Hazard assessment of 2,4-Dichlorophenol

[2,4-Dichlorophenol, CAS No. 120-83-2]

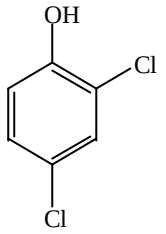
Chemical name: 2,4-Dichlorophenol (2,4-DCP)

Synonym: 4-Hydroxy-1,3-dichlorobenzene, 2,4-DCP

Molecular formula: $C_6H_4Cl_2O$

Molecular weight: 163.0

Structural formula:



Appearance: Colorless Crystals, White or Pale yellow solid[1)]

Melting point: 45°C[1)]

Boiling point: 210.0°C[1)]

Specific gravity: $d_{25}^{60} = 1.383$[1)]

Vapor pressure: 16 Pa (25°C)[1)]

133,000 Pa (53°C)[1)]

Partition coefficient: Log Pow = 3.06 (calculated value)[3)]

Degradability: Hydrolyzability: No report

Biodegradability: Poorly biodegradable (BOD = 0%, 4 weeks)[2)]

Solubility: Water 4.5 g/*l* (20°C)[1)]

Organic solvents: Soluble in carbon tetrachloride, ethanol, benzene and ethyl ether[1)], Soluble in aqueous alkali[1)]

Amount of production/import: No data on production/import[3)]

Usage: Intermediate for dye-stuff and herbicides[1)]

Applied laws and regulations: Water Supply Law, Water Pollution Control Law, Law Marine Pollution Prevention Law, Sewage Water Law

[1)] HSDB, 2001; [2)] Ministry of International Trade and Industry, 1982; [3)] Ministry of International Trade and Industry, 1999

1. Toxicity Data

1) Information on adverse effects on human health

A male worker exposed to hot pressurized steam containing 2,4-DCP (amount and purity are unknown) fell unconscious immediately and died (EPA, 2000).

It is also reported that a worker was splattered with almost 100% pure molten 2,4-DCP over his right thigh through right arm (less than 10% of the body surface) and within 20 min he developed epileptic seizure and died. In his serum, urine, bile and gastric content, 24.3, 5.3, 18.7 and 1.2 mg/*l* of 2,4-DCP, respectively, were detected (Kintz et al., 1992). In another fatality case reported from a chemical factory in England in the same year, 2,4-DCP (amount and purity are unknown) and hot steam spurted out onto the worker's face and neck, resulting in his death 20 min later (EPA, 2000).

In 1998, a male worker who was sprayed with hot pressurized steam containing 2,4-DCP (amount and purity are unknown) fell unconscious and died 1 hr later. The skin contamination involved his forearm, knee, thigh and face, and 2,4-DCP was detected in serum and urine at concentrations of 13.1 and 6.2 mg/ *l*, respectively (EPA, 2000).

These cases indicate that molten or hot 2,4-DCP is immediately absorbed through the skin in amounts which is lethal for humans unless the skin areas are immediately decontaminated by washing with water. The skin exposure to molten 2,4-DCP, in particular, was shown to be fatal even if it involves as little as 1% of the body surface area. The US EPA together with OSHA issued warning of CANPR (Chemical Advisory and Notice of Potential Risk) for 2,4-DCP (EPA, 2000).

As its chronic effects, 29 cases of chloracne and 11 cases of porphyria have been reported from the US plants manufacturing 2,4-DCP and 2,4,5-trichlorophenol (Bleiberg et al., 1964). These cases are considered to be related to trichlorophenols and impurities such as dioxins (BUA, 1988).

2) Information on endocrine system and reproductive system

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

In receptor binding assays, 2,4-DCP did not bind to human and bovine estrogen receptors (ER) up to the concentration of 5×10^{-5}M (Kramer et al., 1999; CERI, 2001). In yeast two-hybrid assay, 2,4-DCP induced gene transcription activation in an ERE (estrogen responsive element)-dependent manner (Nishihara et al., 2000). In an *in vitro* cell proliferation assay with MCF-7 cells (human breast tumor cells), the dose-dependent proliferation of tumor cells was reported (Jones et al., 1998). In a reporter gene assay using yeast cells transfected with human progesterone receptor gene, 2,4-DCP had no agonist or antagonist activities (Tran et al., 1996). In reporter gene assay using cultured recombinant HeLa cells, 2,4-DCP did not induce ERE (estrogen response element)-dependent gene transcription activation with concentration range of 10^{-11}-10^{-5}M (CERI, 2001a).

(2) in vivo test results in mammals (Attachments-2, 3)

In uterotrophic assay, ovariectomized 8-week-old Wistar Hannover rats were treated orally with 2,4-DCP alone at doses of 0, 100, 200 and 400 mg/kg/day (to detect the estrogenic effect) or in combination with 17α-ethinylestradiol subcutaneously at 0.5 μg/kg/day (to detect the anti-estrogenic effect) for 3 days, but no treatment-related abnormal changes were observed in uterine weight in either group (CERI, 2001b).

In Hershberger assay (in accordance with the OECD Draft Guidelines) which is used for screening androgenic and anti-androgenic effects, castrated 8-week-old Wistar Hannover rats were treated orally with 2,4-DCP alone at doses of 0, 50, 100 and 200 mg/kg/day (to assess the androgenic effect) or in combination with testosterone propionate subcutaneously at 0.4 mg/kg/day for 10 days (to assess the anti-androgenic effect), but no treatment-related abnormal changes were observed in the weights of any male accessory reproductive organs in either group (CERI, 2001b).

