Hazard assessment of Di (2-ethylhexyl) phthalate

[Di (2-ethylhexyl) phthalate, CAS No. 117-81-7]

Chemical name: Di (2-ethylhexyl) phthalate

Synonyms: Bis (2-ethylhexyl) phthalate, Di (2-ethylhexyl) ester, 1,2-Benzenedicarboxylic acid, DEHP

Molecular formula: C_{24}H_{38}O_{4}

Molecular weight: 390.56

Structure formula:

![Structure formula image]

Appearance: Colorless oily liquid

Melting point: -55°C

Boiling point: 230°C (666 Pa)

Specific gravity: d^{20}_{4} = 0.9861

Vapor pressure: 9.64 × 10^{-6} Pa (25°C)

Partition coefficient: Log Pow = 7.60 (measured value)

Degradability: Hydrolyzability: No report.

Biodegradability: Easily degradable (BOD=29%, 14 days, BOD=69%, 28 days)

Solubility: Water: 0.285 mg/L (24°C)

Miscible with mineral oil and hexane, and slightly soluble in carbon tetrachloride.

Amount of production/import: 272,988t in 2000 (manufactured: 272,910t, imported: 78t)

Usage: A plasticizer most extensively used to soften the resins of vinyl chloride, nitrocellulose, methacrylic acid and chlorinated rubber. Also used as an additive to lubricant, adhesive, pigment and...
Di(2-ethylhexyl) phthalate

Applied laws and regulations: Law Concerning Reporting, etc. of Release of Specific Chemical Substances to the Environment and Promotion of the Improvement of Their Management, Industrial Safety and Hygiene Law, of Marine Pollution Prevention Law

1) HSDB 2001; 2) "Tsusansho Koho" (daily), 1975; 3) Ministry of Economy, Trade and Industry, 1999;

1. Toxicity Data

1) Information on adverse effects on human health

Oral administration of di(2-ethylhexyl) phthalate (DEHP) to volunteers (2 adults) at 5,000 mg did not induce any symptom, but mild gastrointestinal disorder and diarrhea occurred at 10,000 mg (Shaffer et al., 1945).

Non-specific hepatitis was reported in 3 of 27 hemodialysis patients with renal disorder who used PVC tubes containing DEHP. The symptom disappeared when the tube was replaced with the one that did not contain DEHP (Neergarrd et al., 1971). Though elution of diethyl phthalate was detected from this PVC tube, DEHP was not proven. Accordingly, the relation between exposure to DEHP and onset of hepatitis was not clear.

In three new born infants artificially ventilated with PVC respiratory tubes unusual lung disorders were observed and died during four weeks of life (Roth et al., 1988). But Health Canada pointed out overestimate of the exposure level and FDA was skeptical of the relation between DEHP and lung damage (Health Canada, 2002; FDA, 2001).

According to the results of study on chromosome aberration in 10 workers engaged in DEHP manufacture for 10 - 30 years, no increase in the incidence of chromosome aberration occurred within the exposure concentration range of 0.0006 - 0.01 ppm (Thies & Flieg, 1978).

Premature breast development was noted in young Puerto Rican girls. DEHP and phthalate ester mainly consisting of DBP (dibutylphthalate) were detected from 28 of 41 serum samples taken from the girls with premature breast development (6 months - 8 years old), with DEHP (187-2,098µg/l) in 25 samples and DBP (15-276µg/l) in 13. The DEHP and DBP concentrations were significantly higher than those detected in 35 serum
samples taken from healthy female children of the same age. Though it is possible that phthalate esters including DEHP and DBP caused the premature development of breast, the author reported that further evidence is needed by conducting epidemiological study in human and animal experiments before concluding that this premature development is caused by phthalate esters with estrogenic activity. (Colon et al., 2000).

2) Information on endocrine system and reproductive system

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

No binding to estrogen receptor was observed up to 1 mM of DEHP in a binding test using uterus homogenate of SD female rat (Blair et al., 2000; Zacharewski et al., 1998). On the other hand, weak binding to human estrogen receptor was observed (1/1,400 of 17\(\beta\)-estradiol (E2), CERI, 2001).

In a reporter gene assay in which MCF-7 transiently transfected with Gal4-human estrogen receptor gene and Gal4-modulated luciferase reporter gene as well as HeLa cell stably transfected with these genes, and in another reporter gene assay using *S. cerevisiae* PL3 strain that proliferates in response to binding to human estrogen receptor, and another reporter gene assay conducted by yeast two hybrid assay method, no activity was observed at the maximum concentrations (2 mM by yeast two hybrid method, 10 \(\mu\)M in others) (Zacharewski et al., 1998., Nishihara et al., 2000; CERI, 2001).

As described in the above, no significant response was observed with DEHP in any of the **in vitro** assays.

(2) **in vivo** test results in mammals (Attachment-2)

Though there is no report that focused on the effect of DEHP on endocrine system and reproductive system, the data obtained from the repeated-dose toxicity test and reproductive & developmental toxicity test indicated the effects on these systems. The data obtained in 1980's and thereafter to clarify the no observed adverse effect level (NOAEL) are shown in the attachment-2 under the headings of the repeated-dose toxicity test and reproductive & developmental toxicity test.

When DEHP at 0, 1,000, 5,000, 10,000 and 25,000 ppm (equivalent to 0, 245, 1,209, 2,579 and 6,992 mg/kg/day respectively in the case of males, and 0, 270, 1,427, 2,897 and 7,899 mg/kg/day respectively in the case of females) was mixed in food and administered
Di(2-ethylhexyl) phthalate

to male and female B6C3F1 mice (6 weeks old) for 4 weeks, atrophy of thymus in males and females, decreased testis weight and atrophy of testis in males, and disappearance of corpus luteum of ovary in females were observed (Hazleton, 1992a).

When DEHP at 0, 0.01, 0.1, 0.6, 1.2 and 2.5% (equivalent to 0, 11, 101, 667, 1,224 and 2,101 mg/kg/day respectively in males, and 0, 12, 109, 643, 1,197 and 1,892 mg/kg/day respectively in females) was mixed in food and administered to male and female F344 rats (no description of age) for 21 days, the decreased testis weight and atrophy of testis were observed in males of 2.5% group (BIBRA, 1984).

