HAZARD ASSESSMENT REPORT

CHLOROBENZENE

CAS No. 108-90-7

Chemicals Evaluation and Research Institute (CERI), Japan

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Preface to the English Version of the Hazard Assessment Reports

For six years from April 2001 to March 2007, Chemicals Evaluation and Research Institute (CERI/Japan) was engaged in a project named “Chemical Risk Assessment and Development of Risk Assessment Methods” under "Comprehensive Chemical Substance Assessment and Management Program" funded by New Energy and Industrial Technology Development Organization (NEDO/Japan). Under this project, about 150 chemical substances were selected among those designated as Class-I Chemicals in the Law for Pollutant Release and Transfer Register and Promotion of Chemical Management (hereafter PRTR Law)[1)]. The selection criteria of these chemicals were their priorities for risk assessment based on their production levels and environmental/human health concerns.

CERI developed the hazard assessment reports of these selected chemical substances based on the review and evaluation of the environmental and human health hazard data obtained from the existing evaluation documents released by the regulatory agencies and international organizations as well as those from the published scientific literatures. The data review and compilation of the reports were conducted according to the guidelines[2)] and the guidance manual[2)] developed for this project. The proposed hazard assessment reports by CERI were reviewed by the experts in the relevant scientific fields from both inside and outside this project for accuracy, relevance and completeness. The final reports were published in Japanese after going through the deliberation by the “Council on Chemical Substances” under the Ministry of Economy, Trade and Industry (METI/Japan), which is responsible for regulation of chemical substances in Japan.

This project was the first attempt in Japan to develop comprehensive hazard assessments of chemical substances for application in risk assessment. In order to share the outcomes of the project globally, CERI independently selected the following seven chemical substances and developed the English version of the hazard assessment reports:

(1) Acetaldehyde
(2) Chlorobenzene
(3) Hydrazine
(4) *N*, *N*-Dimethylformamide
(5) Poly(oxyethylene)nonylphenylether
(6) 3,3’-Dichloro-4,4’-diaminodiphenylmethane
(7) Dimethyl-2,2-dichlorovinyl phosphate (Dichlorvos)

We hope that the hazard assessment reports from our project contribute to the risk assessment and management of chemical substances globally, and appreciate your feedback. .

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1) Details of the PRTR Law, the list of designated chemical substances, and release data in Japan are available on Internet at: http://www.prtr.nite.go.jp/index-e.html.

2) Guidelines and the guidance manual in Japanese are available on Internet at: http://www.safe.nite.go.jp/risk/riskhykdl01.html. Also, the initial risk assessment reports in Japanese developed in this project which include calculations of margin of exposure based on the result of hazard assessment and exposure assessment, are available on Internet at: http://www.safe.nite.go.jp/risk/riskhykdl01.html.

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Summary

Chlorobenzene is a colorless liquid having a vapor pressure of 1.2 kPa at 20 ^{o}C and a boiling point of 131-132 ^{o}C. It is soluble in water and miscible with organic solvents. Its water solubility is 500 mg/L. Chlorobenzene is mainly used as raw material for synthesis of triphenylphosphine (catalyst for organic synthesis), phenylsilane and thiophenol (intermediate for pesticides and pharmaceuticals). Domestic supplies of chlorobenzene for 5 years from 1998 to 2002 decreased from 35,000 to 10,000 tons/year in Japan.

It has been estimated that 514 tons of chlorobenzene was released annually into air, and 29 tons into water in Japan.

Chlorobenzene released into the aquatic environment is eliminated mainly by volatilization, but almost not by biodegradation. Low bioaccumulation potential is suggested in aquatic organisms.

Many studies have been conducted to assess the toxic effects of chlorobenzene on organisms in the environment using indices including mortality, immobilization and growth inhibition. In acute toxicity of chlorobenzene to algae, a 96-hr EC_{50} (growth inhibition) for freshwater alga was 12.5 mg/L. The acute toxicity of chlorobenzene to invertebrates is reported in freshwater and seawater crustaceans. A 48-hour EC_{50} (immobilization) for the freshwater water flea was 0.59 mg/L. The long-term toxicity to freshwater water fleas has been reported, and the lowest value was 0.32 mg/L as the 16-day NOEC for reproduction of the water flea. The acute toxicity of chlorobenzene to fish is reported in rainbow trout, bluegill and fathead minnow, and the 96-hr LC_{50} values were 4.7 mg/L for the rainbow trout, 7.4 mg/L for the bluegill and 7.7 mg/L for the fathead minnow. The long-term toxicity to fish in the early life stage has been reported in rainbow trout, goldfish and largemouth bass, and the reliable lowest LC_{50} was the 7.5-day LC_{50} of 0.05 mg/L for 4-day posthatch of the largemouth. The lowest value of toxicity in aquatic organisms is the 7.5-day LC_{50} of 0.05 mg/L for 4-day posthatch of the largemouth.

In experimental animals, chlorobenzene is absorbed mainly through the gastrointestinal and respiratory tracts, and dermal absorption is considered low. Chlorobenzene is lipophilic and has a tendency to accumulate in lipid-rich tissues. Chlorobenzene is metabolized to generate two kinds of epoxides by cytochrome P450, and these epoxides bind to nucleic acids and form covalent bonds with proteins in a nonspecific manner in the liver and lung. In the metabolic process of chlorobenzene, these epoxides are metabolized into mercapturic acid derivatives and excreted into urine. Otherwise, these epoxides are metabolized into either chlorocatechols or chlorophenols and excreted in the urine as highly water-soluble glucuronoconjugates and sulfoconjugates. Most chlorobenzene orally administered is excreted in the urine, and some in the feces, and the unchanged chlorobenzene excreted in exhalation through the lung.

The toxic effects of chlorobenzene on humans were exhaustion, nausea, lethargy, headache and irritation to the upper respiratory tract and eye. Contact of chlorobenzene with the skin induced irritation. No reports were obtained on sensitization by chlorobenzene in this investigation.

The oral LD_{50} values of chlorobenzene were 1,445 mg/kg in mice, 1,427 to 3,400 mg/kg in rats and 2,250 to 2,830 mg/kg in rabbits. The LC_{50}s following 6-hr inhalation exposure were 1,889 ppm in mice and 2,968 ppm in rats.

Slight irritation in the eyes and skin has been reported in studies with rabbits.

The repeated oral administration of chlorobenzene to mice caused suppressed body weight gain, a decrease in spleen weight and hepatocyte necrosis. The LOAEL is 60 mg/kg/day with effects on liver and kidney in mice and rats by 90-day administration. The repeated inhalation exposure to rats from 10 weeks before mating to the completion of lactation resulted in an increase in liver weight (males and females), hypertrophy of the centrilobular hepatocytes (males), renal tubular dilation and interstitial nephritis (males) and degeneration of the seminiferous epithelium in the males. The NOAEL is 50 ppm (234 mg/m^3) with the effects on the liver and kidney.

In a study on reproductive toxicity to rats, inhalation exposures of chlorobenzene to male and female rats from 10 weeks before mating to the completion of lactation caused degeneration of the seminiferous epithelium in males, but rates of mating behavior, pregnancy and fertility in all dose groups were simialr to those of the control group. Exposures of chlorobenzene to pregnant rats from gestation day 6 to 15 and rabbits from gestation day 6 to 18 exhibited no embryotoxic or teratogenic effects on fetuses, except slightly retarded ossification of the fetuses of rat observed at maternal toxic dose. Therefore, it is considered that chlorobenzene has no reproductive toxicity to rats, although it caused adverse effect on male reproductive organ in rats. In addition, chlorobenzene has no developmental toxicity including embryotoxicity and teratogenicity to rats and rabbits.

Chlorobenzene showed negative results in many *in vitro* and *in vivo* tests of *in vitro* gene mutation assays using bacteria (*Salmonella typhimurium*), an *in vitro* chromosomal aberration test using CHO cells, and *in vitro* DNA damage tests with bacteria and unscheduled DNA synthesis tests with rat hepatocytes, and *in vivo* dominant lethal test in mice. Otherwise, chlorobenzene showed positive and/or negative results in other test systems: in *in vivo* micronucleus tests in mice, the results were negative in oral administration, but positive in intraperitoneal injection. In an *in vitro* sister chromatid exchange (SCE) test with Chinese hamster ovary (CHO) cells, chlorobenzene showed positive results without metabolic activation and negative results with metabolic activation , and in an *in vivo* SCE test in mice, negative results are exhibited. As summarized above, negative results were obtained in the majority of genotoxicity tests of chlorobenzene, with some positive results. The overall evaluation of the available data indicates that chlorobenzene is not genotoxic.

With regard to the carcinogenicity of chlorobenzene, tumor incidence was not increased in male and female mice by 103-week oral administration of cholorobenzene. After 103-week oral administration to male and female rats, incidences of neoplastic nodules in the liver of males in the treated groups were increased, but those of hepatocarcinoma were not increased. Therefore, chlorobenzene has no carcinogenicity to mice and rats. The carcinogenicity of chlorobenzene has not been evaluated by the

IARC.

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1. Identification of the substance

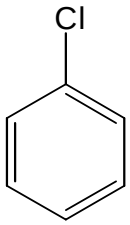
1.1 Chemical name : Chlorobenzene

1.2 Class reference number in Chemical Substance Control Law[1)] : 3-31

1.3 PRTR[2)] number (Law for PRTR and Promotion of Chemical Management) : 1-93

1.4 CAS registry number : 108-90-7

1.5 Structural formula



1.6 Molecular formula : C_6H_5Cl

1.7 Molecular weight : 112.56

2. General Information

2.1 Synonyms

Phenyl chloride, Monochlorobenzene, Benzene chloride

2.2 Purity

>99% (Commercial products) (CERI/Japan, 2002)

2.3 Impurities

Unknown

2.4 Additives/Stabilizers

No additives and stabilizers (Commercial products) (CERI/Japan, 2002)

2.5 Current regulations in Japan[3)]

Law for PRTR and Promotion of Chemical Management:	Class-I designated chemical substance
Fire Service Law:	Dangerous goods class IV second oil division
Labor Standards Law:	A chemical substance resulting in the illness
Industrial Safety and Health Law:	Dangerous substance, Inflammable substance, Second-class organic solvent (more than 5wt%), Harmful substance whose name is to be indicated

[1)] The Low Concerning the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc., Japan. Provisional translation is available on Internet at: http://www.safe.nite.go.jp/english/kasinn/kaiseikasinhou.html

[2)] Pollutant Release and Transfer Register

[3)] As this document covers basic information on Japanese regulations (unofficial translations), you should confirm the details using it.