In a reproduction study in pregnant mice, 2,4-DCP was administered subcutaneously at 74 mg/kg (dissolved in DMSO) to female C57BL/6 and AKR mice (6 dams/group) on gestation days 6-14 and 6-15, respectively, and the dams were cesarean sectioned on gestation days 18 (C57BL/6) and 19 (AKR). The fetal mortality increased in C57BL/6 mice, and in the AKR mice the 2,4-DCP-related toxicities included decrease in relative liver weight in dams, decrease in fetal body weight, excessive extension of four limbs in 4/40 fetuses (untreated control group: 6/251 fetuses, DMSO group: 1/229 fetuses), cystic kidney (1 fetus), short limb (2 fetuses) and thenar dysplasia (1 fetus) (NTIS, 1968a).

In a teratogenicity study, 2,4-DCP (purity 99.2%) was administered by oral gavage to female F344 rats (34 females) at doses of 0, 200, 375 and 750 mg/kg/day (in corn oil) on gestation days 6-15, and dams were cesarean-sectioned on gestation day 20. In the 200 mg/kg/day and higher groups, suppression of body weight gain and soiling of external genitalia were observed in dams. In the 750 mg/kg/day group, maternal toxicities included alopecia, abnormal respiratory sound, adhesion of blood-like substance around the eye, nostrils and mouth and death (4/34 dams), and delayed ossification of sternebrae and vertebral arches were observed in fetuses. The authors concluded that 2,4-DCP had no teratogenic potential, but caused delayed fetal development secondary to maternal toxicities at 750 mg/kg/day (Rodwell et al., 1989).

In another reproduction study, 2,4-DCP (purity 99%) was administered in drinking water to 3-week-old female SD rats at doses of 0, 3, 30 and 300 ppm (corresponding to 0, 0.5, 5 and 50 mg/kg/day), and females were mated with untreated males at the age of 13 weeks. The administration continued until delivery of their pups, and the offsprings were observed until 6 weeks of age. The findings in offsprings included decrease in survival rate at weaning in 30 ppm group and decrease in number of live newborns and increase in spleen and liver weights at the age of 6 weeks in 300 ppm group (Exon et al., 1984; Exon and Koller, 1985).

The results of the *in vitro* fertilization study are shown in Attachment-3. Using the oocytes from superovulated female CB_6F_1 mice aged 6-8 weeks and sperms from male CD-1 mice with proven fertility, *in vitro* fertilization was performed in medium containing DCP at concentrations of 0, 0.1, 0.3 and 1.0 mM. In the groups treated with 2.5-, 3,4- and 3,5-DCP, sperm motility and sperm penetration rate into oocytes decreased, whereas 2,4-DCP had no effect on either of these sperm parameters. In another *in vitro* fertilization test, male CD-1 mice were treated with 2,4-DCP in drinking water at doses of

0, 50, 150 and 500 mg/kg/day for 90 days, and the sperms from these males were collected and fertilized in medium with oocytes from untreated and superovulated females. In this experiment, 2,4-DCP was reported to have no effect on sperm motility or fertilization rate. No reports are available on the effects of 2,5-, 3,4- and 3,5-DCP in the same testing method (Seyler et al., 1984).

3) Information on general toxicity

(1) Acute toxicities (Table 1)

Table 1 shows LD_{50}, LC_{50} and LDLo values following each route of administration in mice, rats, rabbits and guinea pigs. In rats, acute toxicities included disappearance of locomotor activity, gait ataxia, CNS toxicities such as salivation secretion and increased muscle tone with fibrillary twitch, but no histopathological changes were reported (BUA, 1988).

Table 1 Results of acute toxicity studies*

	Mouse	Rat	Rabbit	Guinea pig
Oral LD_{50}	1,276 mg/kg [1)]	580-4,000 mg/kg* [2)] 47 mg/kg [3)]	—	—
Inhalation LD_{50}	—	—	—	—
Percutaneous LD_{50}	—	> 2,000 mg/kg [3)]	—	—
Intraperitoneal LD_{50}	153 mg/kg [1)]	430 mg/kg [1)]	—	—
Subcutaneous LD_{50}	—	1,730 mg/kg [1)]	—	—
Oral LDLo	—	—	—	2,000 mg/kg [1)]
Percutaneous LDLo	—	—	3,160 mg/kg [1)]	—

*: Variable depending on the studies.
[1)] BUA 1988, [2)] EHC 1989, [3)] NTIS, 1968b (Molten 2,4-DCP was used.)

(2) Repeated-dose toxicity (Attachment-4)

In a 13-week feeding study of 2,4-DCP (purity, 99% or more) in $B6C3F_1$ mice (10 mice/sex/group) using dietary concentrations of 0, 2,500, 5,000, 10,000, 20,000 and 40,000 ppm, 2,4-DCP caused rough hair coat in both sexes and appearance of multinuclear hepatocytes in males at 10,000 ppm and above, suppression of body weight gain and decrease in food consumption at 20,000 ppm with cellular necrosis in all males, and at 40,000 ppm death of all mice within 3 weeks and epithelial necrosis of the renal tubules (NTP, 1989).