When DEHP at 0, 1,000, 4,000, 12,500 and 25,000 ppm (equivalent to 0, 63, 261, 850 and 1,724 mg/kg/day respectively in males, and 0, 73, 302, 918 and 1,858 mg/kg/day respectively in females) was mixed in food and administered to male and female F344 rats (8 weeks old) for 13 weeks, decreased uterus weight in females, decreased testis weight and atrophy of testis accompanied with aspermia in males, and histological changes in pituitary and adrenal in males and females were observed at 25,000 ppm (Hazleton, 1992b).

When DEHP at 0, 5, 50, 500 and 5,000 ppm (equivalent to 0, 0.4, 3.7, 37.6 and 375 mg/kg/day respectively in the case of males, and 0, 0.4, 4.2, 42.2 and 419 mg/kg/day respectively in the case of females) was mixed in food and administered to male and female SD rats (young ones, age unknown) for 13 weeks, vacuolation of Sertoli’s cell in testis of males administered 500 ppm or more, decreased relative weight of testis, atrophy of seminiferous tubule, decreased number of sperms or complete disappearance of sperms in males at 5000 ppm, and histological changes in thyroid gland accompanied with reduced follicular size and decreased colloid concentration in males and females at 5,000 ppm were observed (Poon et al., 1997).

When DEHP at 0, 1,600, 3,100, 6,300, 12,500 and 25,000 ppm (equivalent to 0, 160, 320, 641, 1,282 and 2,563 mg/kg/day respectively in males, and 0, 182, 364, 727, 1,454 and 2,908 mg/kg/day respectively in females) was mixed in food and administered to male and female F344 rats (5 - 6 weeks old) for 13 weeks, atrophy of testis was observed in males administered 12,500 ppm or more (NTP, 1982).

After oral administration of DEHP at 0, 100, 500 and 2,500 mg/kg/day to male marmosets (no description of strain and age) for 13 weeks, no influence on testis was observed (Kurata et al., 1998).
Many reports have been available on the results of reproductive and developmental toxicity tests, indicating influence on parent animals as well as fetal toxicity and teratogenicity.

When DEHP at 0, 0.025, 0.05, 0.1 and 0.15% (equivalent to 0, 9, 44, 91, 191, 293 mg/kg) was mixed in food and administered to female CD-1 mice from day 0 to 17 of gestation, lethargy at 91 mg/kg/day and increased liver weight at 191 mg/kg/day or more were noted for the toxicity in parent animals, while increased number of malformed fetuses at 91 mg/kg/day, and resorption of embryos, increased number of dead fetuses, decreased number of surviving fetuses and decreased body weight of surviving fetuses at 191 mg/kg/day or more were noted (Tyl, et al., 1984, 1988).

After oral administration of DEHP at 0, 40, 200 and 1,000 mg/kg/day to female CD-1 mice from day 6 to 15 of gestation, decreased body weight and increased relative weight of liver were noted at 1,000 mg/kg/day as the toxicity in parent animals. As the toxicity in fetus, increased external and visceral malformation at 200 mg/kg/day, decreased fetal survival rate and body weight as well as increased skeletal and visceral malformation at 1,000 mg/kg/day were observed (CERHR, 2000).

When DEHP at 0, 0.05, 0.1, 0.2, 0.4 and 1.0% (equivalent to 0, 70, 190, 400, 830 and 2,200 mg/kg/day respectively) was mixed in food and administered to female ICR-JCL mice from day 0 to 18 of gestation, decreased body weight (day 18 of gestation) occurred at 0.2% or more as the effect on the parent animals. As the toxicity in the fetus, increased fetal mortality at 0.1% or more, decreased fetal body weight and increased fetal malformation at 0.2%, and 100% fetal mortality at 0.4% or more were noted (Shiota et al., 1980; 1985).

When DEHP at 0, 0.01, 0.025 and 0.05% (equivalent to 0, 19, 48 and 95 mg/kg/day respectively) was mixed in food and administered to female CD-1 mice from day 0 to 17 of gestation, increased mortality of fetuses and neonates was observed in 0.05% group, but there was no effect on the growth and development of surviving offspring (Price et al., 1988).

When DEHP at 0, 0.01, 0.1 and 0.3% (equivalent to 0, 14, 141 and 425 mg/kg/day respectively) was mixed in food and administered to male and female CD-1 mice for 106 days of gestation (7 days before and 98 days during co-housing), decreases of fertility, numbers of pups delivered and live pups at 0.1% and infertility at 0.3% were observed.
In cross-over mating, fertility, number of pups delivered and proportion of pups born alive were decreased in males in the highest dose group mated with control females. The complete infertility in females was observed in the highest dose group mated with control males. (Lamb et al., 1987).

When DEHP at 0, 0.25, 0.5 and 1.0% (equivalent to 0, 164, 313 and 573 mg/kg/day respectively) was mixed in food and administered to female F344 rats from day 0 to 20 of gestation, decreased food consumption at 0.5% or more and inhibition on body weight increase at 1.0% were observed as the influence on parent animals. The decreased fetal growth at 0.5% and decreased fetal body weight and growth at 573 mg/kg/day were observed, but there was no effect on growth and development of surviving offspring (Price et al., 1986).

By oral administration of DEHP at 0, 40, 200 and 1,000 mg/kg/day in female Wistar rats from day 6 to 15 of gestation, increased relative weights of liver and kidney, decreased body weight and uterus weight, and increased embryo resorption occurred at 1,000 mg/kg/day as the effect on parents, while decreased body weight and increased deformations occurred at 1,000 mg/kg/day as the toxicity in the fetus (Hellwig et al., 1997).

According to the DEHP assessment report released by the expert panel of NTP (CERHR), the following descriptions are noteworthy: Various malformations including curtailed anogenital distance (AGD), residual papilla, hypospadias, etc. are considered to be attributable to anti-androgen action. However, the report in question was not quoted (CERHR, 2000). In this regard, DEHP and its metabolite MEHP are not bound to human androgen receptors up to the concentration of 10 µM according to the reports subsequently published. Oral administration of DEHP at 750mg/kg/day during gestational period inhibited biosynthesis of testosterone in testis of male fetus and the amount of testosterone in testis decreased to a level similar to that observed in the females, indicating that reproductive toxicity and deformation attributable to DEHP occurred by anti-androgen action without mediated by androgen receptors (Parks et al., 2000; Gray L.E. Jr. et al., 2000).