	(more than 5wt%), Hazardous substance to be notified in terms of whose name, Administrative Control Level 10 ppm
Law Relating to the Prevention of Marine Pollution and Maritime Disasters:	Noxious liquid substance category B
Ship Safety Law:	Flammable liquid
Civil Aeronautics Law:	Flammable liquid
Port Regulation Law:	Flammable liquid

3. Physico-chemical properties

Appearance:	Colorless liquid	(U.S.NLM:HSDB, 2003)
Melting point:	-45°C	(Merck, 2001)
Boiling point:	131-132°C	(Merck, 2001)
Flash point:	27°C (closed-cup)	(IPCS, 1998)
	29°C (closed-cup)	(NFPA, 2002)
Ignition point :	590°C	(IPCS, 1998)
	593°C	(NFPA, 2002)
Explosion limit :	1.3-11 vol % (in air)	(IPCS, 1998)
	1.3-9.6 vol % (in air)	(NFPA, 2002)
Specific gravity:	1.107 (20°C/4°C)	(Merck, 2001)
Vapor density:	3.88 (air = 1)	
Vapor pressure:	1.2 kPa (20°C), 2.0 kPa (30°C), 5.3 kPa (50°C)	(Verschueren, 2001)
Partition coefficient:	log Kow (*n*-octanol/water) = 2.84 (measured), 2.64 (estimated)	(SRC:KowWin, 2003)
Dissociation constant :	No functional groups capable of dissociation.	
Mass spectrum:	Main mass fragments	
	m/z 112 (base peak = 1.0), 77 (0.45), 114 (0.33)	(NIST, 1998)
Soil adsorption coefficient:	Koc = 270 (estimated)	(SRC:PcKocWin, 2003)
Solubility:	Water solubility: 500 mg/L (20°C)	(Verschueren, 2001)
	Freely soluble in alcohols, benzene, chloroform and ethers.	(Merck, 2001)
Henry's constant:	315 $Pa \cdot m^3/mol$ (3.11×10^{-3} $atm \cdot m^3/mol$) (25°C, measured)	(SRC:HenryWin, 2003)
Conversion factor:	(air, 20°C) 1 ppm = 4.68 mg/m^3, 1 mg/m^3 = 0.214 ppm	

4. Sources of release to the environment

4.1 Production, import and domestic supply

The production and import of chlorobenzene in Fiscal Year 2001 ranged from 10,000 to 100,000 tons (METI/Japan, 2003).

Domestic supplies of chlorobenzene for 5 years from 1998 to 2002 in Japan are shown in Table 4-1. The domestic supply has been decreasing over the years with an increase in withdrawals of the domestic chlorobenzene manufacturers from the market (The Chemical Daily, 2001 and 2002).

Table 4-1 Domestic supply of chlorobenzene (tons)

Year	1998	1999	2000	2001	2002
Domestic supply	35,000	30,000	30,000	25,000	10,000

(NITE/Japan, 2004)

4.2 Uses

The estimated use pattern of chlorobenzene is shown in Table 4-2 (NITE/Japan, 2004). Chlorobenzene is mainly used as raw material for the synthesis of chemicals including triphenylphosphine (catalyst for organic synthesis), phenylsilane, and thiophenol (pesticide and pharmaceutical intermediate). It is also used as raw material for the synthesis of solvent for organic synthesis reactions including methylenediphenyldiisocyanate, urethane raw material, agricultural adjuvants, paint and ink, and cleaning solvent for electronics. The domestic market of chlorobenzene has been changing along with the decrease of domestic supply (NITE/Japan, 2003 and 2004). Chlorobenzene was previously used as raw material for the synthesis of *o*- and *p*-nitrochlorobenzene and 2,4-dinitrochlorobenzene. All *p*-nitrochlorobenzene has been imported since 2001 (The Chemical Daily, 2003).

Table 4-2 Estimated use patterns

Uses	Ratio (%)
Raw material for organic synthesis	75
Solvent for organic synthesis reactions	20
Solvent (paint, ink and others)	5
Total	100

(NITE/Japan, 2004)

4.3 Releases

4.3.1 Releases under PRTR system

According to the "Total Release and Transfers for Fiscal Year 2001 (hereafter 2001 PRTR Data)" under the PRTR system (METI/Japan and MOE/Japan, 2003a), 420 tons of chlorobenzene was released into air, 26 tons into public water, 545 kg into sewers and 1,390 tons was transferred as wastes from the business institutions required to report their releases and transfers. No chlorobenzene was reported to have been released into the land. In addition, it is estimated that 53 tons of chlorobenzene was released from the business institutions in the industries designated under the PRTR system but were exempted from notification, and 44 tons from the industries outside the scope of the PRTR system. No estimation was made for the amounts of release from households and those from mobile sources.

a. Release and transfer from the industries within the scope of PRTR system

The amounts of releases into the environmental media (air, water and land) and transfer by the industries designated under the 2001 PRTR system are shown in Table 4-3. METI/Japan and MOE/Japan (2003a)

do not provide the amounts of release by environmental media for the estimations of releases from the business institutions exempted from notification. The ratio for each environmental medium of the releases estimated for the business institutions exempted from notification was calculated based on the assumption that the ratios of releases into air, water and land were the same as those obtained by notification (NITE/Japan, 2004).

Table 4-3 Releases and transfer of chlorobenzene to environmental media by industries (tons/year)

Industries	By Notification					Notification Exempted			Total amount of releases by notification and by estimation	
	Release			Transfer		Release (estimated)[1)]				
	Air	Water	Land	Sewer	Wastes	Air	water	Land	Total release[3)]	Ratio (%)
Chemical and allied products	337	26	0	1	1,021	49	3	0	415	83
Plastic products	43	0	0	0	2	-	-	-	43	9
General machinery	31	0	0	0	366	0	0	0	31	6
Other Industries	5	0	0	0	1	-	-	-	5	1
Transportation equipment	4	0	0	0	<0.5	0	0	0	4	1
Publishing, printing and allied industries	-	-	-	-	-	1	<0.5	0	1	0
Pulp, paper and paper products	0	0	0	0	0	<0.5	<0.5	0	<0.5	0
Leather tanning, leather products and fur skins	-	-	-	-	-	<0.5	<0.5	0	<0.5	0
Advanced educational organizations	-	-	-	-	-	<0.5	<0.5	0	<0.5	0
Others[2)]	<0.5	0	0	0	<0.5	<0.5	<0.5	0	<0.5	0
Total [3)]	420	26	0	1	1,390	50	3	0	499	100

(NITE/Japan, 2004)

1) Based on the assumption that ratios of releases into the air, water and land were the same as those of the releases obtained by notification, the amounts of releases from the business institutions exempted from notification were estimated.

2) "Others" indicates the industries other than those shown above.

3) The total may not correspond with the sum of rounded values in each column of the table.

-: Not notified or estimated

"<0.5" indicates less than 0.5 tons.

Based on the production volume and the emission factor at manufacturing sites of chlorobenzene in 2001 (Japan Chemical Industry Association, 2002), the amount of release into the air is estimated to be 2 tons per

year, and no releases into water or land (NITE/Japan, 2004). Therefore, the releases of chlorobenzene are considered to occur mostly not during the manufacturing process but in use.

b. Releases from the non-designated industries, households, and mobile sources

Based on the 2001 PRTR Data, the amount of release as agricultural adjuvants into the environment from the non-designated industries is estimated to be 44 tons (METI/Japan and MOE/Japan, 2003b). METI/Japan and MOE/Japan (2003a) do not provide the amounts of release by environmental media for the estimation of release. It is assumed that 44 tons of chlorobenzene was released into air considering its use and physico-chemical properties (NITE/Japan, 2004). The amounts of chlorobenzene release from households and those from mobile sources are outside the scope of estimation required under PRTR (METI/Japan and MOE/Japan, 2003b).

4.3.2 Releases from other sources

Generation in landfill sites is one of the possible sources of chlorobenzene other than those included in the 2001 PRTR data. The National Institute for Environmental Studies (NIES/Japan) estimated that 0.01 to 700 g of chlorobenzene is released from gas drainage pipes in 6 landfill sites into the air per year but concluded that the amounts of release from landfill sites are extremely small compared to those from other sources (NIES/Japan, 1999).

4.4 Estimated routes of releases

Judging from the use information that chlorobenzene is used mainly as raw material for synthesis and based on the 2001 PRTR Data, the main release route is assumed to be through emissions in the use process of chlorobenzene and products including chlorobenzene. Releases from landfill sites are not considered.

As the scenario of chlorobenzene releases in Japan, it is estimated that 514 tons of chlorobenzene is released annually into the air, and 29 tons into water. Releases into the environment after processing of waste at waste disposal facilities are not considered for estimation of the amount transferred as waste and that transferred into sewers.

5. Environmental fate

5.1 Stability in the atmosphere

a. Reaction with OH radical

The reaction rate constant of chlorobenzene with OH radical is 7.70×10^{-13} cm^3/molecule-sec (25°C, measured value) in tropospheric air (SRC: AopWin, 2003). Assuming an OH radical level of 5×10^5 to 1×10^6 molecule /cm^3, the half-life is calculated to be 10 to 20 days.

b. Reaction with ozone

The reaction rate constant of chlorobenzene with ozone is not more than 5.00×10^{-21} cm^3/molecule-sec (25°C, measured value) in tropospheric air (SRC: AopWin, 2003). Assuming an ozone level of 7×10^{11}

molecule /cm^3, the half-life is calculated to be 6 years or longer.

c. Reaction with nitrate radical

No reports were obtained on the reaction of chlorobenzene with nitrate radical in this investigation.

d. Direct degradation by sunlight

Chlorobenzene absorbs light at and above 290 nm. Therefore, chlorobenzene is degraded directly by light in air. Monochlorobiphenyl has been identified as a photoproduct of chlorobenzene (U.S. NLM:HSDB, 2003).

5.2 Stability in water

5.2.1 Abiotic degradation

It is reported that the half-life of photolysis for chlorobenzene in distilled water was 17.5 hours. Since chlorobenzene has no chemical bonds that are subject to hydrolysis (US. NLM:HSDB, 2003), it is not hydrolyzed in the aquatic environment.

5.2.2 Biodegradation

Chlorobenzene is ranked as a persistent substance based on the result of the aerobic biodegradation study using an improved culture flask for volatile materials which is required under the Chemical Substance Control Law, Japan. The study result indicated that the degradation rate of chlorobenzene was 0% in biological oxygen demand (BOD) determination under the conditions of 30 mg/L of test substance concentration, 100 mg/L of activated sludge concentration and 4 weeks of test period. The degradation rate determined by ultraviolet (UV) absorption spectrometry was 5% (MITI/Japan, 1976).

In an aerobic biodegradation study using domestic wastewater as the source of microorganism, 5 and 10 mg/L of chlorobenzene were biodegraded by 89% and 30%, respectively, for 7 days, and finally to 100% by conditioned continuous subcultures (Tabak et al., 1981). Another aerobic biodegradation study was conducted with a sewage settling tank model, using an aerated flow-through system, in which 3 mg/L of chlorobenzene was continuously added to the sewage tank at a rate of 2 L/day with artificial sewage water including milk powder at 23°C for 40 days. It was reported that 63% of chlorobenzene was biodegraded, 29.9% was emitted, 0.2% was adsorbed on algae, etc., 1.4% remained in water, and 5.5% was flowed out (Davis et al., 1983a, b).

In an anaerobic biodegradation study with digestion sludge in methane fermentation, 8.1 to 72 μg/L of chlorobenzene was not degraded at all for 12-week incubation period (Rittmann et al., 1980). In another study with a biofilm column in anaerobic methane fermentation, 0% to 15% of chlorobenzene (concentration: 22 μg/L) was eliminated after 2-day retention in the column (Bouwer, 1985). It is reported in an anaerobic biodegradation study with digestion sludge of a sewage facility treating urban wastewater and industrial wastewater that chlorobenzene (initial concentration: 78 mg/L) was not degraded at 30°C for 60 days (Battersby and Wilson, 1989).

These results suggest that chlorobenzene is biodegraded at low concentrations in specific aerobic conditions associated with acclimazation, but not in anaerobic conditions.

5.2.3 Removal in sewage treatment

No reports were obtained on chlorobenzene removal in sewage treatment in this investigation.