In a six-month feeding study of 2,4-DCP in ICR mice (7 males/group) using dietary concentrations of 0, 0.02, 0.05, 0.1 and 0.2% (corresponding to 18, 45, 100 and

230 mg/kg/day), relative liver weight decreased in 230 mg/kg/day group, with hepatocellular swelling in one, small round cell infiltration in interstitial of liver in two and thinning of adrenal cortex in two males. Based on these, the authors estimated that the NOEL of 2,4-DCP was 100 mg/kg/day (Kobayashi et al., 1972).

When 2,4-DCP was administered in diet to B6C3F_1 mice (50 mice/sex/group) at the dietary concentrations of 0, 5,000 and 10,000 ppm (males; 0, 800 and 1,300 mg/kg/day, females; 0, 430 and 820 mg/kg/day) for two years, body weight gain was suppressed in the 10,000 ppm group, and incidence of multinuclear hepatocytes increased dose-dependently in males (control group: 11/50, 5,000 ppm group: 33/49, 10,000 ppm group: 42/48) (NTP, 1989).

In a 13-week feeding study of 2,4-DCP (purity, 99% or more) in F344 rats (10 rats/sex/group) using the dietary concentrations of 0, 2,500, 5,000, 10,000, 20,000 and 40,000 ppm, atrophy of bone marrow and marked decreases in erythrocytes and myelocytes were observed in 6/10 females in 10,000 ppm group and all rats in 20,000 ppm and higher groups and retardation of body weight gain, hunchback posture, rough hair coat and decrease in food consumption in 40,000 ppm group (NTP, 1989). The NOEL was estimated to be 10,000 ppm (corresponding to 1,000 mg/kg/day) for males and 5,000 ppm (corresponding to 500 mg/kg/day) for females under the conditions tested (BUA, 1996).

In a 4-week feeding study of 2,4-DCP in F344 rats (5 rats/sex/group) using the dietary concentrations of 0, 200, 1,000, 5,000 and 20,000 ppm (corresponding to 0, 20, 101, 493 and 1,782 mg/kg/day) (OECD TG407), suppression of body weight gain, increase in γ-GTP activity and prolongation of clotting time were observed in both sexes in 20,000 ppm group (BUA, 1996).

In a two-year feeding study of 2,4-DCP (purity, 99% or more) in F344 rats using dietary concentrations of 0, 5,000 and 10,000 ppm (corresponding to 0, 210 and 400 mg/kg/day) for males and those of 0, 2,500 and 5,000 ppm (corresponding to 0, 120 and 250 mg/kg/day) for females, 2,4-DCP had no effect on survival rate at any dose levels, but caused retardation of body weight gain in both sexes in high dose group. In males in 2,4-DCP groups, the incidence of diffuse degeneration of respiratory epithelium tended to increase (control group: 25/45, 5,000 ppm group: 38/48, 10,000 ppm group: 42/46) (NTP, 1989).

In a succeeding generation study in SD rats, 2,4-DCP was administered in drinking water to 3-week old females (10 females/group) at the concentrations of 0, 3, 30 and 300 ppm (corresponding to 0, 0.5, 5, and 50 mg/kg/day), and administration was continued during mating with untreated males at the age of 13 weeks through gestation and lactation periods until weaning. The offsprings were weaned at the age of 3 weeks and treated with 2,4-DCP in drinking water until the age of 12 weeks. In offsprings in 300 ppm group, spleen and liver weights increased, and, hematologically, increases in red blood cells and hemoglobin were observed. In this study liver and spleen showed no histopathological changes despite increases in their weights (Exon et al., 1985). Based on these, US EPA set up NOEL and NOAEL 2,4-DCP at 3 ppm (corresponding to 0.5 mg/kg/day) and 30 ppm (corresponding to 5 mg/kg/day), respectively (IRIS, 1988).

WHO has adopted 200 μg/kg/day as TDI for humans, derived from NOEL of 100 mg/kg/day (Kobayashi et al., 1972) divided by 500, uncertainty factor concerning test period (EHC, 1989).

4) Information on mutagenicity/genotoxicity and carcinogenicity

(1) Mutagenicity/genotoxicity (Table 2)

2,4-DCP was reported to be negative in reverse mutation test with *Salmonella typhimurium* strains but positive in the assay with the mouse lymphoma L5178 cells and sister chromatid exchange test using CHO cells. In chromosomal aberration tests with CHO cells, 2,4-DCP was reported to be both positive and negative (Hilliard et al., 1998; NTP, 1981, 1989). In a mutation assay with Chinese hamster V79 cells without metabolic activation, 2,4-DCP was reported to be cytotoxic for V79 cells and not to induce any marked increase in 6-thioguanine-resistant mutants (Jansson, 1986).

2,4-DCP has been reported to be positive for mutagenic potential in DNA double strand breakage test with SD rat primary hepatocyte cultures (Elia et al., 1994).

No *in vivo* test data are available.