3) Information on general toxicity

(1) Acute toxicity (Table 1) (IARC, 1982; IARC, 2000; EHC, 1992)
Di(2-ethylhexyl) phthalate

Diarrhea was observed as a major symptom by oral and intraperitoneal administration (Hodge, 1943). The spontaneous movement decreased and abnormal behavior was observed in rats after intraperitoneal administration (Rubin & Jaeger, 1973), while swelling of lung occurred after intravenous administration, indicating histologically edema and thickening of alveolar wall and marked infiltration of neutrophils (Schulz et al., 1975). Intravenous administration caused decreased blood pressure and increased respiration rate in rabbits (Calley et al., 1966).

Table 1  Results of acute toxicity test

<table>
<thead>
<tr>
<th></th>
<th>Mouse</th>
<th>Rat</th>
<th>Rabbit</th>
<th>Guinea pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral LD₉₀</td>
<td>33,500 mg/kg</td>
<td>30,600 mg/kg</td>
<td>33,900 mg/kg</td>
<td>26,300 mg/kg</td>
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<tr>
<td>Inhalation LC₉₀</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Percutaneous LD₅₀</td>
<td>-</td>
<td>-</td>
<td>25,000 mg/kg</td>
<td>10,000 mg/kg</td>
</tr>
<tr>
<td>Intraperitoneal LD₅₀</td>
<td>14,000 – 75,000 mg/kg*</td>
<td>30,700 mg/kg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Intravenous LD₅₀</td>
<td>-</td>
<td>200 – 250 mg/kg*</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*: There are differences between the literatures cited.

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

DEHP at 0, 1,000, 5,000, 10,000 and 25,000 ppm (equivalent to 0, 245, 1,209, 2,579 and 6,992 mg/kg/day respectively in males, and 0, 270, 1,427, 2,897 and 7,899 mg/kg/day respectively in females) mixed in food and administered to male and female B6C3F₁ mice (6 weeks old) for 4 weeks increased liver weight accompanied with necrosis occurred in males and females, and decreased kidney weight accompanied by inflammation and anemia occurred in males at 5,000 ppm or more (Hazleton, 1992a).

When DEHP at 0, 800, 1,600, 3,100, 6,300, 12,500 ppm (equivalent to 0, 144, 289, 578, 1,156 and 2,311 mg/kg/day respectively in males, and 0, 157, 314, 629, 1,258 and 2,516 mg/kg/day respectively in females) was mixed in food and administered to male and female B6C3F₁ mice (5 - 6 weeks old) for 13 weeks, suppression of body weight increase was noted in males at 3,100 ppm or more (NTP, 1982).

When DEHP at 0, 0.01, 0.1, 0.6, 1.2 and 2.5% (equivalent to 0, 11, 101, 667, 1,224 and 2,101 mg/kg/day respectively in males, and 0, 12, 109, 643, 1,197 and 1,892 mg/kg/day respectively in females) was mixed in food and administered to male and female F344 rats (no description of age) for 21 days, increased liver weight accompanied with histological changes was noted in males and females at 0.6% or more (BIBRA, 1984).
When DEHP at 0, 1,000, 4,000, 12,500 and 25,000 ppm (equivalent to 0, 63, 261, 850 and 1,724 mg/kg/day respectively in males, and 0, 73, 302, 918 and 1,858 mg/kg/day respectively in females) was mixed in food and administered to male and female F344 rats (8 weeks old) for 13 weeks, liver weight increased in males of 1,000 ppm group as well as in males and females of 4,000 ppm group. In addition, kidney weight increased and RBC decreased in males of 4,000 ppm group. The liver and kidney weights increased and as histological changes hypertrophy of hepatocytes and hyperplasia of proximal convoluted tubule occurred in males and females administered 12,500 ppm or more (Hazleton, 1992b).

When DEHP at 0, 5, 50, 500 and 5,000 ppm (equivalent to 0, 0.4, 3.7, 37.6 and 375 mg/kg/day respectively in males, and 0, 0.4, 4.2, 42.2 and 419 mg/kg/day respectively in females) was mixed in food and administered to male and female SD rats (5 - 6 weeks old) for 13 weeks, increased liver and kidney weights, hypertrophy of hepatocytes and hepatic peroxisome proliferation were observed in males and females of 5,000 ppm group. In addition, anemia occurred in males of this group (Poon et al., 1997).

When DEHP at 0, 1,600, 3,100, 6,300, 12,500 and 25,000 ppm (equivalent to 0, 160, 320, 641, 1,282 and 2,563 mg/kg/day respectively in males, and 0, 182, 364, 727, 1,454 and 2,908 mg/kg/day respectively in females) was mixed in food and administered to male and female F344 rats (5 - 6 weeks old) for 13 weeks, inhibition of body weight increase occurred in males and females of 25,000 ppm group (NTP, 1982).

After oral administration of DEHP at 0, 100, 500 and 2,500 mg/kg/day to male and female marmosets for 13 weeks, decreased body weight in males of 2,500 mg/kg/day group was observed as well as tendency of increase of cytochrome P450 and slight increase in mean peroxisome volume in males at 100mg/kg/day or more, and males and females at 500 mg/kg/day or more. However, it is considered that DEHP does not proliferate peroxisome, because increase of liver mass and hypertrophy of hepatocytes were not detected in organ weight measurements and histopathological examination and peroxisome enzymatic activities, number of peroxisomes, volume density and peroxisome morphology were not different from those in the control group. (Kurata et al., 1998).

No hepatic peroxisome proliferation was observed after oral administration of DEHP at 0, 100 and 500 mg/kg/day to cynomolgus monkeys for 25 days (Short et al., 1987).
4) Information on mutagenicity/genotoxicity and carcinogenicity

(1) Mutagenicity/genotoxicity (Table 2)

The result of *in vitro* reverse mutation test using *Salmonella typhimurium* and *E. coli* was negative (Ashby et al., Yoshikawa et al., Zeiger et al., 1985). The results of chromosome aberration test and sister chromatid exchange test using established cell line of rat hepatocytes (Priston & Dean, 1985), unscheduled DNA synthesis (UDS) test using primary culture of rat hepatocytes were all negative (Probst & Hill, 1985). The results of chromosome aberration test and sister chromatid exchange test using established Chinese hamster cell line were also negative (Phillips et al., 1982; Douglas et al., 1986).