5.3 Behavior in the aquatic environment

Chlorobenzene has a solubility of 500 mg/L (20°C), and its vapor pressure is high (1.2 kPa at 20°C) and Henry's constant is large (315 $Pa \cdot m^3/mol$ at 25°C) (see Chapter 3). Regarding the volatilization of chlorobenzene from water into air using Henry's constant, the half-life in a model river (water depth: 1 m; flow velocity: 1 m/sec; wind velocity: 3 m/sec) was estimated to be 3.3 hours, and that in a model lake (water depth: 1 m; flow velocity: 0.05 m/sec; wind velocity: 0.5 m/sec) to be 4.3 days (Lyman et al., 1990). The half-lives of chlorobenzene in river water and sediment were 150 and 75 days, respectively (Lee and Ryan, 1979). Considering a soil adsorption coefficient, Koc, of 270 (see Chapter 3), it is assumed that chlorobenzene is adsorbed to suspended solids in water and sediment to some extent. Therefore, chlorobenzene is assumed to be easily released from the aquatic environment into the air.

Based on the information summarized here and in Section 5.2, it is assumed that chlorobenzene released into the aquatic environment is eliminated mainly by emission, and almost none by biodegradation.

5.4 Bioaccumulation

Chlorobenzene is ranked as a non- or low bioaccumulative substance based on the result of a 6-week bioaccumulation study using carp under the provisions of the Chemical Substance Control Law, Japan. The study result indicated that the bioconcentratrion factors of chlorobenzene were 4.3 to 40 and 3.9 to 23 at 0.15 and 0.015 mg/L of chlorobenzene concentration in water, respectively (MITI/Japan, 1976).

6. Effects on organisms in the environment

6.1 Effects on aquatic organisms

6.1.1 Microorganisms

Toxicity data of chlorobenzene to microorganisms are shown in Table 6-1.

In bacteria, the 16-hr hazardous threshold (EC_3) in growth inhibition of *Pseudomonas* was 17 mg/L and the 8-day hazardous threshold (EC_3) in growth inhibition of blue-green bacteria was 120 mg/L (Bringmann and Kuhn, 1976, 1977a, 1978). In protozoa, it has been reported that the 72-hr toxic threshold (EC_5) in growth inhibition of flagellata *Entosiphon sulcatum* was over 390 mg/L (Bringmann, 1978).

Table 6-1 Toxicity of chlorobenzene to microorganisms

Species	Temperature (°C)	Endpoint		Concentration (mg/L)	Reference
Bacteria *Pseudomonas putida* (Pseudomonas)	25	16-hr toxic threshold [1)]	Growth inhibition	17 (n)	Bringmann & Kuhn, 1976, 1977a
Microcystis aeruginosa (blue green bacteria)	27	8-day toxic threshold [1)]	Growth inhibition	120 (n)	Bringmann & Kuhn, 1976, 1978
Protozoa *Entosiphon sulcatum* (flagellata)	25	72-hr toxic threshold [2)]	Growth inhibition	>390 (n)	Bringmann, 1978
Chilomonas paramaecium (flagellata)	20	48-hr toxic threshold [2)]	Growth inhibition	>196 (n)	Bringmann et al, 1980
Uronema parduczi (ciliata)	25	20-hr toxic threshold [2)]	Growth inhibition	>390 (n)	Bringmann & Kuhn, 1980

(n): Nominal concentration

1) Concentration giving 3% effect compared to the control (EC_3)

2) Concentration giving 5% effect compared to the control (EC_5)

6.1.2 Algae and aquatic plants

Toxicity data of chlorobenzene to algae are shown in Table 6-2.

Of growth inhibition studies, studies with closed systems considering volatility of chlorobenzene and estimation of results with measured concentrations were referred to as reliable studies. The values of the toxicity were 12.5 mg/L (Calamari et al., 1983; Galassi and Vighi, 1981) and 224 to 232 mg/L (U.S. EPA, 1980) as the 96-hr EC_{50} in the freshwater green alga *Selenastrum capricornutum* and 341 mg/L (U.S. EPA, 1980) as the 96-hr EC_{50} in the marine diatom *Skeletonema costatum*. The values of the toxicity were 56.6 mg/L obtained as the 3-hr EC_{50} in photosynthesis inhibition of *Chlamydomonas angulosa* (Hutchinson et al., 1980) and 50 mg/L as the 4-hr EC_{50} for *Ankistrodesmus falcatus* (Wong et al., 1984).

The NOECs for growth inhibition of freshwater alga *Selenastrum* and duckweed *Lemna* and marine diatom *Skeletonema* were obtained, but reliability of those results is low because volatility of chlorobenzene was not considered for the toxicity studies.

Table 6-2 Toxicity of chlorobenzene to algae and aquatic plants

Species	Method/ Condition	Temperature (°C)	Endpoint		Concentration (mg/L)	Reference
Freshwater species						
Selenastrum capricornutum [1)] (green alga)	Static, closed	ND	96-hr EC_{50}	Growth inhibition chlorophyll *a*	232 (n)	U.S. EPA, 1980
			96-hr EC_{50}	Growth inhibition	224 (n)	
	Static, closed	ND	96-hr EC_{50} 3-hr EC_{50}	Growth inhibition Photosynthesis inhibition	12.5 (n) 33.0 (n)	Calamari et al., 1983
	Static	20	96-hr EC_{50}	Growth inhibition	12.5 (m)	Galassi & Vighi, 1981
	ND	ND	24-hr EC_{50}	Growth inhibition	298	U.S. EPA,

Species	Method/ Condition	Temperature (°C)	Endpoint		Concentration (mg/L)	Reference
			48-hr EC_{50}	chlorophyll	239	1978
			96-hr EC_{50}		210	
			96-hr NOEC		<100	
			96-hr EC_{50}	Photosynthesis inhibition	343 (n)	
	ND	ND	96-hr EC_{50}	Growth inhibition	202 (n)	U.S. EPA, 1978
Scenedesmus quadricauda (green alga)	Static, closed	27	8-day toxic threshold [2)]	Growth inhibition	>390 (n)	Bringmann & Kuhn, 1977a, 1978
Chiorella vulgaris (green alga)	Static, closed	19	3-hr EC_{50}	Photosynthesis inhibition	99.1 (n)	Hutchinson et al., 1980
Chlamydomonas angulosa (green alga)	Static, closed	19	3-hr EC_{50}	Photosynthesis inhibition	56.6 (n)	
Ankistrodesmus falcatus (green alga)	Static, closed	20	4-hr EC_{50}	Photosynthesis inhibition	50 (n)	Wong et al., 1984
Lemna gibba (G-3) (duckweed)	U.S. EPA, Static	25± 0.7	7-day EC_{50} 7-day NOEC	Growth inhibition frond number	581 294 (n)	Cowgill et al., 1991
Lemna minor (7136) (duckweed)	U.S. EPA, Static	25± 0.7	7-day EC_{50} 7-day NOEC	Growth inhibition frond number	353 294 (n)	
Marine species						
Skeletonema costatum (diatom)	Static, closed	ND	96-hr EC_{50}	Growth inhibition	341 (n)	U.S. EPA, 1980
			96-hr EC_{50}	Photosynthesis inhibition	343 (n)	
	Static pH 7.7-9.0	19.9-20.6	5-day EC_{50} 5-day NOEC	Growth inhibition	203 100 (n)	Cowgill et al., 1989
			5-day EC_{50} 5-day NOEC	Growth inhibition biomass	201 100 (n)	

ND: No data available, (n): Nominal concentration, (m): Measured concentration

Closed system: a test container and water bath is closed with a cover such as a lid, but a headspace is kept.

1) Current scientific name: *Pseudokirchneriella subcapitata*

2) Concentration giving 3% effect compared to the control (EC_3)

6.1.3 Invertebrates

Toxicity data of chlorobenzene to invertebrates are shown in Table 6-3.

The acute toxicity of chlorobenzene to the freshwater crustacea, water fleas (*Daphnia magna* and *Ceriodaphnia dubia*), and lavae of insecta, *Chironomus* species (Chironomidae) has been reported. The data were obtained from reliable studies that were conducted in closed/sealed systems considering volatility of chlorobenzene or estimation of results with measured concentrations. Consequently, the values of acute

toxicity were 0.59 to 26.0 mg/L for the 24- to 48-hr EC_{50} (immobilization) (Bobra et al., 1985; Calamari et al., 1983; Hermens et al., 1984; Rose et al., 1998) and 5.8 to 86 mg/L for the 48-hr LC_{50} (Abernathy et al., 1986; LeBlanc, 1980) in water fleas. The 96 to 98-hr NOEC in the bloodworm was 0.72 mg/L (van der Zandt et al., 1994). The acute toxicity of chlorobenzene to marine crustacea, mysid shrimp and fleshy prawn, has been reported, and the 96-hr LC_{50} for the fleshy prawn was 1.72 mg/L (Yin and Lu, 1993).

Long-term toxicity in the water fleas, *Daphnia magna* and *Ceriodaphnia dubia*, has been reported and the lowest value is 0.32 mg/L in *Daphnia magna* as the 16-day NOEC for reproduction (Hermens et al., 1984).

Table 6-3 Toxicity of chlorobenzene to invertebrates

Species	Growth Stage	Method/ Condition	Temper-ature (°C)	Hardness (mg $CaCO_3$/L)	pH	Endpoint	Concent-ration (mg/L)	Reference
Acute toxicity: freshwater species								
Daphnia magna (crustacea, water flea)	<24hr	Static, closed	22	72	6.7-8.1	24-hr LC_{50} 48-hr LC_{50} 48-hr NOEC Death	140 86 10 (n)	LeBlanc, 1980
	ND	AFNOR[1)] Static sealed	ND	ND	ND	24-hr EC_{50} Immobilization	4.3 (m)	Calamari et al., 1983
	4-6-days	Static, sealed	23±2	ND	ND	48-hr LC_{50}	5.8 (n)	Abernathy et al., 1986
	Larva 1.5 mm	Static, sealed	ND	ND	6-7	48-hr EC_{50} Immobilization	0.59 (n)	Bobra et al., 1985
	Larva	Static	19.8-20.9	157	7.7-9.9	24-hr LC_{50}	13.9-14.2	Gersich et al., 1986
						48-hr LC_{50}	10.7-15.4 (n)	
	<12 hours	Static, feed	25±2	160-180	8.2±0.2	48-hr LC_{50}	31 (n)	Cowgill & Milazzo, 1991
	Larva	Static	20.2-20.9	ND	8.00-8.60	48-hr LC_{50}	10.7- 15.4 (n)	Cowgill et al., 1985
			24.1-24.8	ND	8.15-8.50	48-hr LC_{50}	8.60- 21.3 (n)	
	<48 hours	Static	20-22	70	7.6-7.7	24-hr LC_{50}	310 (n)	Bringmann & Kuhn, 1977b
	ND	ND	ND	ND	7	24-hr EC_{50} Immobilization	16 (m)	Bazin et al., 1987
	<48hr	NEN[2)] Static, sealed	22±1	Approx. 100	ND	48-hr EC_{50} Immobilization	26.0 (m)	Hermens et al., 1984
Ceriodaphnia dubia (crustacea)	<48 hours	U.S. EPA, Static, sealed vehicle [3)]	25	65.2	7.7	48-hr EC_{50} Immobilization	5.3 (m)	Rose et al., 1998
	<24 hours	Static	25	45.5	7.6	24-hr LC_{50}	7.6 (m)	Marchini et al., 1993