Table 2 Results of mutagenicity/genotoxicity tests

Test method		Test conditions	Results*	References
in vitro	Reverse mutation test	*Salmonella typhimurium* strains TA98, TA1535, TA100 and TA1537 S9(-) 500, 1,000 μg/plate	−	BUA, 1988
		Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 Rat S9(+/-) 3.3-333 μg/plate	−	NTP, 1989
		Salmonella typhimurium strains TA98, TA 100 and TA1537 Hamster S9(+/-) 3.3-333 μg/plate	−	NTP, 1989
		Salmonella typhimurium strains TA98, TA100, TA1537 and TA1535 S9(-) 3.3-333μg/plate	−	NTP, 1989
		Salmonella typhimurium strains TA98, TA100, YG1021, YG1024, YG1026 and YG1029 S9(+) 100 μg/plate	−	Tanaka, et al. 1996
	Gene mutation test	Chinese hamster V79 cells (6-TG-resistant cells) S9(-) 12.5-50 mg/*l*	−	Jansson et al., 1986
	Mouse lymphoma cell TK+/- assay	Mouse lymphoma L5178Y cells S9(-) 10-60 mg/*l*	+	NTP, 1989
	Chromosomal aberration test	CHO cells S9(+/-) S9(-): 40.2-75 mg/*l* S9(+): 100.5-176 mg/*l*	−	NTP, 1988
		CHO cells S9(+/-) S9-: 75 mg/*l* S9+: 150 mg/*l*	−	Anderson et al., 1990
		CHO cells S9(+/-) S9-: 0.8-1.4 mM S9+: 0.6-1.0 mM	+	Hilliard et al., 1998
		Human lymphoblast TK6 cells S9(-) 0.8-1.2 mM	−	
		CHO cells S9(-) 1.2 mM, 1.4 mM	+	Galloway et al., 1998
	Sister chromatid exchange test	CHO cells S9(+/-) S9-: 0.167-12.6 mg/*l* S9+: 99.7-160 mg/*l*	+	NTP, 1989
	Aneuploidy test	Chinese hamster V79 cells 500 μmol/*l*	+	Onfelt, 1987
	Unscheduled DNA synthesis test	Rat liver cells 50-1,000 mmol/m*l*	−	Probst, et al., 1981
	DNA double strand breakage test	SD rat primary hepatocyte cultures, 0.2-0.8 mM	+	Elia et al., 1994

*−: Negative +: Positive

(3) Carcinogenicity (Table 3, Attachments -5, 6)

In a 2-year carcinogenicity study in which 2,4-DCP (purity, 99% or more) was administered in diet to 8-week-old $B6C3F_1$ mice (50 mice/sex/group) at dietary concentrations of 0, 5,000 and 10,000 ppm (corresponding to 0, 800 and 1,300 mg/kg/day in males and to 0, 430 and 820 mg/kg/day in females), body weight gain was suppressed in females in the 10,000 ppm group, and incidence of multinuclear hepatocytes increased markedly in males in a dose-dependent manner (control group: 11/50, 5,000 ppm group: 33/49, 10,000 ppm group: 42/48). In females in the treated groups, incidence of malignant lymphoma decreased (control group: 12/50, 5,000 ppm group: 6/50, 10,000

ppm group: 4/50). However, the authors concluded that this change was not related to 2,4-DCP since these incidences were within the range of background fluctuation for this strain of mice. One male (1/50) in the 10,000 ppm group had squamous cell carcinoma of forestomach which is rare in this strain of mice (control group: 8%). However, since 2,4-DCP did not promote hyperplasia of forestomach, the authors concluded that the results showed no evidence of carcinogenicity (NTP, 1989).

In another 2-year carcinogenicity study in F344/N rats in which 2,4-DCP (purity, 99% or more) was administered in diet to males at the dietary concentrations of 0, 5,000 and 10,000 ppm (corresponding to 0, 210 and 440 mg/kg/day) and to females at 0, 2,500 and 5,000 ppm (corresponding to 0, 120 and 250 mg/kg/day), incidence of mononuclear cell leukemia decreased in treated males (control group: 62%, 5,000 ppm group: 34%, 10,000 ppm group: 34%). However, since these figures were almost similar to the incidence of untreated males in background fluctuation (36.3%), the authors concluded that these decreases were unrelated to 2,4-DCP (NTP, 1989).

No adequate evidence is available for carcinogenicity of monochlorophenol and 2,4-DCP possessing one and two chlorine atom(s) respectively in each molecule, but 2,4,6-trichlorophenol which has three chlorine atoms in its molecule is classified into B2, b and category 3 by the EPA, NTP and EU, respectively. 2,4-D (2,4-dichlorophenoxy acetic acid) which is metabolized into 2,4-DCP in living body (EHC, 1989) is contained in polychlorophenols and their sodium salts (mixed exposure), and exposure to the mixture of these chemicals is classified into group 2B in 1977 by the IARC.

In a study in Sutter mice (8-12 weeks of age) by topical application, 25 μ*l* of 0.3% DMBA (dimethylbenzanthracene) (DMBA: 75 μg) was topically applied to the back skin of mice for 1 week, followed by topical application of 25 μ*l* of 20% 2,4-DCP (corresponding to 5 mg/mouse) to back skin twice weekly for 15-24 weeks. Since development of papilloma was observed at application site in 13/27 (48%) and 12/16 mice (75%) at weeks 15 and 24 of application, respectively (control group at week 24 of application: 3/27 mice, 11%), 2,4-DCP was reported to act as a promoter (Attachment-6). At week 24 of application, skin cancer was identified at application site in one mouse (Boutwell and Bosch, 1959).