However, positive reaction was observed within the dose range of 7.5 - 20 µg/ml in the absence of metabolic activation system in a genetic mutation test using mouse lymphoma cell (Ashby et al., 1985). Aneuploid was reportedly positive within the dose range of 25 - 50 µg/ml in hepatocyte of Chinese hamster (Ashby et al., 1985).

As to *in vivo* studies, the results were mostly negative in a dominant lethal test in which single intraperitoneal administration at 12.5, 25 g/kg was conducted in CD male mice (Hamano et al., 1979). The results of sex-linked recessive lethal test using *Drosophila melanogaster* and micronucleus test of mouse peripheral blood were also negative (Yoon et al., 1985; Douglas et al, 1986).
Table 2 Results of mutagenicity/genotoxicity tests

<table>
<thead>
<tr>
<th>Test method</th>
<th>Cell and animal species used</th>
<th>Result</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse mutation test</td>
<td><em>Salmonella typhimurium</em> TA98, TA100, TA1535, TA1537, TA1538 (+/- S9)</td>
<td>-</td>
<td>Ashby et al., 1985; Yoshikawa et al., 1983; Zeiger et al. 1985</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em> WP2 (+/- S9)</td>
<td>-</td>
<td>Yoshikawa et al., 1983</td>
</tr>
<tr>
<td>Chromosome aberration test</td>
<td>Rat hepatocyte established cell line</td>
<td>-</td>
<td>Priston &amp; Dean, 1985</td>
</tr>
<tr>
<td></td>
<td>Chinese hamster CHO cell</td>
<td>-</td>
<td>Phillips et al., 1982</td>
</tr>
<tr>
<td>unscheduled DNA synthesis test</td>
<td>Primary culture of rat hepatocyte</td>
<td>-</td>
<td>Probst &amp; Hill, 1985</td>
</tr>
<tr>
<td>Sister chromatid exchange test</td>
<td>Rat hepatocyte established cell line</td>
<td>-</td>
<td>Priston and Dean, 1985</td>
</tr>
<tr>
<td></td>
<td>Chinese hamster CHO cell (+/- S9)</td>
<td>-</td>
<td>Douglas et al., 1986</td>
</tr>
<tr>
<td>Genetic mutation test</td>
<td>Mouse lymphoma cell (L5178Y) (-S9) at 7.5 - 20 µg/ml</td>
<td>+</td>
<td>Ashby, et al., 1985</td>
</tr>
<tr>
<td></td>
<td>Hepatocyte of Chinese hamster at 25 - 50 µg/ml</td>
<td>+</td>
<td>Ashby, et al., 1985</td>
</tr>
<tr>
<td>Dominant lethal test</td>
<td>CD mice, single dose of 12.5, 25 g/kg i.p.</td>
<td>-</td>
<td>Hamano et al., 1979</td>
</tr>
<tr>
<td>Sex-linked recessive lethal test</td>
<td><em>Drosophila melanogaster</em></td>
<td>-</td>
<td>Yoon et al., 1985</td>
</tr>
<tr>
<td>Micro nucleus test</td>
<td>Mouse (peripheral blood)</td>
<td>-</td>
<td>Douglas et al., 1986</td>
</tr>
</tbody>
</table>

* -: Negative, +: Positive, +W: Weakly positive, ±: False positive

(2) Carcinogenicity (Table 3)

When DEHP at 0, 3,000 and 6,000 ppm (male: equivalent to 0, 672, 1,325 mg/kg/day, female: equivalent to 0, 799, 1,821 mg/kg/day) was mixed in food and administered to male and female B6C3F1 mice (6 weeks old) for 103 weeks, the incidence of hepatocellular carcinoma significantly increased in 1,325 mg/kg/day group (male) and in 799 mg/kg/day or more group (female), compared with the control group (NTP, 1982).

Similarly, after administration of DEHP at 0, 6,000 and 12,000 ppm (male: equivalent to 0, 322, 674 mg/kg/day, female: equivalent to 0, 394, 774 mg/kg/day) mixed in food for 103 weeks to male and female F344 rats (5-6 weeks old), the incidence of hepatocellular adenoma increased in male and female of all groups. The incidence of hepatocellular carcinoma was significantly increased in the females of 774 mg/kg/day group (NTP, 1982).

DEHP at 0, 100, 500, 1,500 and 6,000 ppm (equivalent to 0, 19, 99, 292 and 1,266 mg/kg/day respectively in males, and 0, 24, 117, 354 and 1,458 mg/kg/day respectively in females)
Di(2-ethylhexyl) phthalate (DEHP) was mixed in food and administered to male and female B6C3F₁ mice, and when DEHP at 0, 100, 500, 2,500 and 12,500 ppm (equivalent to 0, 5.8, 29, and 147 mg/kg/day respectively in males, and 0, 7.3, 36, 182 and 939 mg/kg/day respectively in females) was mixed in food and administered to male and female F344 rats for 104 weeks. In both cases, increase in the incidence of liver tumor was observed in male and female mice and rats of high dose DEHP groups. However, no significant increase in the onset of liver tumor was observed at the doses of 100 ppm (equivalent to 19 mg/kg/day) or less in mice and in rats at the doses of 500 ppm (equivalent to 29 mg/kg/day) or less (CERHR, 2000; David, et al., 1999).

Concerning the carcinogenicity of DEHP, in view of hepatic peroxisome proliferation of liver observed in the repeated administration toxicity tests, many tests have been conducted. There is also a report that growth of hepatocytes was promoted in association with liver peroxisome proliferation to induce tumorigenic change, thereby promoting hepatocellular carcinoma in the rat (Cattley and Popp, 1989). DEHP was observed to have weak promoter action in an experimental system when hepatocellular carcinoma was induced by diethylnitrosamine in B6C3F₁ mice and F344 rats as well as in another experimental system in which skin cancer was induced by DMBA in SENCAR mice (Ward et al., 1983; 1986).