Species	Growth Stage	Method/ Condition	Temper-ature (°C)	Hardness (mg $CaCO_3$/L)	pH	Endpoint	Concent-ration (mg/L)	Reference
	Larva	Static	20.4-20.9 24.1-24.7	ND	8.05-8.66 8.20-8.58	48-hr LC_{50}	7.9-11.4 10.4-11.8 (n)	Cowgill et al., 1985
	<12 hours	Static, feeding	25±2	90-110	8.2±0.2	48-hr LC_{50}	47 (n)	Cowgill & Milazzo, 1991
Chironomus thummi (insecta, chironomidae)	4th instar larva	Semi-static	19	150	8	96 to 98-hr NOEC	0.72 (n)	van der Zandt et al., 1994
Acute toxicity: marine species								
Americamysis bahia (crustacea, mysid)	ND	ND	ND	ND	ND	96-hr LC_{50}	16.4 (n)	U.S. EPA, 1978
Penaeus chinensis (crustacea, fleshy prawn)	ND	ND	23-27	ND	7.53-8.95	96-hr LC_{50}	1.72 (n)	Yin & Lu, 1993
Long-term toxicity: freshwater species								
Daphnia magna (crustacea, water flea)	<24 hours	NEN[2] Semi-static	19±1	Approximately 100	ND	16-day LC_{50} 16-day NOEC Death 16-day EC_{50} 16-day NOEC Reproduction	4.0 1.0 1.1 0.32 (m)	Hermens et al., 1984
	Approx. 12 hours	Semi-static, sealed	20	ND	ND	14-day EC_{50} Reproduction	2.5 (m)	Calamari et al.,1983
	<12 hours	Semi-static	25±2	160-180	8.2±0.2	10-day LC_{50} 9 to 11-day EC_{50} 9 to 11-day NOEC Reproduction	16 15 6.5 (n)	Cowgill & Milazzo, 1991
Ceriodaphnia dubia (crustacea, water flea)	<12 hours	Semi-static	25±2	90-110	8.2±0.2	7-day LC_{50} 7 to 10-day EC_{50} 7 to 10-day NOEC Reproduction	24 14 12 (n)	

ND: No data available, (n): Nominal concentration, (m): Measured concentration
Closed system: a test container and water bath is closed with a cover such as a lid, but a headspace is kept.
Sealed: a test container is filled up to the top edge with test solution without headspace.
1) Test guideline by the Association francaise de normalization
2) Test guideline by the Netherlands Normalistie Institute
3) Acetone

6.1.4 Fish

Toxicity data of chlorobenzene to fish are shown in Table 6-4.

Results of acute toxicity to fish were obtained from reliable studies that were conducted in a closed system in flow-through/semi-static conditions or those with results estimated from determination concentrations. In freshwater fish, the 96-hr LC_{50} values were 4.7 mg/L for rainbow trout (Dalich et al., 1982), 7.4 mg/L for bluegill (Bailey et al., 1985) and 7.7 mg/L for fathead minnow (Marchini et al., 1993). In marine fish, the 96-hr LC_{50} for sheepshead minnow was 6.2 mg/L (Heitmuller et al., 1981). However, the effect of volatilization was not considered in this study.

Long-term toxicity to fertilized eggs at an early life stage has been reported. The 28-day NOEC with endpoints of death, hatching success and growth was 4.8 mg/L for zebra fish (van Leeuwen et al., 1990) and the 27-day LC_{50} was 0.11 mg/L for rainbow trout (Black and Birge, 1982). In the latter, the LC_{10} was reported to be 0.0361 mg/L. However, this value is considered to be inaccurate in this assessment, because a geometric ration of concentration in the concentration region around LC_{10} was larger than that in other regions. In other studies of toxicity at the early life stage from fertilization to 4 days posthatch, it was reported that the 8-day LC_{50} was 0.88 to 1.04 mg/L for goldfish and that the 7.5-day LC_{50} was 0.05 to 0.06 mg/L for largemouth bass (Birge et al., 1979).

Table 6-4 Toxicity of chlorobenzene to fish

Species	Growth stage	Method/ Condition	Temperature (°C)	Hardness (mg $CaCO_3$/L)	pH	Endpoint	Concentration (mg/L)	Reference
Acute toxicity: freshwater species								
Oncorhynchus mykiss (rainbow trout)	ND	Static, closed	15	320	7.4	48-hr LC_{50}	4.1 (m)	Calamari et al., 1983
	ND	Flow-Through	15	ND	ND	96-hr LC_{50}	4.7 (m)	Dalich et al., 1982
	4.6-6.4 cm 1.2-3.8 g	Flow-through	14.1-16.5	ND	7.60-8.19	96-hr LC_{50}	7.46 (m)	Hodson et al., 1984
Lepomis macrochirus (bluegill)	Fry 0.32-1.2 g	Static vehicle [1)]	21-23	32-48	6.7-7.8	24-hr LC_{50} 96-hr LC_{50}	17 16 (n)	Buccafusco et al., 1981
	3.8-6.4 cm 1-2 g	APHA[2)] Static	25	20	7.5	24-hr LC_{50} 48-hr LC_{50} 96-hr LC_{50}	24 24 24 (n)	Pickering & Henderson, 1966
	Fry 3.65 cm 0.90 g	Static	22±1	31.2	6-9	24-hr LC_{50} 48-hr LC_{50} 72-hr LC_{50} 96-hr LC_{50}	4.5 4.5 4.5 4.5 (m)	Bailey et al., 1985
		Flow-through			6-8	24-hr LC_{50} 48-hr LC_{50} 72-hr LC_{50} 96-hr LC_{50}	8.0 7.7 7.4 7.4 (m)	
Danio rerio (zebra fish)	ND	Static, closed	23	320	7.4	48-hr LC_{50}	10.5 (m)	Calamari et al., 1983

Species	Growth stage	Method/ Condi-tion	Temp-erature (°C)	Hardness (mg $CaCO_3$/L)	pH	Endpoint	Concen-tration (mg/L)	Reference
Poecilia reticulata (guppy)	6-months 1.9-2.5 cm 0.1-0.2 g	APHA[2)] Static	25	20	7.5	24-hr LC_{50}	45.53	Pickering & Henderson, 1966
						48-hr LC_{50}	45.53	
						96-hr LC_{50}	45.53 (n)	
	ND	ND	ND	ND	ND	24-hr LC_{50}	5.63 (n)	Benoit-Guyod et al., 1984
Pimephales promelas (fathead minnow)	31-days 1.78 cm 0.083 g	Flow-through	25.7	43.8	7.5	96-hr LC_{50}	16.9 (m)	Geiger et al., 1990
	3.8-6.4 cm 1-2 g	APHA[2)] Static	25	20	7.5	24-hr LC_{50}	29.12	Pickering & Henderson, 1966
						48-hr LC_{50}	29.12	
						96-hr LC_{50}	29.12 (n)	
			25	20	7.5	24-hr LC_{50}	33.93	
						48-hr LC_{50}	33.93	
						96-hr LC_{50}	33.93 (n)	
			25	360	8.2	24-hr LC_{50}	39.19	
						48-hr LC_{50}	34.98	
						96-hr LC_{50}	33.93 (n)	
	Fry <24 hours after hatch	Flow through	25	45.5	7.6	96-hr LC_{50}	7.7 (m)	Marchini et al., 1993
	Fry 10-15 days 9.5 mm 11.6 mg	Static	21-23	96-125	7.2-8.5	96-hr LC_{50}	22.3 (n)	Mayes et al., 1983
	Fry 30-35 days 14.9 mm 76.8 mg	Static	21-23	96-125	7.2-8.5	96-hr LC_{50}	35.4 (n)	Mayes et al., 1983
	Immature fish 65-94 days 28 mm 391 mg	Static	21-23	96-125	7.2-8.5	96-hr LC_{50}	22.2 (n)	Mayes et al., 1983
Carassius auratus (goldfish)	3.8-6.4 cm 1-2 g	APHA[2)] Static	25	20	7.5	24-hr LC_{50}	73.03	Pickering & Henderson, 1966
						48-hr LC_{50}	56.00	
						96-hr LC_{50}	51.62 (n)	
Acute toxicity: marine species								
Cyprinodon variegatus (sheepshead minnow)	14-28 days 8-15 mm	U.S. EPA, Static	25-31	Salinity: 10-31	ND	24-hr LC_{50}	>20	Heitmuller et al., 1981
						48-hr LC_{50}	8.9	
						96-hr LC_{50}	6.2 (n)	

Species	Growth stage	Method/ Condition	Temperature (°C)	Hardness (mg $CaCO_3$/L)	pH	Endpoint	Concentration (mg/L)	Reference
Platichthys flesus (European flounder)	56.2 g	Semi-static, closed, aeration vehicle [3]	6	Salinity: 5‰	ND	96-hr LC_{50}	6.6 (a, n)	Furay & Smith, 1995
Solea solea (Dover sole)	45.0 g	Semi-static, closed, aeration vehicle [3]	6	Salinity: 22‰	ND	96-hr LC_{50}	5.8 (a, n)	
Long-term toxicity: freshwater species								
Danio rerio (zebra fish)	Fertile egg	Semi-static	24±2	210	7.4-8.4	28-day LC_{50} 28-day LOEC 28-day NOEC Death, hatching success and growth	10.3 8.5 4.8 (m)	Van Leeuwen et al., 1990
Oncorhynchus mykiss (rainbow trout)	Egg within 0.5 hours after fertilization	Flow-through, closed	14.3± 0.2	103.6±1.2	7.8 ±0.02	23-day LC_{50} (0-day posthatch)	0.11	Black & Birge, 1982
						27-day LC_{50} 27-day LC_{10} (4-day posthatch)	0.11 0.0361 (m)	
Carassius auratus (goldfish)	Egg within 1-2 hours after laying	Flow-through, closed	18.2-25.8	51.2	7.6	4-day LC_{50} (0-day posthatch)	3.48	Birge et al., 1979
						8-day LC_{50} (4-day posthatch)	0.88 (m)	
				203.4		4-day LC_{50} (0-day posthatch)	2.37	
						8-day LC_{50} (4-day posthatch)	1.04 (m)	
Micropterus salmoides (largemouth bass)	Egg within 1-2 hours after laying	Flow-through, closed	18.2-25.8	51.2	7.6	3.5-day LC_{50} (0-day posthatch)	0.34	Birge et al., 1979
						7.5-day LC_{50} (4-day posthatch)	0.05	
				203.4		3.5-day LC_{50} (0-day posthatch)	0.39	
						7.5-day LC_{50} (4-day posthatch)	0.06 (m)	

ND: No data available, (n): Nominal concentration, (m): Measured concentration

Closed system: a test container and water bath is closed with a cover such as a lid, but a headspace is kept.

1) The type of adjuvant is unknown.

2) Test guideline by the American Public Health Association

3) Acetone

6.1.5 Other aquatic organisms

Toxicity data of chlorobenzene to other aquatic organisms are shown in Table 6-5.

In toxicity studies for amphibian vertebrates exposed to chlorobenzene for embyo to larval stages, leopard frogs were exposed from 5 days before hatching to 0-day or 4-days posthatch. The LC_{50} values for exposure periods of 5 and 9 days were 1.53 and 1.20 mg/L, respectively. Northwestern salamanders were exposed from 5.5 days before hatching to 0-day or 4-days posthatch..The LC_{50} values for exposure period of 5.5-day and 9.5-days were 1.65 and 1.15 mg/L, respectively (Black and Birge, 1982).

Table 6-5 Toxicity of chlorobenzene for other aquatic organisms

Species	Growth stage	Method/ Condition	Temp-erature (°C)	Hardness (mg $CaCO_3$/L)	pH	Endpoint	Concent-ration (mg/L)	Reference
Rana pipiens (leopard frog)	Egg within 0.5 hours after fertiliza-tion	Flow-through, closed	20.2± 0.5	98.8±0.7	7.8± 0.02	5-day LC_{50} (0-day posthatch) 9-day LC_{50} (4-day posthatch)	1.53 1.20 (m)	Black & Birge, 1982
Arabystoma gracile (Northwestern salamander)	Egg within 0.5 hours after fertiliza-tion	Flow-through, closed	20.2± 0.5	98.8±0.7	7.8± 0.02	5.5-day LC_{50} (0-day posthatch) 9.5-day LC_{50} (4-day posthatch)	1.65 1.15 (m)	Black & Birge, 1982

(m): Measured concentration

Closed system: a test container and water bath is closed with a cover such as a lid, but a headspace is kept.