2,4-DCP was administered in drinking water to female SD rats from 3 weeks of age at

the concentrations of 0, 3, 30 and 300 ppm. After mating at the age of 13 weeks, females received together with 2,4-DCP EU (ethyl urea), a precursor of ENU (ethyl nitrosurea) as initiator, and nitrogen dioxide in drinking water at concentrations of 0.150% and 1 ppm, respectively, on gestation days 14 through 21. And then, the weanlings were ① treated or ② untreated with 2,4-DCP in drinking water. In experiment ③, dams were treated with the initiator alone in drinking water on gestation days 14-21, and the pups with 2,4-DCP in drinking water at concentrations of 0, 3, 30 and 300 ppm. To evaluate the tumor-promoting action of 2,4-DCP, the results of these experiments were compared with the control group which received the initiator alone. 2,4-DCP exhibited no tumor-promoting activity on the ENU-initiated cells (Exon and Koller, 1985).

Table 3 Carcinogenicity assessment by national and international organizations

Organization	Category	Significance	References
EPA	—	Carcinogenicity of 2,4-DCP is not assessed.	JETOC, 1999
EU	—	Carcinogenicity of 2,4-DCP is not assessed.	JETOC, 2000
NTP	—	Carcinogenicity of 2,4-DCP is not assessed.	NTP, 2000
IARC	—	Carcinogenicity of 2,4-DCP is not assessed.	IARC, 2001
ACGIH	—	Carcinogenicity of 2,4-DCP is not assessed.	ACGIH, 2000
Japan Society for Occupational Health	—	Carcinogenicity of 2,4-DCP is not assessed.	Japan Society for Occupational Health, 2001

5) Information on immune system (Attachment-7)

2,4-DCP was administered to 3-week-old female SD rats (10 females/group) in drinking water at concentrations of 0, 3, 30 and 300 ppm (corresponding to 0, 0.5, 5 and 50 mg/kg/day), and females were mated with untreated males at the age of 13 weeks. ① Administration in drinking water to dams was continued through parturition until weaning, and offsprings were also treated with 2,4-DCP in drinking water from weaning at the age of 3 weeks until 12 weeks of age. ② Separately dams were treated until parturition, and offsprings were maintained until the age of 6 weeks. In experiment ①, Suppression of DTH (delayed-type hypersensitivity) reaction, increases in immunoglobulin levles (quantitated by ELISA) and dose-related suppression of cellular immune response were observed in offsprings in 30 ppm and higher groups, and increase in spleen and liver weights and increase in anti-KLH antibody level in offsprings in 300 ppm group. In experiment ②, spleen weight increased in offsprings in 300 ppm group, but the treatment

had no effect on immune function (Exon et al., 1984).

In a 4-week feeding study in which 2,4-DCP was administered to F344 rats (5 rats/sex/group) at dietary concentrations of 0, 200, 1,000, 5,000 and 20,000 ppm (corresponding to 0, 20, 101, 493 and 1,782 mg/kg/day) (OECD TG 407), IgG and IgM levels were slightly decreased (BUA, 1996).

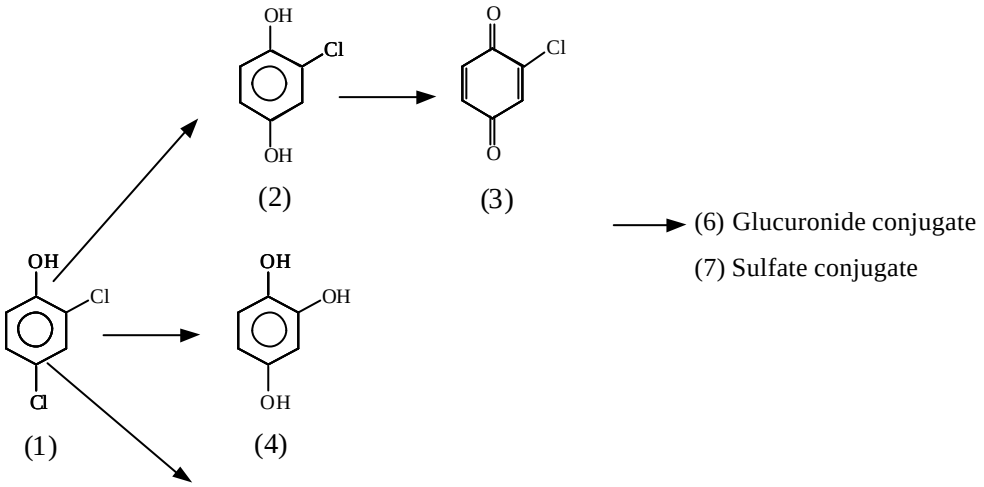
6) Fate and Metabolism

2,4-DCP is relatively rapidly absorbed from the digestive tract, skin and respiratory organs (IARC, 1986).

After single intravenous administration at 10 mg/kg to SD rats (males; 250-300 g), 2,4-DCP was rapidly transformed into a glucuronide conjugate or other conjugates (not specified). The half-lives of 2,4-DCP and its metabolites (not specified) in brain, liver, kidney and plasma was 4-30 minutes. Within 10-15 minutes after administration, 2,4-DCP and its conjugates were detected in brain, liver, kidney and plasma, and 2,4-DCP alone in adipose tissues. At 1 hour after administration, 76% of the total administered dose was detected in kidney, with the maximal concentration in renal tissues of 17.7 mg/kg kidney weight (Somani & Khalique, 1982).