Even though peroxisome proliferation occurred in the repeated-dose toxicity tests in rats and mice, this did not necessarily occur in the primates and the reaction related to hepatic peroxisome proliferation in rat did not occur in human in various in vitro experiments using cultured cell isolated from human liver. Accordingly, IARC changed the classification of DEHP from Group 2B (possible carcinogenicity in human) to Group 3 (cannot be classified as to the carcinogenicity in human) in February 2000 (IARC, 2000).
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Table 3  Carcinogenicity assessment by national and international organizations

<table>
<thead>
<tr>
<th>Organizations</th>
<th>Classification</th>
<th>Significance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA</td>
<td>Group B2</td>
<td>A substance fully evidenced to have carcinogenicity in animals, and according to the epidemiological study have the insufficient evidence for carcinogenicity in human or no evidence in human.</td>
<td>IRIS, 2002</td>
</tr>
<tr>
<td>EU</td>
<td>-</td>
<td>No evaluation.</td>
<td>ECB, 2000</td>
</tr>
<tr>
<td>NTP</td>
<td>R</td>
<td>Reasonably anticipated to be human carcinogens.</td>
<td>NTP, 2000</td>
</tr>
<tr>
<td>IARC</td>
<td>Group 3*</td>
<td>Unclassifiable as to carcinogenicity in human.</td>
<td>JARC, 2001</td>
</tr>
<tr>
<td>ACGIH</td>
<td>A3</td>
<td>Animal carcinogen.</td>
<td>ACGIH, 2001</td>
</tr>
<tr>
<td>Japan Society for Occupational Health</td>
<td>Group 2B</td>
<td>Substance assumed to have carcinogenicity in human but the evidence is insufficient.</td>
<td>Japan Society for Occupational Health, 2001</td>
</tr>
</tbody>
</table>

*: Changed from Group 2B to Group 3 in 2000

5) Information on immune system

At present, no report has been made on the influence on immune system.

6) Fate and Metabolism

After hydrolysis by lipase secreted from pancreas, DEHP generates mono (2-ethylhexyl) phthalate (MEHP). Most of DEHP is hydrolyzed to generate MEHP in rat. During the metabolism of MEHP, 2-ethylhexyl side chain is processed by $\omega$-oxidation or by $\omega$-1 oxidation. In the case of rat and guinea pig, $\omega$-oxidation is the major metabolic pathway of MEHP. However, $\omega$-oxidation is not a major metabolic pathway in mice, hamster, green monkey, cynomolgus monkey and marmoset. Though the metabolism of DEHP by $\omega$- and $\omega$-1 oxidation is reported in humans, $\omega$-oxidation is not a major pathway. Compared with intravenous administration, a large amount of carboxyl derivative was observed in the 2-ethylhexyl side chain after oral administration, but whether or not this difference is attributable to the difference in administration route is not clear (WHO, 1992).

Though a large amount of glucronic acid conjugate of MEHP was detected in the urine of human, marmoset, green monkey and mouse, none was detected in the urine of rat (IARC, 1982).

It is known that the peroxisome proliferating action of DEHP in liver markedly differs depending on the animal species. Though MEHP and another metabolite 2-ethyl-5-oxohexyl phthalate demonstrate high peroxisome proliferating activity in the incubated
hepatocyte of rat, they hardly demonstrate such activity in hepatocyte of human, cynomolgus monkey, marmoset and guinea pig (WHO, 1992).

![Diagram of metabolic pathways of di(2-ethylhexyl) phthalate]

1) Di(2-ethylhexyl) phthalate  2) 2-ethylhexyl hydrogen phthalate  3) Phthalic acid  
4) 2-ethylhexanol  5) 2-(2-hydroxyethyl)hexyl hydrogen phthalate  
6) 2-(1-hydroxyethyl)hexyl hydrogen phthalate  7) 2-ethyl-6-hydroxyhexyl hydrogen phthalate  
8) 2-ethyl-5-hydroxyhexyl hydrogen phthalate  9) 2-ethyl-4-hydroxyhexyl hydrogen phthalate  
10) 2-(carboxymethyl)hexyl hydrogen phthalate  11) 2-(methylcarbonyl)hexyl hydrogen phthalate  
12) 5-carboxy-2-ethylpentyl hydrogen phthalate  13) 2-ethyl-5-oxohexyl hydrogen phthalate  
14) 2-ethyl-4-oxohexyl hydrogen phthalate  15) 2-carboxyhexyl hydrogen phthalate

Fig. 1: Metabolic pathways of di(2-ethylhexyl) phthalate

2. **Hazard Assessment at present**

There is no report that clarifies the relation of exposure to DEHP with effects on the human endocrine system and reproductive system.
The results of in vitro experiments conducted to investigate the effects of DEHP on endocrine system indicated that the binding to estrogen receptors and the response mediated by receptor binding were weak or negative in most cases. In other words, the endocrine disruption mediated by estrogen receptor is unlikely.

On the other hand, as to major effects on endocrine system and reproductive system observed in animal experiments, decreased testis weight and atrophy of testis were observed in rats and mice in the repeated administration toxicity tests at doses equivalent to 375mg/kg/day. As to reproductive & developmental toxicity, decrease on survival rate and growth of fetuses as well as induction of external and visceral malformation were observed. As well, toxicity in fetus were observed with administration to the dams at doses equivalent to 91mg/kg/day and more.

According to the assessment published by the expert panel of CERHR, various malformations and abnormalities including curtailed anogenital distance (AGD), residual nipple, hypospadias, etc. were noted in F1 pups after oral administration of DEHP to pregnant rats, and inhibition of testosterone biosynthesis system is involved in the mechanism of inducing malformations including hypospadias, etc., which is attributable to the anti-androgen action not mediated by androgen receptors.

As the information related to the harmful effects of DEHP, digestive symptoms after oral ingestion of a large quantity was observed in humans. For animal experiments, the effects on liver including peroxisome proliferation was noted after repeated administration to rodents. Despite some positive results, the results of mutagenicity test are mostly negative. As to the carcinogenicity tests, the onset of hepatocellular adenoma/carcinoma was reported in mice and rats. However, the peroxisome proliferation observed in rodents did not occur in primates, and the reactions related to peroxisomal proliferation were not noted in various in vitro experiments using cultured liver cell isolated from human liver. Accordingly, IARC changed the classification of DEHP from Group 2B (have possible carcinogenicity in human) to Group 3 (cannot be classified as to the carcinogenicity in human) in February 2000.