6.2 Effects on terrestrial organisms

6.2.1 Microorganisms

No reports on toxicity of chlorobenzene to microorganisms were obtained in this investigation.

6.2.2 Plants

Toxicity data of chlorobenzene to plants are shown in Table 6-6.

In studies of growth and death observation with lettuce in chlorobenzene-treated soil and nutrient solution, the 7-day NOEC of growth was 1 mg/kg dry soil and the 16-day NOEC of growth was 3.2 mg/mL nutrient solution (Adema and Henzen, 2001; Hulzebos et al., 1993).

Table 6-6 Toxicity of chlorobenzene for plants

Species	Method/Condition	Endpoint		Concentration	Reference
Lactuca sativa L (dicotyledon)	Soil test: Addition to soil (clay: 12%, organic component: 1.4%, pH: 7.5, humidity: 25-30%)	7-day EC_{50} 7-day NOEC	Growth	(mg/kg dry clay) >1000 1	Adema & Henzen, 2001; Hulzebos et al., 1993
		14-day EC_{50} 14-day NOEC	Growth	>1000 3.2	
		14-day NOEC	Death	≧1 (n)	
	Hydroponic culture: Addition to nutrient solution Exchange frequency of test nutrient solution 3 times/week	16-day EC_{50} 16-day NOEC	Growth	(mg/mL) 9.3 3.2	
		16-day EC_{50} 16-day NOEC	Growth	14 3.2	
		16-day NOEC	Death	100 (n)	

(n): Nominal concentration

6.2.3 Animals

Toxicity data of chlorobenzene to animals are shown in Table 6-7.

In a contact test, the 48-hr LC_{50} for manure worms exposed to chlorobenzene-treated filter-paper was 0.029 mg/cm^2 (Neuhauser et al., 1985). Studies with different soils in red tigers (*Eisenia andrei*) and red marsh worms (*Lumbricus rubellus*) have also been reported. The 2-week LC_{50}s for red tigers and red marsh worms exposed to chlorobenzene-treated wild soil were 240 and 547 mg/kg dry soil, respectively (van Gestel et al., 1991).

Table 6-7 Toxicity of chlorobenzene for animals

Species	Method/Condition	Endpoint	Concentration	Reference
Eisenia fetida (red tiger)	Contact test to filter paper	48-hr LC_{50}	0.02 mg/cm^2 (n)	Neuhauser et al., 1985
Eisenia andrei (red tiger)	Natural soil in the field environment pH 4.8	2-week LC_{50}	240 mg/kg dry soil (n)	van Gestel et al., 1991
	Artificial soil (OECD) pH 5.9		446 mg/kg dry soil (n)	
Lumbricus rubellus (red marsh worm)	Natural soil in the field environment pH 4.8	2-week LC_{50}	547 mg/kg dry soil (n)	
	Artificial soil (OECD) pH 5.9		1,107 mg/kg dry soil (n)	

(n): Nominal concentration

6.3 Summary of effects on organisms in the environment

Many studies have been conducted to assess the hazardous effects of chlorobenzene on organisms in the environment using indices including mortality, immobilization and growth inhibition.

In microorganisms, the 16-hr toxic threshold (EC_3) in growth inhibition of *Pseudomonas putida* was 17 mg/L.

In algae growth inhibition studies, values of acute toxicity to algae differed approximately 10 fold. The lowest value for algae is 12.5 mg/L of 96-hr EC_{50} in the freshwater green alga *Selenastrum capricornutum*. The acute toxicity of chlorobenzene to invertebrates is reported in the crustacean water flea. The 48-hr EC_{50} (immobilization) was 0.59 mg/L. Long-term toxicity in water fleas has been reported, and the lowest value is 0.32 mg/L in the water flea *Daphnia magna* as the 16-day NOEC for reproduction.

The acute toxicity of chlorobenzene to fish is reported in the rainbow trout. The 96-hr LC_{50} was 4.7 mg/L. Long-term toxicity to fish at the early life stage has been reported. The 27-day LC_{50} was 0.11 mg/L for the rainbow trout exposed from fertilization to 4-days posthatch, the 8-day LC_{50} in the goldfish is reported to be 0.88 to 1.04 mg/L, and the 7.5-day LC_{50} in the largemouth bass is reported to be 0.05 to 0.06 mg/L.

In toxicity studies for amphibian vertebrates, leopard frogs were exposed from 5 days before hatching to 0-day or 4-days posthatch. The LC_{50} values for exposure periods of 5 and 9 days were 1.53 and 1.20 mg/L, respectively.

In terrestrial organisms, studies with lettuce in chlorobenzene-treated soil and nutrient solution were conducted, and the 7- and 16-day NOEC for growth were 1 mg/kg dry soil and 3.2 mg/mL nutrient solution, respectively. In addition, the 48-hr LC_{50} for manure worms exposed to chlorobenzene-treated filter-paper was 0.029 mg/cm^2.

The long-term NOECs in crustacea and fish are 0.32 and 0.05 mg/L, respectively.
The lowest value of toxicity in aquatic organisms is 0.05 mg/L of the 7.5-day LC_{50} for the largemouth bass at the early life stage.

Although formal classification criteria is not used in this investigation, it can be considered that the acute toxicity values of chlorobenzene to aquatic organisms is corresponding to the GHS acute toxicity hazard category I (very toxic).

7. Effects on human health

7.1 Kinetics and metabolism

Studies on kinetics and metabolism of chlorobenzene are summarized in Table 7-1, and metabolic pathway of chlorobenzene is shown in Figure 7-1.

a. Absorption

Chlorobenzene is absorbed mainly through the gastrointestinal tract (Ogata and Shimada, 1983; Smith et

al., 1972) and the respiratory tract (Ogata and Shimada, 1983; Sullivan et al., 1983). Dermal absorption is estimated to be low, as slight toxicity was found in rats applied to the skin at high doses of chlorobenzene (Kinkead and Leahy, 1987; Oettel et al., 1936).

b. Distribution

In experimental animals, chlorobenzene was accumulated mainly in the adipose tissue and some in the liver and other organs. As chlorobenzene is lipophilic, its distribution in the organisms depends on the lipid distribution in their organs (Shimada 1988; Sullivan et al., 1983, 1985).

c. Metabolism/Excretion

In oral, inhalation, dermal and intraperitoneal studies of chlorobenzene in various mammals (humans, rhesus monkeys, capuchin monkeys, rats, mice, guinea pigs, dogs, rabbits, cats, gerbils and hedgehogs), ten metabolites listed below were detected in the urine (Azouz et al., 1953; Baumann, 1883; Gessner and Smith, 1960; Hele, 1924; Jaffe, 1879; Jerina et al., 1967; Knight and Young, 1958; Nishimura, 1929; Ogata and Shimada, 1983; Shimada, 1981; Smith et al., 1950; Smith et al., 1972; Spencer and Williams, 1950a, b; Sullivan et al., 1983, 1985; Yoshida and Hara, 1985b; Yoshida et al., 1986):

1) 4-chlorophenyl-mercapturic acid
2) 4-chlorocatechol and 4-chlorophenol and their glucuronoconjugates and sulfoconjugates
3) 2- and 3-chlorophenols and their glucuronoconjugates and sulfoconjugates (trace amount)
4) chlorocatechols
5) 2-chloroquinol
6) monophenols
7) 2- and 3-chlorophenyl-mercapturic acids
8) quinol
9) 3,4-dihydro-3,4-dihydroxychlorobenzene
10) chlorophenyl sulfides.

The first phase of chlorobenzene metabolism, regardless of administration route, animal species or *in vivo/in vitro* system, is oxidization by the cytochrome P450 system (Brandt and Brittebo, 1983; Brittebo and Brandt, 1984). Chlorobenzene-3,4-epoxide (Brodie et al., 1971; Kerger et al., 1988; Selander et al., 1975; Smith et al., 1972) and a small amount of chlorobenzene-2,3-epoxide (Lau and Zannoni, 1979; Selander et al., 1975) and 3-chlorophenol (Selander et al., 1975) are formed through oxidization.

Both epoxides of chlorobenzene-3,4-epoxide and chlorobenzene-2,3-epoxide are reported to bind covalently to nucleic acids of DNA and RNA and to proteins in a nonspecific manner in liver and lung (Brodie et al., 1971; Grilli et al., 1985; Jergil et al., 1982; Prodi et al., 1986; Reid and Krishna, 1973; Reid et al., 1973a; Tunek et al., 1979). Similarly, these epoxides are formed in the kidney and adrenal

cortex other than the liver and lung (Brandt and Brittebo, 1983; Brittebo and Brandt, 1984; Dalich and Larson, 1985; Grilli et al., 1985; Jergil et al., 1982; Prodi et al., 1986; Reid, 1973; Reid and Krishna, 1973; Reid et al., 1973b; Selander et al., 1975; Tunek et al., 1979).

In the second phase, epoxides, which are related to the toxicity of chlorobenzene, are enzymatically converted into water-soluble mercapturic acid derivatives by glutathione *S*-transferase (Brodie et al., 1971; Chadwick et al., 1984; Zampaglione et al., 1973) or chlorocatechols via 3,4-dihydro-dihydroxy chlorobenzene by epoxide hydratase (Billings, 1985; Chadwick et al., 1984; Oesch et al., 1973).