In a metabolism study with isolated rat liver, 2,4-DCP was reported to be conjugated with glucuronide or metabolized into dichloromethoxyphenols (Fig. 1) (Somani & Khalique, 1982). In an *in vitro* study on human P450 3A4-mediated metabolism of 2,4-DCP, 2-chloro-1,4-hydroxyquinone, 2-chloro-1,4-benzoquinone and 1,2,4-hydroxybenzene were detected by thin-layer chromatography (Fig. 1) (Mehmood et al., 1997).

In rabbits, 2,4-DCP is excreted mainly as its glucuronide conjugate, but 16% or less of the administered dose was converted into its sulfate conjugate (HSDB). In cattles, the entire amount of 2,4-DCP (20 g) administered in diet was excreted within 24 hours after administration (HSDB, 2001).



(1) 2,4-Dichlorophenol (2,4-DCP)
(2) 2-Chloro-1,4-hydroxyquinone
(3) 2-Chloro-1,4-benzoquinone
(4) 1,2,4-Hydroxybenzene

Fig. 1 Metabolic pathways for 2,4-dichlorophenol

2. Hazard assessment at present

Effects on endocrine and reproductive systems in humans have not been reported. In experiments on potential effects of 2,4-DCP on endocrine system, the results are contradictory: i.e., 2,4-DCP is reported not to bind to estrogen receptors and not to cause consequent estrogen receptor-mediated activation of gene transcription, and it is also reported to bind to estrogen receptors. Any way, since the results of *in vivo* studies including uterotrophic assay and rodent Hershberger assay were negative, there seems to be little possibility that 2,4-DCP has sex hormone receptor-mediated endocrine disrupting effect.

In studies on the effects on reproductive system, 2,4-DCP has been reported to have toxic effects on fetuses secondary to maternal toxicities such as decrease in litter size and increase in organ weights, or to have intrinsic fetotoxicities. However, since multigeneration reproductive toxicity studies have not yet been performed, the effects of this compound on reproductive performance and development of next generation remain unclear. Thus, it is difficult to conclude that enough scientific findings are available to evaluate the effects on reproductive performance in multi-generations and on development

of offspring.

As for the information on hazardous effects of this compound in humans, it is warned that skin exposure to molten or hot 2,4-DCP may result in death. In the repeated oral toxicity studies in animals, the toxic effects of 2,4-DCP have been reported particularly on liver and hematopoietic and immune systems. In the assessment of mutagenicity, 2,4-DCP is not mutagenic in bacterial reverse mutation tests, but there are reports giving positive results in other test systems. For the evaluation of carcinogenicity, no adequate reports are available.

3. Risk assessment and other necessary future measures

2-Generation reproductive toxicity study for 2,4-DCP is now under way. 2,4-DCP is unlikely to have endocrine disrupting effect mediated by sex hormone receptors. But the findings on effects on fertility in multi-generations and development of offspring is not necessarily sufficient. By incorporating the results of the above 2-generation reproductive study into existing findings, the endocrine-disrupting effect of 2,4-DCP and its toxcities associated with disruption of endocrine system will be comprehensively assessed.

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Attachment-1 Results of *in vitro* studies on receptor binding

Item	Test methods and conditions	Results	Conclusion	References
ER binding assay	Methods: Competitive binding assay using [^{3}H]-E2 as a ligand. Receptor: Bovine uterine homogenate ER	IC50 value: $>5\times10^{-5}$M ($E2: 6.89\times10^{-9}$ M)	Does not bind to ER.	Kramer et al., 1999
	Methods: Binding assay on human ER (Recombinant ERα ligand domain)	IC50 value: $>10^{-4}$M ($E2: 1.2\times10^{-9}$ M)	Does not bind to ER.	CERI, 2001a
Yeast two-hybrid assay	Cells: Yeast cells transfected with Gal4 DNA binding domain/human ER ligand binding domain genes, Gal4 activation domain/coactivator TIF2 genes and β-galactosidase reporter genes	REC10: 4×10^{-5} M ($E2: 3\times10^{-10}$ M)	Induce ER related gene transcription activation.	Nishihara et al., 2000
Reporter-gene assay using recombinant yeast cells	Cells: Yeast cells transfected with human progesterone receptor genes and β-galactosidase reporter genes Incubation concentrations: 1.0×10^{-6} M (2,4-DCP) 1.0×10^{-8} M (progesterone) 1.0×10^{-6} M (2,4-DCP)+ 1.0×10^{-8} M (progesterone) Incubation period: 12 hr	2,4-DCP did not induce any significant activity at 10^{-6}M. The simultaneous presence of 1.0×10^{-8}M of progesterone and 10^{-6}M of 2,4-DCP had no effect on progesterone activity.	Does not induce progesterone receptor related gene transcription activation.	Tran et al., 1996
Reporter-gene assay with cultured recombinant cells	Cells: HeLa cells transfected with human ER genes and estrogen responsive element concentration: 10^{-11} – 10^{-5} M (2,4-DCP)	Negative for the estrogenic agonist activity within a concentration range of 10^{-11}-10^{-5}M ($E2: PC50: <10^{-11}$ M)	Does not induce ER related gene transcription activation.	CERI, 2001a
Human mammary tumor cell proliferation assay	Cells: Human mammary tumor cells (MCF-7) concentrations: 10^{-9}-10^{-4} M (2,4-DCP) 10^{-14}-10^{-11} M (E2) Test period: 6 days	Concentration-dependent weak cell proliferation response within a concentration range of 10^{-9}-10^{-4}M. (E2-dependent proliferation activity within a concentration range of 10^{-14}-10^{-11}M)	Induces cell proliferation.	Jones et al., 1998

