mammalian endocrine and reproductive systems

<table>
<thead>
<tr>
<th>Animal species (SD, female)</th>
<th>Administration method</th>
<th>Administration period</th>
<th>Dose</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>s.c. (uterotrophic assay)</td>
<td>3 days from day 20 after birth</td>
<td>0, 2, 20, 200 mg/kg/day</td>
<td>No effect on uterine weight.</td>
<td>Yamasaki et al., 2001</td>
</tr>
</tbody>
</table>

| Animal species (SD, female, 6 rats/group) (ovariectomized rats) | Oral gavage (uterotrophic assay) | Rats were ovariectomized at the age of 6 weeks and given DCHP for 7 days from the age of 8 weeks, and uterus was removed 24 hr after final dose | 0, 10, 100, 1,000 mg/kg/day | No effect on uterine weight. | CERI, 2001b |

| Animal species (SD, male) Castrated at the age of 6 weeks | Oral gavage (Hershberger assay) | After 10 days of treatment from the age of 7 weeks, rats were necropsied about 24 hr after final dose. | 0, 10, 100, 1,000 mg/kg/day | No effect on male accessory reproductive organs. | CERI, 2001b |

| Animal species (SD, male) (age, unspecified) | Oral gavage | 7 days | 0, 500, 1,000, 1,500, 2,000, 2,500 mg/kg/day | Atrophy of testis (seminiferous tubules) at 2,500 mg/kg/day (1/5) | Lake et al., 1982 |

| Animal species (male) (age and strain, unspecified) | Oral gavage | 21 days | 4,170 mg/kg/day | Atrophy and reduced spermatogenesis of testis (seminiferous tubules) | BIBRA, 1994 |

| Animal species (Wistar, male and female) (age, unspecified) | By feeding | 18 months | 100 ppm (Corresponding to 5 mg/kg/day) | No effect. | BIBRA, 1994; Lefaux, 1968 |

| Animal species (female) (strain and age, unspecified) | Oral gavage | Females were treated for 6 weeks and then mated with untreated males. | 25% (Corresponding to 2 m/kg/day and 600 mg/kg/day) | No effect on litter size. No effects on postnatal development and fertility of F1 animals. | BIBRA, 1994 |

<p>| Animal species (Wistar, male and female) (age, unspecified) | By feeding | 4-Generation | 100 ppm (Corresponding to 5 mg/kg/day) | No effect. | BIBRA, 1994; Lefaux, 1968 |</p>
<table>
<thead>
<tr>
<th>Animal species</th>
<th>Administration method</th>
<th>Administration period</th>
<th>Dose</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rat</em> (SD, male) (30 days of age)</td>
<td>Oral gavage</td>
<td>7 days</td>
<td>DCHP 0, 500, 1,000, 1,500, 2,000, 2,500 mg/kg/day</td>
<td>1,500 mg/kg group: Centrilobular swelling of hepatocytes, induction of hepatic enzymes. 2,500 mg/kg group: Centrilobular swelling of hepatocytes and marked proliferation of smooth endoplasmic reticulum in liver lobules.</td>
<td>Lake et al., 1982</td>
</tr>
<tr>
<td>Major metabolites of DCHP: Monocyclohexyl phthalate 1,130 mg/kg/day; cyclohexanol 455 mg/kg/day (dose equivalent to 1,500 mg/kg/day of DCHP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rat</em> (strain, age and sex, unspecified)</td>
<td>Oral gavage</td>
<td>21 days</td>
<td>4,170 mg/kg/day</td>
<td>Hepatomegaly, squamous cell proliferation in forestomach and alopecia</td>
<td>BIBRA, 1994</td>
</tr>
<tr>
<td><em>Rat</em> (Albino) (age and sex, unspecified)</td>
<td>Oral gavage</td>
<td>90 days</td>
<td>25-200 mg/kg/day</td>
<td>Increased liver weight without histopathological changes in 25 mg/kg/day or higher groups.</td>
<td>BIBRA, 1994</td>
</tr>
<tr>
<td><em>Rat</em> (male and female) (strain and age, unspecified)</td>
<td>By feeding</td>
<td>90 days</td>
<td>Diet admixed with 8.8% DCHP-containing plastic film at concentration of 10% (equivalent to 800 mg/kg/day as DCHP)</td>
<td>Suppressed body weight gain, decreased adrenal and spleen weights and slight histopathological changes in various organs except for testis and liver are reported, but their causal relationship with DCHP is not documented.</td>
<td>BIBRA, 1994</td>
</tr>
<tr>
<td><em>Rat</em> (male and female) (strain and age, unspecified)</td>
<td>Oral gavage (in olive oil)</td>
<td>6 weeks, 1 year (twice weekly)</td>
<td>25% DCHP 1, 2 ml/rat (Corresponding to 100 and 200 mg/kg/day)</td>
<td>No effect. (body weight, hematology and histopathology).</td>
<td>BIBRA, 1994</td>
</tr>
</tbody>
</table>