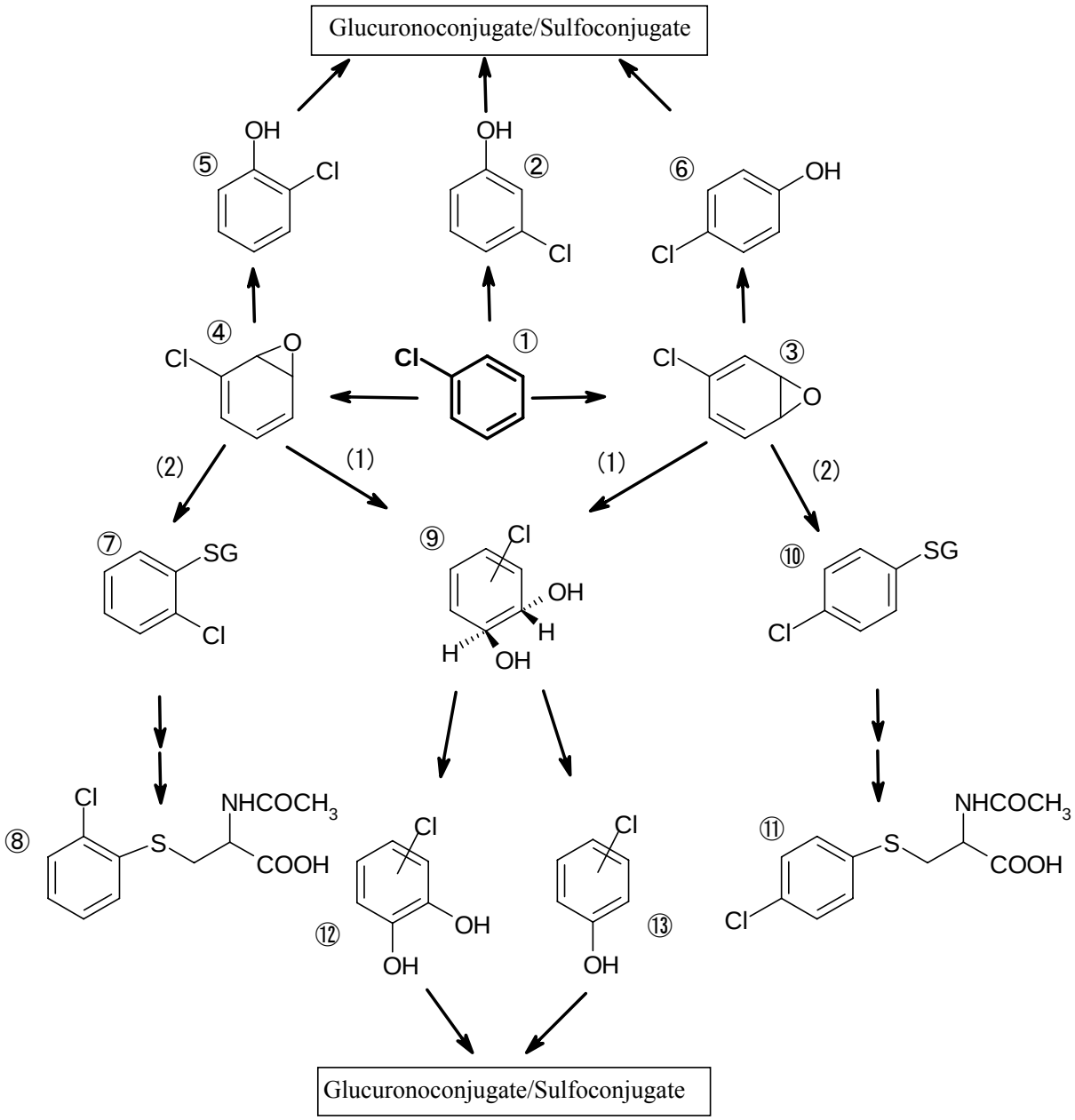


Figure 7-1 Metabolic pathway of chlorobenzene

(GDCh BUA, 1990, Recasting)

1) : Chlorobenzene
2) : 3-Chlorophenol
9) : 3,4-Dihydro-3,4-dihydroxychlorobenzenes
10) : 4-Chlorophenylglutathion (conjugate)

3) : Chlorobenzene-3,4-epoxide
4) : Chlorobenzene-2,3-epoxide
5) : 2-Chlorophenol
6) : 4-Chlorophenol
7) : 2-Chlorophenylglutathione (conjugate)
8) : 2-Chlorophenyl-mercapturic acid
11) : 4-Chlorophenyl-mercapturic acid
12) : Chlorocatechols
13) : Chlorophenols
(1) : 5-Epoxide hydratase
(2) : Glutathione *S*-transferase

Without the involvement of enzymes, chlorophenol is formed from epoxides by intramolecular rearrangement (Selander et al., 1975). Most chlorophenols and chlorocatechols are metabolized and excreted in the urine as highly water-soluble glucuronoconjugates and sulfoconjugates (Spencer and Williams, 1950a,b), and some poorly water-soluble metabolites of chlorophenols and chlorocatechols are also excreted in the urine (Spencer and Williams, 1950a).

In an oral study of chlorobenzene in rabbits, chlorobenzene was excreted as metabolites mainly in the urine and slightly in the feces. Unmetabolized chlorobenzene was detected in the expired air (Smith et al., 1972).

The percentages of metabolites that were excreted in the urine in 24 hours after administration in humans and experimental animals are shown in Table 7-2. Some species differences in content of metabolites in the urine were observed : for example, the content of 4-chlorophenyl-mercapturic acid was lower in humans, guinea pigs and rabbits than those in other species such as monkey, dog, rat, mice and hamster (Ogata and Shimada, 1983; Williams et al., 1975).

In an inhalation study, ^{14}C-chlorobenzene was exposed to rats at doses of 100, 400 and 700 ppm (469, 1,871 and 3,235 mg/m^3) for 8 hour/day once or 5 times (5 days). Dose-dependent increases of radioactivity were measured in all tissues of blood, liver, kidney, lung and adipose tissue around the epididymis. Especially in the adipose tissue, radioactivity was rapidly increased at more than 400 ppm, suggesting that most of the detected radioactivity was unmetabolized chlorobenzene. Furthermore, in the groups of 400 ppm and above, the content of unchanged chlorobenzene in the urine was increased, while the content of mercapturic acid was decreased. These results suggest that metabolism of chlorobenzene may be saturated at 400 ppm and above (Sullivan et al., 1983, 1985).

In a single 8 hours inhalation exposure study of chlorobenzene in rats, the half-life of the early phase of expiration ranged from 0.8 to 1.1 h in the 100 to 400 ppm groups without any significant differences; however, it was 3.7 h in the 700-ppm group. In repeated-dose and high-dose studies, the excretion ratio of unchanged chlorobenzene into expiration was higher than that in a single-dose study, and the excretion ratios of mercapturic acid conjugate, 4-chlorophenol, 4-chlorophenol sulfoconjugate and 4-chlorophenol glucuronoconjugate were decreased (Chadwick et al., 1984; Sullivan et al., 1983, 1985). These results show that metabolism of chlorobenzene is saturated at repeated and high doses.

Table7-1 Kinetic and Metabolism of Chlorobenzene

Species/ sex/number of animals	Route	Dose	Results	Reference
Mouse 4 animals/ group	Inhalation	100, 300, 500 ppm (469, 1,407, 2,345 mg/m^3) Exposure for 3 hours at 100 ppm; 1 hour at 300, 500 ppm	500 ppm: Concentrations in organs/tissues after 1-hour exposure: intra-abdominal adipose tissue > liver > kidney > blood > heart > brain Half-life in organs: intra-abdominal adipose tissue > brain > liver > spleen > kidney > blood	Shimada, 1988
Rabbit 4 animals	Oral gavage 0.5 g/twice/day 4 days	[U-^{14}C]chlorobenzene (purity: 99%)	Absorption: Mainly through the gastrointestinal tract Metabolism: Metabolite by the cytochrome P-450 system Main : chlorobenzene-3,4-epoxide Minor : chlorobenzene-2,3-epoxide The following metabolites detected in the urine (radioactivity ratio (%)) 3,4-dihydro-3,4-dihydroxy chlorobenzenes: 0.6 Monophenols: 2.8 Dinophenols : 4.17 Mercapturic acids: 23.8 Sulfoconjugates: 33.9 Glucuronoconjugates: 33.6 Excretion: Chlorobenzene metabolites in the urine >> the feces. Unmetabolized chlorobenzene detected in the expired air	Smith et al., 1972
Rat SD Male 15 animals/ group	Inhalation Exposure period: Up to 5 days 8 hours/day	[U-^{14}C]chlorobenzene 100, 400 and 700 ppm (469, 1,871 and 3,275 mg/m^3)	Distribution: Dose-dependent increases of ^{14}C-radioactivity in blood, liver, kidney, lung and adipose tissue around the epididymis. Especially, rapid increase of radioactivity in the adipose tissue beyond 400 ppm Excretion: Mainly in the urine and slightly in the feces. Unmetabolized chlorobenzene in the exhaled air. Half-life of chlorobenzene in expiration: Rapid phase: 100-400 ppm: 0.8-1.1 hr (no clear difference); 700 ppm: 3.7 hr Slower phase: 100 ppm: 9 hr; 700 ppm: 6 hr Repeated exposure (compared with those at a single exposure): Increase in content of unchanged chlorobenzene in the expired air, derease of mercapturic acid conjugates, 4-chlorophenol, 4-chlorophenol sulfoconjugate and 4-chlorophenol In the urine It was assumed that chlorobenzene metabolism is saturated at 400 ppm and above (8-h exposure). At repeated exposure, metabolism was enhanced more than at a single exposure.	Sullivan et al., 1983

Species/ sex/number of animals	Route	Dose	Results	Reference
Mouse BALB/c Male Rat Wistar Male	Intraperitoneal	[U-^{14}C]chlorobenzene (purity: >98%) 0.714 mg/kg	Metabolism: *In vivo* covalent binding with DNA,RNA, proteins in the liver, kidney and lung in mice and rats In specific activity (pmol/mg), Proteins>RNA>DNA	Grilli et al., 1985; Prodi et al., 1986
Rat SD Male	Intraperitoneal Single injection	[^{14}C]chlorobenzene 255, 552, 1,103 and 1,655 mg/kg	Excretion: Dose-dependent decrease in radioactivity in the urine collected within 24 hours: Dose Recovery (%) 255 mg/kg: 59% 1,655 mg/kg: 19%	Dalich & Larson, 1985
Rat Long-Evans	*in vitro* study Liver microsome	[^{14}C]chlorobenzene 3 µmol/2 mL Incubated with liver microsome (1-20 mg/mL), 37°C, 30 min	Formation of 2- and 4-chlorophenols from chlorobenzene-2,3-epoxide and chlorobenzene- 3,4-epoxide, respectively, by intramolecular rearrangement (nonenzymatic reaction) Formation of 3-chlorophenol from chlorobenzene by enzymatic reaction	Selander et al., 1975
Rat SD Female 5 animals/ group (30 animals in total)	Oral gavage Administration period: 7 days/week 8 days	^{14}C chlorobenzene (purity: 96%) 300 mg/kg/day	Metabolism: Reaction of epoxides into mercapturic acid derivatives (water-soluble) by glutathione *S*-transferase and excreted in the urine. Reaction of epoxides into chlorocatechols via dihydro-dihydroxy chlorobenzene by epoxide hydratase. At repeated and high dose exposures, increase in unchanged chlorobenzene in the expired air, decreases in mercapturic acid conjugates, 4-chlorophenol, 4-chlorophenol sulfoconjugate and 4-chlorophenol glucuronoconjugate	Chadwick et al., 1984
Rat SD 2 males	Intraperitoneal	[U-^{14}C]chlorobenzene 1,126 mg/kg	Metabolism: Reaction of epoxides into chlorocatechols via dihydro-dihydroxy chlorobenzene Excretion: Major metabolites in the urine: glucuronoconjugates of chlorophenols and chlorocatechols	Oesch et al., 1973
Rabbit	ND	ND	Excretion: Major metabolites in the urine: glucuronoconjugates of chlorophenols and chlorocatechols (water-soluble). Minor metabolites in the urine: chlorophenols and chlorocatechols (poorly water-soluble)	Spencer & Williams, 1950a