3. Risk assessment and other necessary future measures

Though DEHP is unlikely to demonstrate endocrine disrupting activity mediated by estrogen receptor, the reproductive and developmental toxicity were observed in the tests
Di(2-ethylhexyl) phthalate

orally administered at the doses of about 91mg/kg/day and more of DEHP. CERHR suggests that the influence of DEHP on reproductive and developmental systems, especially on male reproductive system, is attributable to anti-androgen activity not mediated by androgen receptors. It is necessary to demonstrate the presence or absence of anti-androgen action of DEHP and its involvement in androgen receptors on the basis of in vitro tests currently conducted to clarify the binding to androgen receptors and the results of Hershberger assay.

Since the effects on reproductive/developmental toxicity were observed in the repeated-dose toxicity tests of DEHP regardless of the presence or absence of endocrine disrupting activity, it is thought necessary to carry out risk assessment based on the results of hazard assessment and exposure assessment and to explore an appropriate method for risk control.
REFERENCE


Di(2-ethylhexyl) phthalate exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat., Toxcol. Sci., 58, 350 - 365 (2000).


http://www.iarc.fr


Di(2-ethylhexyl) phthalate


NTP (1982) NTP Carcinogenicity bioassay of di(2-ethylhexyl) phthalate (CAS No. 117-82-7) in F344 rats and B6C3F1 mice (feed study). PB82-184011: NTIS.


Di(2-ethylhexyl) phthalate

investigation/research on environment-compatible technology development on behalf of the Ministry of Environment and Industry.
"Tsusansho Koho" (daily) (1975)
### Attachment-1  Results of in vitro test on receptor binding

<table>
<thead>
<tr>
<th>Item</th>
<th>Test methods and conditions</th>
<th>Result</th>
<th>Remark</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding to estrogen receptor (ER)</td>
<td>Method: Competitive binding test using [3H]-E2 as a ligand</td>
<td>IC50: &gt;10^{-3} M (E2: 8.99 × 10^{-10} M) Relative binding affinity to E2 (E2 = 1) is &lt;9.0 × 10^{7}</td>
<td>Demonstrates no binding</td>
<td>Blair et al., 2000</td>
</tr>
<tr>
<td></td>
<td>Receptor: Uterine homogenate of ovariectomized female SD rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temperature: 4° C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH: 7.4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Method: Competitive binding test using [3H]-E2 as a ligand</td>
<td>IC50: &gt;10^{-3} M (E2: 1.3 × 10^{-9} M) Relative binding affinity to E2 (E2 = 1) is &gt; 1.3 × 10^{6}</td>
<td>Demonstrates no binding</td>
<td>Zacharewski et al., 1998</td>
</tr>
<tr>
<td></td>
<td>Receptor: Uterine homogenate of female SD rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Concentrations: 10^{-3} µM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temperature: 30° C</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>pH: 7.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Binding to human (recombinant ER α ligand domain)</td>
<td>IC50: 9.49 × 10^{-7} M (E2: 6.74 × 10^{-10} M) RBA: 0.071%</td>
<td>The binding affinity is weak</td>
<td>CERI, 2001</td>
<td></td>
</tr>
<tr>
<td>Reporter gene assay</td>
<td>Cells: MCF-7 transiently transfected with Gal4-human estrogen receptor gene and Gal4-modulated luciferase reporter gene</td>
<td>The activity was negative within the dose range of 10^{-1} - 10^{-3} M. The transcription activity rate increase was dependent on the exposure concentration within the range of 10^{-12} - 10^{-9} M. 23-fold activation rate at E2 = 10^{-9} M.</td>
<td>No gene transcriptional activity</td>
<td>Zacharewski et al., 1998</td>
</tr>
<tr>
<td></td>
<td>Concentrations: 10^{-7}, 10^{-6}, 10^{-5} M (DEHP), 10^{-12} M-10^{-8} M (E2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cell: HeLa cell in which Gal4-human estrogen receptor gene and Gal4 regulator luciferase reporter gene were steadily expressing. Concentrations: 10^{-7}, 10^{-6}, 10^{-5} M (DEHP) 10^{-12} M-10^{-9} M (E2)</td>
<td>The activity was negative within the dose range of 10^{-1} - 10^{-3} M. The transcription activity rate increase was dependent on the concentration within the range of 10^{-12} - 10^{-9} M. 11-fold activation rate at E2 = 10^{-9} M.</td>
<td>No gene transcriptional activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cell: HeLa cell incorporated with human ER expression gene and ER response sequence</td>
<td>PC50: &gt;10^{-7} M (E2: &lt;10^{-11} M)</td>
<td>No gene transcriptional activity</td>
<td>CERI, 2001</td>
</tr>
<tr>
<td>Human estrogen receptor responsive yeast proliferation test</td>
<td>Cell: S. cerevisiae PL3 strain transfected with human estrogen receptor</td>
<td>No growth Definite proliferation was detected with E2 from day 3 onward.</td>
<td>No influence on the cell growth activity</td>
<td>Zacharewski et al., 1998</td>
</tr>
<tr>
<td></td>
<td>Concentration: 10^{-3} M (DEHP), 10^{-9} M(E2) Incubation: 5 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeast two hybrid assay</td>
<td>Cell: Yeast, which carry a β-galactosidase receptor gene, transfected with ERLBD (estrogen receptor ligand domain) and TLF2(coactivator) expression plasmids.</td>
<td>REC10: &gt;2 × 10^{-3} M (E2: 3 × 10^{-8} M) Relative activity ratio to E2 (E2=1) was &gt;1.5 × 10^{7}</td>
<td>No gene transcriptional activity</td>
<td>Nishihara et al., 2000</td>
</tr>
</tbody>
</table>

ER: Estrogen receptor  E2: 17 β-estradiol

REC10: Concentration equivalent to 10% of E2 activity level; PC50: Concentration equivalent to 50% of the maximum activity by E2; IC50: 50% inhibition concentration; RBA: Relative binding affinity (%)
**Attachment-2**  Results of tests on the endocrine system and reproductive system of mammals