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 against E2

IC50: 50% inhibition concentration

Attachment-2 Results of studies on mammalian endocrine and reproductive systems

Animal species	Administration method	Administration period	Dose	Results	References
Rat (Wistar Hannover, female) 6 rats/group Ovariectomized rats Ovariectomized at the age of 6 weeks	Gavage (uterotrophic assay)	For 3 days from the age of 8 weeks. The uterus was removed 24 hr after the end of administration and weighed.	0, 100, 200, 400 mg/kg/day	No effect on uterine weight.	CERI, 2001b
			0, 100, 200, 400 mg/kg + Ethinyl estradiol 0.5 μg/kg/day, s.c.	No effect on uterine weight.	
Rat (Wistar Hannover, male) Castrated at the age of 6 weeks	Gavage (Hershberger assay)	For 10 days from the age of 8 weeks. Necropsied 24 hr after the final dose.	0, 50, 100, 200 mg/kg/day	No effect on accessory reproductive organ weights.	CERI, 2001b
			0, 50, 100, 200 mg/kg/day + Testosterone propionate (TP) 0.4 mg/kg/day, s.c.	No effect on accessory reproductive organ weights.	
Mouse (C57BL/6) 6 dams/group	s.c. (DMSO)	Gestation days 6-14 (Cesarean section: gestation day 18)	0, 74 mg/kg/day	Dams: No effect	NTIS, 1968a
				Fetuses: Increase in mortality rate	
Mouse (AKR) 6 dams/group		Gestation days 6-15 (Cesarean section: gestation day 19)		Dams: Decrease in relative liver weight	
				Fetuses: Low body weight, Overextension of four limbs (4/40 fetuses)	
Rat (F344) 34 dams/group	Gavage (in corn oil)	Gestations days 6-15 (Cesarean section: gestation day 20)	0, 200, 375, 750 mg/kg/day	Dams: 200 mg/kg/day and higher groups Suppression of body weight gain 750 mg/kg/day group Death (4/34)	Rodwell et al., 1989.
				Fetuses: 750 mg/kg/day group Low fetal body weight, delayed ossification	
Rat (SD) 10 dams/group	In drinking water	Dams: 3 weeks of age -parturition	0, 3, 30, 300 ppm (corresponding to 0, 0.5, 5, 50 mg/kg/day) (Mated with untreated males at the age of 13 weeks)	Dams: 300 ppm Decrease in live born pups	Exon, et al., 1984, Exon, and Koller, 1985
				Offsprings (6 weeks of age): 30 ppm Suppression of survival rate at weaning 300 ppm Increases in spleen and liver weights	

Attachment-3 Results of *in vitro* fertilization tests in mammals

Animal species	Administration method	Administration period	Dose	Results	References
Mouse (Male: CD-1, Female: CB_6F_1)	In drinking water	90 days	Males: 0, 50, 150, 500 mg/kg/day	No effect on sperm motility and penetration rate into ovum.	Seyler et al., 1984
	Addition to incubation medium	—	0.1, 0.3, 1.0 mM (0.4 m*l*, Added to medium)	No effect on sperm motility and penetration rate into ovum.	

Attachment-4 Results of repeated-dose toxicity studies

Animal species	Administration method	Administration period	Dose	Results	References
Mouse ($B6C3F_1$, male and female) 10 mice/group	Feeding	13 weeks	0, 2,500, 5,000, 10,000, 20,000, 40,000 ppm	2,500 ppm and above: Hepatocellular necrosis 10,000 ppm and above: Appearance of multinuclear hepatocytes in all males 20,000 ppm: Suppression of body weight gain 20,000 ppm and above: Decrease in food consumption, hepatocellular necrosis in females 40,000 ppm: Death in all animals, epithelial necrosis in urinary tubules (8/9 males, 3/10 females) NOEL: Female － 10,000 ppm (corresponding to 1,500 mg/kg/day)	NTP, 1989
Mouse (ddN, Male) 7 mice/group	Feeding	6 months	0.02, 0.05, 0.1, 0.2% (The concentrations in three higher dose groups correspond to 45, 100 and 230 mg/kg/day, respectively)	0.2%: Decrease in relative liver weight, thinning of adrenal cortex (2 mice), hepatocellular hypertrophy (1 mouse), small round cell infiltration in hepatic interstitium (2 mice) NOEL=100 mg/kg/day	Kobayashi et al., 1972
Mouse ($B6C3F_1$, male and female) 50 mice/group	Feeding	2 years	0, 5,000, 10,000 ppm (Males: Corresponding to 800 and 1300 mg/kg/day, Females: Corresponding to 430 and 820 mg/kg/day)	10,000 ppm: Suppression of body weight gain, decrease in food consumption, dose-dependent appearance of multinuclear hepatocytes in males (control group: 11/50, treated groups: 33/49, 42/48)	NTP, 1989
Rat (F344, male and female) 5 rats/group	Feeding	4 weeks	200, 1,000, 5,000, 20,000 ppm (Corresponding to 20, 101, 493 and 1,782 mg/kg/day)	20,000 ppm: Suppression of body weight gain, increase in γ-GT activity, prolongation of clotting time	BUA, 1996
Rat (F344, male and female) 10 rats/group	Feeding	13 weeks	0, 2,500, 5,000, 10,000, 20,000, 40, 000 ppm	Bone marrow atrophy and marked decreases in red blood cells and myelocytes in females (6/10) in 10,000 ppm group and all animals in the 20,000 ppm and higher groups 40,000 ppm: Rough hair coat, hunchback posture, suppression of body weight gain, decrease in food consumption in males NOEL Male: 10,000 ppm Female: 5,000 ppm	NTP, 1989