Species/ sex/number of animals	Route	Dose	Results	Reference
Rabbit Chinchilla	Oral gavage (forced)	150 mg/kg	Excretion: Major metabolites in the urine: glucuronoconjugates of chlorophenols and chlorocatechols, sulfoconjugates of mercapturic acid (water-soluble conjugates) Ratio of content of glucuronoconjugate, sulfoconjugate and mercapturic acid conjugate in the urine: 25:27:20	Spencer & Williams, 1950b
Rat Wistar	Oral gavage	33.8 mg/kg	Metabolism: *p*-Chlorophenyl-mercapturic acid (MA) and 4-chlorocatechol (CC) detected in the urine of humans and rats Content ratio of MA to CC in the urine (MA/CC): rat: 2.85, human: 0.002 Species difference in metabolism was found between rats and humans.	Ogata & Shimada, 1983
Humans Male Volunteer	Ingestion	33.8 mg/kg 3 times		
Mouse C57B1 2-6 animals/	Intravenous Intraperitoneal	[U-^{14}C]chlorobenzene (purity: 98%) 1.2 mg/kg (i.v.) 1.7 mg/kg (i.p.) Removed organs: nasal mucosa, lung, liver	Distribution: Non-volatile binding of [^{14}C]chlorobenzene to the subepithelial glands (Bowman's glands) underneath the olfactory epithelium, olfactory epithelium in the nose, tracheo-bronchial mucosa, liver, cortex of kidney and adrenal cortex *in vivo*	Brandt & Brittebo, 1983; Brittebo et al., 1984
B6C3F$_1$ Mouse	*In vitro* study Liver microsome	ND	Metabolism: Oxidization of chlorobenzene into chlorobenzene-3,4-epoxide and a small amount of chlorobenzene-2,3-epoxide by cytochrome P-450.	Kerger et al., 1988
Rabbit 6 animals	Oral gavage (forced)	12 g/animal	Excretion: Metabolites detected in the urine: Glucuronoconjugate of 4-chlorocatechol Sulfoconjugate of 4-chlorocatechol 4-chlorophenyl mercapturicacid	Smith et al., 1950
Rat	Dermal	225 mg/kg	Metabolites detected in the urine: *p*-chlorophenyl-mercapturic acid, 4-chlorocatechol	Shimada, 1981
Rat Rabbit Cat Ferret	Oral gavage (cat and ferret: capsule; others: gastric tube)	[^{14}C]chlorobenzene 255, 552, 1,103 and 1,655 mg/kg	Metabolites of phenol compounds (4-chlorocatechol,2-chloroquinol and chlorophenol) detected in the urine.	Gessner & Smith, 1960
Rat Wistar Male	Intraperitoneal	56, 233 mg/kg	Chlorophenyl methylsulfides (volatile) were detected in the urine.	Yoshida & Hara, 1985b

ND : No data available

Table 7-2 Rate (%) of main metabolites[1)] of chlorobenzene detected in urine[2)]

species	4-chlorophenyl-mercapturic acid	4-chlorocatechol	4-chlorophenol
Humans	19	31	33
Rhesus monkeys	40	37	19
Dogs	42	45	13
Rats	49	22	23
Mice	42	31	20
Hamsters	43	23	15
Guinea pigs	21	35	27
Rabbits	26	38	19

1) calculated as ^{14}C-labeled compound. 4-Chlorocatechol and 4-chlorophenol were assumed to be excreted in the urine as glucronoconjugetes or sulfonoconjugetes.
2) collected within 24 hours after treatment.

7.2 Epidemiological studies and case reports

No reports of epidemiological studies of chlorobenzene were obtained in this investigation.

General symptoms of acute toxicity caused by occupational exposure in humans are exhaustion, nausea and lethargy (Henschler, 1972-1987). The minimum concentration that caused slight irritation to the human eye and nasal mucosa was 200 ppm (936 mg/m^3) and the odor threshold was 60 ppm (281 mg/m^3) (Henschler, 1972-1987).

It is reported that a chemical plant worker aged 60 years, who had been handling DDT for 30 years, and subsequently handled chlorobenzene, *o*-dichlorobenzene and trichlorobenzene for 3 years, showed slight anemia. (Girard et al., 1969). This worker was simultaneously exposed to chemical substances other than chlorobenzene, of which the amounts of exposure were not reported

7.3 Studies in experimental animals and *in vitro* studies

7.3.1 Acute toxicity

A summarized acute toxicity data of chlorobenzene to experimental animals is shown in Table 7-3.

In oral administration, the LD_{50} values were 1,445 mg/kg in mice and 1,427 to 3,400 mg/kg in rats, and in 6-hour inhalation exposure, the LC_{50}s were 1,889 ppm (8,822 mg/m^3) in mice and 2,968 ppm (13,870 mg/m^3) in rats.

The symptoms observed in the oral administration and inhalation exposure of chlorobenzene were body weight loss, sanguineous lacrimation, unkempt integument, hypertonia, tremor, twitch, hyposthesia, somnolence, narcosis, ataxia, hyposthenia of hind limb and dyspnea (Bonnet et al., 1982; Gotzmann, 1931; Loser, 1982a,b; U.S. NTP, 1985).

In rats injected intraperitoneally with chlorobenzene, degeneration and necrosis were observed in the hepatocytes (Dalich and Larson, 1985). Following oral administration at a lethal dose, no abnormality was observed at autopsy (Loser, 1982a, b).

Table 7-3 Acute toxicity of chlorobenzene

Route	Mouse	Rat	Rabbit	Guinea pig
Oral LD_{50} (mg/ kg)	1,445	1,427-3,400	2,250-2,830	5,060
Inhalation LC_{50} (ppm, (mg/ m^3))	1,889 (8,822) (6 hours)	2,968 (13,870) (6 hours)	ND	ND
Dermal LD_{50} (mg/ kg)	ND	ND	ND	ND
Intraperitoneal LD_{50} (mg/kg)	1,355	570-1,655	ND	ND

ND : No data available

7.3.2 Irritation and corrosion

Studies on irritation and corrosion of chlorobenzene to experimenal animals are summarized in Table 7-4.

In a skin irritation test of chlorobenzene for rabbits according to the OECD test guideline, moderate irritation was observed (Suberg, 1983a, b).

In a study of local application to skin of rabbit under occlusive and non-occlusive conditons, slight reddening of the skin was observed. Dermal application of chlorobenzene for one week, moderate erythema and slight necrosis in the epidermis were found (Irish, 1962).

In a test in which chlorobenzene was applied to the eye of rabbit according to the OECD test guideline, no irritation was found (Suberg, 1983a,b). After application to the eye, conjunctivitis dissappeared within 48 hours, and no corneal damage was observed (Irish, 1962).

Table 7-4 Irritation and corrosion of chlorobenzene

Species/ sex/number of animals	Test method Guidelines	Period	Results	Reference
Rabbit	Skin irritation test OECD: 404	ND	Moderate irritation	Suberg, 1983a, b
Rabbit	Skin irritation test occulusive application	ND	Slight reddening of the skin	Irish, 1962
	non-occulusive application	Continuously 1 week	Moderate erythema and slight necrosis of the epidemis	
Rabbit	Eye irritation test OECD: 405	ND	No irritation	Suberg, 1983a, b
Rabbit	Eye irritation test	ND	Recovery of conjunctivitis within 48 hours after application, no corneal damage)	Irish, 1962

ND: No data available

7.3.3 Sensitization

In a skin sensitization study using the maximization method for guinea pigs, no sensitization was reported to be found (Mihail, 1984), but the details are unknown. No reliable data on sensitization were obtained in this investigation.

7.3.4 Repeated dose toxicity

Studies on repeated dose toxicity of chlorobenzene to experimental animals are summarized in Table 7-5.

a. Oral administration

Chlorobenzene was orally administered by gavage to male and female B6C3F$_1$ mice at doses of 0, 30, 60, 125, 250 and 500 mg/kg/day for 5 days/week, for 14 days. In male mice, suppression of the body weight gain was observed at 30 mg/kg/day and above. In female mice, an increase in body weight was found at 250 mg/kg/day and above. At autopsy, no abnormality was observed at all doses (Kluwe et al., 1985;U.S. NTP,1985).

Male and female B6C3F$_1$ mice were orally administered chlorobenzene by gavage at doses of 0, 60, 125, 250, 500 and 750 mg/kg/day for 5 days/week, for 13 weeks. In male mice, suppression of the body weight gain, a decrease in the spleen weight and necrosis in the hepatocytes were observed at 60 mg/kg/day and above. Increases in the mortality, the urine volume and the kidney weight (slight), vacuolar degeneration and coagulative necrosis in the proximal renal tubule, necrosis or defeciency of thymus lymphocytes and defeciency of the spleen lymphocytes and the myelocytes at 250 mg/kg/day and above. In female mice at 250 mg/kg/day and above, an increase in the mortality, increases in the urine volume, the urinary porphyrin excretion and liver and kidney weight (slight), and a decrease in the spleen weight were observed. Histopathologically, vacuolar degeneration and coagulative necrosis in the proximal renal tubule were observed at 250 mg/kg/day, degeneration and necrosis in the liver, necrosis or defeciency of thymus lymphocytes, defeciency of the spleen lymphocytes and the myelocytes and decrease in the bone marrow myelocytes were found at 250 mg/kg/day and above. All female mice died at 750 mg/kg/day (Kluwe et al., 1985;U.S. NTP,1985). Based on the suppression of the body weight gain, decrease in the heart weight, and degeneration and necrosis of the hepatocytes observed in the male mice, the LOAEL of this study is considered to be 60 mg/kg/day in this assessment.

Oral (gavage) administration of chlorobenzene to female rats was carried out at 0, 250 mg/kg/day for 3 days. Increases in the relative liver weight, the hepatic phospholipids and the activity of δ-aminolevulinic acid synthetase (δALS) and decreases in cytochrome P450, the aminopyrine demethylase and aniline hydroxylase activities were found (Ariyoshi et al., 1975).

Oral (gavage) administration of chlorobenzene to male rats at 0, 1,140 mg/kg/day for 5 days caused body weight loss, increase in the urinary porphyrin excretion and histopathological changes in the liver and (Rimington and Ziegler, 1963).

Male and female F344 rats were administered chlorobenzene at doses of 0, 125, 250, 500, 1,000 and 2,000 mg/kg/day for 14 days. Iincreases in body weight gain in male rats at 125 mg/kg/day and above, and suppression of body weight gain in the female rats were observed. At autopsy, however, no changes were found. At 1,000 mg/kg/day and above, reduced responses to the external stimulation were observed in male and female mice, and all of them wasted and died (Kluwe et al., 1985; U.S. NTP,1985).

In male rats administered orally by gavage at doses of 0, 200, 400 and 800 mg/kg/day, increases in

glucuronoconjugate activity was observed at 200 mg/kg/day and above. Suppressed weight gain and a decrease in hepatic cytochrome P450 activity were observed at 800 mg/kg/day (Carlson and Tardiff, 1976).

Following 14-day oral (gavage) administration to rats at 0, 12.5, 50 and 250 mg/kg/day, increases in liver and kidney weight were found at 50 mg/kg/day and above. Suppression of body weight gain was found at 250 mg/kg/day. However, no histopathological changes were observed at either dose (Knapp et al., 1971).

A 13-week oral (gavage) study in male and female F344 rats at doses of 0, 60, 125, 250, 500 and 750 mg/kg/day for 5 days/week was carried out. In the male rats, a decrease in spleen weight was observed at 60 mg/kg/day and above, an increase in the liver weight at 125 mg/kg/day and above, suppression of body weight gain, degeneration/necrosis in the hepatocytes and the proximal renal tubule at 250 mg/kg/day and above, death, a decrease in the bone marrow myelocytes and increases in total porphyrin in the liver and urinary porphyrin excretion at 500 mg/kg/day and above, and decreases in thymus and the spleen lymphocytes and an increase in urine volume at 750 mg/kg/day. In the females, an increase in liver weight was observed at 125 mg/kg/day and above, degeneration and necrosis in the hepatocytes and the proximal renal tubule at 250 mg/kg/day and above, death, suppression of body weight gain, an increase in kidney weight, a decrease in bone marrow myelocytes, increases in total porphyrin in the liver, urinary porphyrin excretionand γ-glutamyl transpeptidase (γ-GTP) and alkaline phosphatase (ALP) activities at 500 mg/kg/day and above, and decreases in thymus and spleen lymphocytes, an increase in urine volume and a decrease in the leukocytes at 750 mg/kg/day (Kluwe et al., 1985; U.S. NTP,1985). From the result of a decrease in spleen weight in the male rats observed at 60 mg/kg/day, the LOAEL of this study is considered to be 60 mg/kg/day in this assessment.