(1) **Repeated-dose toxicity test**

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Administration method</th>
<th>Administration period</th>
<th>Dose</th>
<th>Target organs</th>
<th>References</th>
</tr>
</thead>
</table>
| Mouse (B6C3F1, male and female) 6 weeks old | By feeding | 4 weeks | 0, 1,000, 5,000, 10,000, 25,000 ppm  
Male: Equivalent to 0, 245, 1,209, 2,579, 6,992 mg/kg/day  
Female: Equivalent to 0, 270, 1,427, 2,897, 7,899 mg/kg/day | Atrophy of thymus in males and females, decreased testis weight and atrophy of testis in males, and disappearance of ovary corpus luteum in females at 25,000 ppm | Hazleton, 1992a |
| Mouse (B6C3F1, male and female) 5 - 6 weeks old | By feeding | 13 weeks | 0, 800, 1,600, 3,100, 6,300, 12,500 ppm  
(Male: Equivalent to 0, 144, 289, 578, 1,156, 2,311 mg/kg/day  
Female: Equivalent to 0, 157, 314, 629, 1,258, 2,516 mg/kg/day) | No effect | NTP, 1982 |
| Rat (F344, male and female) No description of age | By feeding | 21 days | 0, 0.01, 0.1, 0.6, 1.2, 2.5%  
(Male: Equivalent to 0, 11, 101, 667, 1,224, 2,101 mg/kg/day  
Female: Equivalent to 0, 12, 109, 643, 1,197, 1,892 mg/kg/day) | Decreased testis weight and atrophy of testis in males at 2.5% | BIBRA, 1984 |
| Rat (F344, male and female) 8 weeks old | By feeding | 13 weeks | 0, 1,000, 4,000, 12,500, 25,000 ppm  
(Male: Equivalent to 0, 63, 261, 850, 1,724, 3,448 mg/kg/day  
Female: Equivalent to 0, 73, 302, 918, 1,858 mg/kg/day) | Decreased uterus weight in females, decreased testis weight and atrophy of testis accompanied with aspermia in males, and histological changes in pituitary and adrenal in males and females at 25,000 ppm | Hazleton, 1992b |
| Rat (SD, male and female) Unknown age | By feeding | 13 weeks | 0, 5, 50, 500, 5,000 ppm  
(Male: Equivalent to 0, 0.4, 3.7, 37.6, 375 mg/kg/day  
Female: 0, 0.4, 4.2, 42.2, 419 mg/kg/day) | Vacuolation of Sertoli cells in males at 500 ppm or more  
Decreased relative weight of testis, atrophy of seminiferous tubule, the decreased number of sperms or complete disappearance of sperms in males at 5000 ppm.  
Histological changes in thyroid gland accompanied with reduced follicular size and decreased colloid concentration in males and females at 5,000 ppm | Poon et al., 1997 |
<table>
<thead>
<tr>
<th>Animal species</th>
<th>Administration method</th>
<th>Administration period</th>
<th>Dose</th>
<th>Target organs</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (F344, male and female) 5-6 weeks old</td>
<td>By feeding</td>
<td>13 weeks</td>
<td>0, 1,600, 3,100, 6,300, 12,500, 25,000 ppm (Male: Equivalent to 0, 160, 320, 641, 1,282, 2,563 mg/kg/day Female: Equivalent to 0, 182, 364, 727, 1,454, 2,908 mg/kg/day)</td>
<td>Atrophy of testis in males at 12,500 ppm or more.</td>
<td>NTP, 1982</td>
</tr>
<tr>
<td>Marmoset (male and female, Callithrix jacchus) (no description of age)</td>
<td>gavage p.o.</td>
<td>13 weeks</td>
<td>0, 100, 500, 2,500 mg/kg/day</td>
<td>No effect</td>
<td>Kurata et al., 1998</td>
</tr>
</tbody>
</table>

(2) Reproductive and developmental toxicity tests

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Administration method</th>
<th>Administration period</th>
<th>Dose</th>
<th>Target organs</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse (CD-1, female)</td>
<td>By feeding</td>
<td>Day 0 - 17 of gestation</td>
<td>0, 0.025, 0.05, 0.1, 0.15 % (Equivalent to 0, 9, 44, 91, 191, 293 mg/kg/day)</td>
<td>Toxicity in parent animals: Lethargy condition at 91 mg/kg/day or more Increased liver weight at 191 mg/kg/day or more Toxicity in fetuses: Increased number of malformed fetuses at 91 mg/kg/day Increased embryo resorption and dead fetuses, decreased number of surviving fetuses and body weight of surviving fetuses at 191/mg/kg/day or more NOAEL = 44 mg/kg/day</td>
<td>Tyl et al, 1984; 1988</td>
</tr>
<tr>
<td>Mouse (CD-1, female)</td>
<td>gavage p.o.</td>
<td>Day 6 - 15 of gestation</td>
<td>0, 40, 200, 1,000 mg/kg/day</td>
<td>Toxicity in parent animals: Decreased body weight and increased relative weight of liver at 1,000 mg/kg/day Toxicity in fetuses: External and visceral malformations at 200 mg/kg/day Decreased survival rate of fetuses and fetal body weight, increased skeletal and visceral malformations at 1,000 mg/kg/day NOAEL = 200 mg/kg/day (Parent animal) NOAEL = 40 mg/kg/day (Fetus)</td>
<td>CERHR, 2000</td>
</tr>
<tr>
<td>Animal species</td>
<td>Administration method</td>
<td>Administration period</td>
<td>Dose</td>
<td>Target organs</td>
<td>References</td>
</tr>
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<tr>
<td>Mouse (ICR-JCL, female)</td>
<td>By feeding</td>
<td>Day 0 - 18 of gestation</td>
<td>0, 0.05, 0.1, 0.2, 0.4, 1.0 % (Equivalent to 0, 70, 190, 400, 830, 2,200 mg/kg/day)</td>
<td>Toxicity in parent animal: Decreased body weight at ≥ 0.2% (day 18 of gestation). Toxicity in fetus: Increased fetal mortality at ≥ 0.1%, decreased fetal body weight and increased fetal malformations at 0.2%; 100% fetal death at 0.4% or more NOAEL = 190 mg/kg/day (Parent animal) NOAEL = 70 mg/kg/day (Fetus)</td>
<td>Shiota, et al., 1980, 1985</td>
</tr>
<tr>
<td>Mouse (CD-1, female)</td>
<td>By feeding</td>
<td>Day 0 - 17 of gestation</td>
<td>0, 0.01, 0.025, 0.05% (Equivalent to 0, 19, 48, 95 mg/kg/day)</td>
<td>Increased fetal and neonatal mortality at 0.05% NOAEL = 48 mg/kg/day</td>
<td>Price et al., 1988</td>
</tr>
<tr>
<td>Mouse (CD-1, male and female)</td>
<td>By feeding</td>
<td>11 weeks of age 106 days</td>
<td>0, 0.01, 0.1, 0.3% (Equivalent to 0, 14, 141, 425 mg/kg/day)</td>
<td>Decrease in fertility, number of pups delivered and number of live pups at 0.1%. Infertility at 0.3%. In cross-over mating, decrease in fertility, number of pups delivered and proportion of pups born alive in males in the highest dose group mated with control females. Infertility in females in the highest dose group mated with control males.</td>
<td>Lamb et al., 1987</td>
</tr>
<tr>
<td>Rat (F344, female)</td>
<td>By feeding</td>
<td>Day 0 - 20 of gestation</td>
<td>0, 0.25, 0.5, 1.0 % (Equivalent to 0, 164, 313, 573 mg/kg/day)</td>
<td>Toxicity in parent animal: Decreased food consumption at ≥ 0.5%, inhibition on body weight increase at 1.0% Toxicity in fetus: Decreased fetal growth at 0.5%. Decreased fetal body weight and growth at 1.0%. NOAEL = 164 mg/kg/day</td>
<td>Price et al., 1986</td>
</tr>
<tr>
<td>Rat (Wistar, female)</td>
<td>gavage p.o.</td>
<td>Day 6 - 15 of gestation</td>
<td>0, 40, 200, 1,000 mg/kg/day</td>
<td>Toxicity in parent animals: Increased relative weights of liver and kidney, decreased body weight and uterus weight at 1,000 mg/kg/day, increased embryo resorption Toxicity in fetuses decreased body weight at 1,000 mg/kg/day, Increased malformations NOAEL = 200 mg/kg/day (Parent animal, Fetus)</td>
<td>Hellwig et al., 1997</td>
</tr>
</tbody>
</table>
### Attachment-3  Repeated-dose toxicity test results