Animal species	Administration method	Administration period	Dose	Results	References
Rat (F344, male and female) 50 rats/group	Feeding	2 years	Males: 0, 5,000, 10,000 ppm (Corresponding to 0, 210 and 440 mg/kg/day) Females: 0, 2,500, 5000 ppm (Corresponding to 0, 120 and 250 mg/kg/day)	Suppression of body weight gain in males in 440 mg/kg group and females in 250 mg/kg group. Increase in diffuse degeneration of respiratory epithelium in males in all treated groups (control group: 25/45, treated groups: 38/48, 42/46)	
Rat (SD) 10 dams/group	In drinking water	Dams: 3 weeks of age to lactation period. Offspring: 3 weeks of age to 15-18 weeks of age	0, 3, 30, 300 ppm (Corresponding to 0, 0.5, 5 and 50 mg/kg/day) (Mated with untreated males at the age of 13 weeks)	Offsprings: 300 ppm Increases in spleen and liver weights, increases in red blood cell count and hemoglobin NOEL 3 ppm (Corresponding to 0.5 mg/kg/day) NOAEL 30 ppm (Corresponding to 5 mg/kg/day)	Exon and Koller, 1985

Attachment-5 Results of carcinogenicity studies

Animal species	Administration method	Administration period	Dose	Results	References
Mouse ($B6C3F_1$, male and female) 50 mice/group	Feeding	2 years	0, 5,000, 10,000 ppm (Males: Corresponding to 0, 800 and 1300 mg/kg/day, Females: Corresponding to 0, 430 and 820 mg/kg/day)	Suppression of body weight gain in females in the 10,000 ppm group. Appearance of multinuclear hepatocytes in males in all treated groups (control group: 11/50, 5,000 ppm group: 33/49, 10,000 ppm group: 42/48). No evidence of carcinogenicity.	NTP, 1989
Rat (F344, male and female) 50 rats/group	Feeding	2 years	Males: 0, 5,000, 10,000 ppm (Corresponding to 0, 210 and 440 mg/kg) Females: 0, 2,500, 5000 ppm (Corresponding to 0, 120 and 250 mg/kg)	Suppression of body weight gain in both sexes in the highest dose group. Decrease in incidence of mononuclear cell leukemia (control group: 62%, treated group: 34%, background data: 36.3%). No evidence of carcinogenicity.	NTP, 1989

Attachment-6 Results of bioassays for tumor promoting effect

Animal species	Administration method for initiator	Administration method	Administration period	Test method	Tumor	Results	References
Mouse (Sutter, female, 8-12 weeks of age)	0.3% DMBA* 25 μl Topically applied (75 μg) for 1 week	Topical application (back skin)	15 weeks	Twice weekly, 20% (25 μl; Corresponding to 5 mg/animal)	Papilloma in dorsal skin DMBA/Vehicle group: 1/14 (7%), DMBA/2,4-DCP group: 13/27 (48%)	Positive for tumor-promoting activity.	Boutwell & Bosch, 1959
			24 weeks		Papilloma in the dorsal skin DMBA/Vehicle group: 3/27 (11%), DMBA/2,4-DCP group: 12/16 (75%)	Positive for tumor-promoting activity.	
Rat (SD) Dams: 12-22 dams/group Offsprings: 48-60 rats/group	EU: 0.150% and NO_2: 1 ppm were administered in drinking water on gestation days 14-21	In drinking water	Dams: 3 weeks of age to parturition	0, 3, 30, 300 ppm (Mated with untreated males at the age of 13 weeks)	No effect on tumor incidence and latency period.	Negative for tumor-promoting activity.	Exon & Koller 1985
			Offsprings: 2 years from 3 weeks of age		No effect on the tumor incidence and latency period.	Negative for tumor-promoting activity.	
			Dams: 3 weeks of age to weaning Offsprings: 2 years from 3 weeks of age		No effect on the tumor incidence and latency period.	Negative for tumor-promoting activity.	

*: DMBA = 9,10-Dimethyl-1,2-benzanthracence

Attachment-7 Results of studies on effects on immune system

Animal species	Administration method	Administration period	Dose	Results	References
Rat (SD) 10 dams/group	In drinking water	Dams: 3 weeks of age to parturition (Mated with untreated males at the age of 13 weeks) (Offsprings: Observed until 6 weeks of age)	0, 3, 30, 300 ppm (Corresponding to 0, 0.5, 5 and 50 mg/kg/day)	Dams: Decrease in litter size Offsprings: Increase in spleen weight in 300 ppm group. No significant difference in immune function as compared with the control group.	Exon et al., 1984, Exon & Koller, 1985
		Dams: 3 weeks of age to lactation period (Mated with untreated males at the age of 13 weeks). Offsprings: 3 weeks of age to 13 weeks of age		Dams: Decrease in the litter size Offsprings: Increases in spleen and liver weights, suppression of cellular immunity 300 ppm: Increase in humoral immune response, increase in anti-KLH antibody level	