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Administration method</th>
<th>Administration period</th>
<th>Dose</th>
<th>Result</th>
<th>References</th>
</tr>
</thead>
</table>
| Mouse (B6C3F₁, male and female) 6 weeks old | By feeding | 4 weeks | 0, 1,000, 5,000, 10, 000, 25,000 ppm  
(Male: Equivalent to 0, 245, 1,209, 2,579, 6,992 mg/kg/day  
Female: Equivalent to 0, 270, 1,427, 2,897, 7,899 mg/kg/day) | Increased liver weight accompanied with necrosis in males and females, and decreased kidney weight accompanied with inflammation and anemia in males at 5,000 ppm or more.  
NOAEL = 1,000 ppm | Hazleton, 1992a |
| Mouse (B6C3F₁, male and female) 5 – 6 weeks old | By feeding | 13 weeks | 0, 800, 1,600, 3,100, 6,300, 12,500 ppm  
(Male: Equivalent to 0, 144, 289, 578, 1,156, 2,311 mg/kg/day  
Female: Equivalent to 0, 157, 314, 629, 1,258, 2,516 mg/kg/day) | suppressed body weight increase in males at 3,100 ppm or more.  
NOAEL = 1,600 ppm | NTP, 1982 |
| Rat (F344, male and female) No description of age | By feeding | 21 weeks | 0, 0.01, 0.1, 0.6, 1.2, 2.5 %  
(Male: Equivalent to 0, 11, 101, 667, 1,224, 2,101 mg/kg/day  
Female: Equivalent to 0, 12, 109, 643, 1,197, 1,892 mg/kg/day) | Increased liver weight accompanied with histological changes in males and females at ≥ 0.6%  
NOAEL = 1.2 % | BIBRA, 1984 |
| Rat (F344, male and female) 8 weeks old | By feeding | 13 weeks | 0, 1,000, 4,000, 12,500, 25,000 ppm  
(Male: Equivalent to 0, 63, 261, 850, 1,724, 3,448 mg/kg/day  
Female: Equivalent to 0, 73, 302, 918, 1,838 mg/kg/day) | Increased liver weight in males at 1,000 ppm  
Increased liver weight in males and females, increased kidney weight and decreased RBC in the males at 4,000 ppm  
Increased liver and kidney weights and histological changes in males and females at ≥ 12,500 ppm  
LOAEL = 1,000 ppm | Hazleton, 1992b |
| Rat (SD, male and female) 5 – 6 weeks old | By feeding | 13 weeks | 0, 5, 50, 500, 5,000 ppm  
(Male: Equivalent to 0, 0.4, 3.7, 37.6, 375 mg/kg/day  
Female: Equivalent to 0, 0.4, 4.2, 42.2, 419 mg/kg/day) | Increased liver and kidney weights, hypertrophy of hepatocytes and hepatic proxisome proliferation in males and females, anemia in males  
NOAEL = 500 ppm | Poon et al., 1997 |
| Rat (E344, male and female) 5 – 6 weeks old | By feeding | 13 weeks | 0, 1,600, 3,100, 6,300, 12,500, 25,000 ppm  
(Male: Equivalent to 0, 160, 320, 641, 1,282, 2,563 mg/kg/day  
Female: Equivalent to 0, 182, 364, 727, 1,454, 2,908 mg/kg/day) | Inhibition on body weight increase in males and females at 25,000 ppm.  
NOAEL = 6,300 ppm | NTP, 1982 |
<table>
<thead>
<tr>
<th>Animal species</th>
<th>Administration method</th>
<th>Administration period</th>
<th>Dose</th>
<th>Result</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marmoset (male and female, Callithrix jacchus) (No description of age)</td>
<td>p.o.</td>
<td>13 weeks</td>
<td>0, 100, 500, 2,500 mg/kg/day</td>
<td>Body weight decrease in males at 2,500 mg/kg/day and tendency of increase of cytochrome P450 content in males at ≥ 100 mg/kg/day. No peroxisome proliferation in males and females at ≥ 500 mg/kg/day.</td>
<td>Kurata et al., 1998</td>
</tr>
<tr>
<td>Monkey (cynomolgus monkey)</td>
<td>p.o.</td>
<td>25 days</td>
<td>0, 100, 500 mg/kg/day</td>
<td>No peroxisome proliferation (only peroxisome was examined).</td>
<td>Short, 1987</td>
</tr>
</tbody>
</table>