In dogs orally administered by gavage chlorobenze at doses of 27, 55 and 273 mg/kg/day for 5 days/week, for 93 days, an increase in blood immature leukocytes, a decrease in blood glucose, increases in serum alanine aminotransferase (ALT) and ALP activities, increases in total bilirubin and cholesterol, and gross and histopathological changes in the liver, kidney, stomach and gastrointestinal mucosa (details unknown) were observed at 273 mg/kg/day (Knapp et al., 1971).

b. Inhalation exposure

Male and female mice (strain unknown) were exposed to chlorobenzene by inhalation at 0, 535 ppm (2,500 mg/m^3) for 7 hours/day, for 3 weeks. Drowsiness, suppression of body weight gain, decreases in food consumption and neutrophil ratio were observed in the treatment group. After exposure at 0, 21 ppm (100 mg/m^3) for 7 hours/day, for 3 months, decreases in exciting symptoms and neutrophil ratio were found (Zub, 1978).

Inhalation exposure of chlorobenzene to male and female SD rats was carried out at doses of 0, 50, 150 and 450 ppm (0, 234, 702 and 2,106 mg/m^3) for 6 hours/day, 7 days/week, from 10 weeks before mating to the completion of lactation: females were not exposed from gestation day 20 to lactation day 4. No effects on body weight, food consumption or death were found in the male and female parent rats of all groups. An increase in liver weight in males and females, hypertrophy of the centrilobular hepatocytes and renal

tubular dilation and interstitial nephritis in males were observed at 150 ppm and above. Degeneration of the seminiferous epithelium in males was observed at 450 ppm (Nair et al., 1987). From these results, the NOAEL of this study is considered to be 50 ppm (234 mg/m^3) in this assessment.

Male SD rats were exposed to chlorobenzene at doses of 0, 75 and 250 ppm for 7 hours/day, for 5, 11 and 24 weeks. For 5-week exposure, a decrease in food consumption was observed at 75 ppm and above, and an increase in kidney weight, decreases in serum aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities were found at 250 ppm. For 11-week exposure, increases in hematocrit and platelet count and decreases in reticulocytes, and increases in food consumption and liver weight, vacuolated adrenal reticular cells, degeneration of renal tubules were observed at 75 ppm and above. Decreases in the leukocyte and monocyte ratio, an increase in the neutrophil ratio and a decrease in serum AST activity were found at 250 ppm. For 24-week exposure, an increase in food consumption was observed at 75 ppm and above, and increases in liver and kidney weights, decreases in the reticulocytes and serum AST activity were found at 250 ppm (Dilley, 1977; Dilley and Lewis, 1978).

Male rabbits were exposed to chlorobenzene at 0, 75 and 250 ppm for 7 hours/day, 5 days/week, for 24 weeks. After 5 weeks, the following results were found: an increase in LDH activity at 75 ppm, a decrease in serum uric acid at 75 ppm and above, and liver and kidney congestion and an increase in the leukocytes at 250 ppm. After 11 weeks, decreases in serum uric acid and AST activity were observed at 75 ppm and above. After 24 weeks, a decrease in serum LDH activity was observed at 75 ppm, and increases in lung and liver weight and neutrophil ratio and a decrease in serum AST activity were found at 250 ppm (Dilley, 1977; Dilley and Lewis, 1978).

From the results described above, oral administration of chlorobenzene to mice for 13 weeks caused suppression of body weight gain, a decrease in spleen weight and necrosis of hepatocyte at the lowest dose of 60 mg/kg/day. Inhalation exposure of chlorobenzene to rats from 10 weeks before mating to the completion of lactation resulted in an increase in liver weight in males and females, hypertrophy of the centrilobular hepatocytes and renal tubular dilation and interstitial nephritis in males at 150 ppm and above. Therefore, the LOAEL for oral administration is 60 mg/kg/day, and the NOAEL for inhalation exposure is 50 ppm (234 mg/m^3).

Table 7-5 Repeated dose toxicity of chlorobenzene

Species/ sex/number of animals	Route	Period	Dose	Results	Reference
Mouse B6C3F$_1$ Male and Female 5 animals/ group	Oral gavage	14 days 5 days /week	0, 30, 60, 125, 250, 500 mg/kg/day	30 mg/kg/day and above: Male: Suppression of body weight gain 60 mg/kg/day and above: Female: Increase in body weight gain No abnormality at autopsy at all doses	Kluwe et al., 1985; U.S. NTP, 1985

<table>
<tr><th>Species/ sex/number of animals</th><th>Route</th><th>Period</th><th>Dose</th><th>Results</th><th>Reference</th></tr>
<tr><td>Mouse
B6C3F$_1$
Male and Female
10 animals/ group</td><td>Oral gavage</td><td>13 weeks

5 days /week</td><td>0, 60, 125, 250, 500, 750 mg/kg/day</td><td>Male:
60 mg/kg/day and above: suppression of body weight gain, decrease in spleen weight, hepatocyte necrosis
250 mg/kg/day and above: Increases in mortality, urine volume, liver weight and kidney weight (slight), decrease in spleen weight (slight)
Histopathological changes:
degeneration/necrosis of the liver, vacuolar degeneration /coagulative necrosis in the proximal renal tubule, necrosis or defect of the thymus lymphocytes

Female:
250 mg/kg/day:
vacuolar degeneration/coagulative necrosis in the proximal renal tubule
250 mg/kg/day and above:
Increase in mortality, increases in urine volume, liver weight and kidney weight (slight), decrease in spleen weight (slight)
Histopathological changes:
degeneration/necrosis in the liver, necrosis or defect of the thymus lymphocytes, defects of spleen lymphocytes and myelocytes, decreased myelocytes of the bone marrow
750 mg/kg/day: death of all animals

LOAEL : 60 mg/kg/day (in this assessment)</td><td>Kluwe et al., 1985; U.S. NTP, 1985</td></tr>
<tr><td>Mouse
B6C3F$_1$
Male and Female
50 animals/ group</td><td>Oral gavage</td><td>103 weeks

5 days /week</td><td>Male: 0, 30, 60 mg/kg/day

Female: 0, 60, 120 mg/kg/day (Vehicle:corn oil)</td><td>30 mg/kg/day and above:
Male and female:
No abnormality in symptom, autopsy and histopathological observations
Male: increase in mortality

Mortality:
<table><tr><td>mg/kg/day</td><td>0</td><td>30</td><td>60</td></tr><tr><td>Male</td><td>11/50</td><td>20/48</td><td>20/49</td></tr><tr><td></td><td></td><td></td><td></td></tr><tr><td>mg/kg/day</td><td>0</td><td>60</td><td>120</td></tr><tr><td>Female</td><td>10/50</td><td>9/50</td><td>11/49</td></tr></table></td><td>Kluwe et al., 1985; U.S. NTP, 1985</td></tr>
<tr><td>Rat
Female</td><td>Oral gavage</td><td>3 days</td><td>0, 250 mg/kg/day</td><td>250 mg/kg/day:
Increases in relative liver weight, hepatic phospholipids and activity of δ-aminolevulinic acid synthetase (δALS) , decreases in cytochrome P450, aminopyrine demethylase and aniline hydroxylase activities</td><td>Ariyoshi et al., 1975</td></tr>
<tr><td>Rat
2 animals/ group
Male</td><td>Oral gavage</td><td>5 days</td><td>0, 1,140 mg/kg/day</td><td>1,140 mg/kg/day:
Body weight loss, histopathological changes in the liver (details unkown), increase in urinary porphyrin (copro-, proto-, uro-) excretion</td><td>Rimington & Ziegler, 1963</td></tr>
</table>

Species/ sex/number of animals	Route	Period	Dose	Results	Reference
Rat F344 Male and Female 5 animals/ group	Oral gavage	14 days 7 days /week	0, 125, 250, 500, 1,000, 2,000 mg/kg/day	125 mg/kg/day and above: Male: increased body weight gain Female: suppression of body weight gain No abnormality at autopsy 1,000 mg/kg/day and above: Prone status, reduced responses to external stimulation (after administration), waste, death of all animals	Kluwe et al., 1985; U.S. NTP, 1985
Rat Male 6 animals/ group	Oral gavage	14 days	0, 200, 400, 800 mg/kg/day	200 mg/kg/day and above: increase in glucuronoconjugates in the urine 800 mg/kg/day: suppression of body weight gain, decrease in hepatic cytochrome P450 activity	Carlson & Tardiff, 1976
Rat	Oral gavage	93 to 99 days 7 days /week	12.5, 50, 250 mg/kg/day	No histopathological changes at all doses 50 mg/kg/day and above: increases in liver and kidney weight 250 mg/kg/day: suppression of body weight gain	Knapp et al., 1971
Rat F344 Male and Female 10 animals/ group	Oral gavage	13 weeks 5 days /week	0, 60, 125, 250, 500, 750 mg/kg/day	Male: 60 mg/kg/day and above: decrease in spleen weight 125 mg/kg/day and above: increase in liver weight 250mg/kg/day and above: suppression of body weight gain, degeneration/necrosis in the hepatocytes and the proximal renal tubule 500 mg/kg/day and above: death (4/10), decrease in bone marrow myelocytes, increases in urinary porphyrin excretion and porphyrin in the liver 750 mg/kg/day: death (9/10), decreases in thymus and spleen lymphocytes Female: 125 mg/kg/day and above: increase in liver weight 250 mg/kg/day and above: degeneration/necrosis in the hepatocytes and the proximal renal tubule 500 mg/kg/day and above: death (3/10), suppression of body weight gain, increase in kidney weight, decrease in bone marrow myelocytes, increases in urinary porphyrin excretion, porphyrin in the liver, serum γ-GTP and alkaline phosphatase activities 750 mg/kg/day: death (2/10), decreases in thymus and spleen lymphocytes and leukocytes LOAEL : 60 mg/kg/day (in this assessment)	Kluwe et al., 1985; U.S. NTP, 1985

<table>
<tr><th>Species/ sex/number of animals</th><th>Route</th><th>Period</th><th>Dose</th><th>Results</th><th>Reference</th></tr>
<tr><td>Rat

Male and Female</td><td>Oral gavage</td><td>192 days

5 days /week (137 times)</td><td>14.4, 144, 288 mg/kg/day</td><td>144 mg/kg/day and above:
increases in liver and kidney weight, histopathological changes in the liver (details unknown)</td><td>Irish, 1962</td></tr>
<tr><td>Rat
F344
Male and Female
50 animals/ group
103 weeks</td><td>Oral gavage</td><td>103 weeks

5 days /week</td><td>0, 60, 120 mg/kg/day
(Vehicle: corn oil)</td><td>120 mg/kg/day:
Male: increase in mortality
No abnormality in symptom, autopsy and histopathological observations

Mortality:)<table><tr><td>mg/kg/day</td><td>0</td><td>60</td><td>120</td></tr><tr><td>Male</td><td>9/48</td><td>12/44</td><td>15/41</td></tr><tr><td>Female</td><td>13/42</td><td>11/50</td><td>12/43</td></tr></table></td><td>Kluwe et al., 1985; U.S. NTP, 1985</td></tr>
<tr><td>Dog
93 days</td><td>Oral gavage</td><td>93 days

5 days /week</td><td>0, 27, 55, 273 mg/kg/day</td><td>273 mg/kg/day:
increase in blood immature leukocytes, decrease in blood glucose, increases in serum ALT and ALP activities, total bilirubin and cholesterol changes in the liver, kidney, stomach and gastrointestinal mucosa at autopsy and histopathology (details unknown)</td><td>Knapp et al., 1971</td></tr>
<tr><td>Mouse
Male and Female
5 animals/ group</td><td>Inhala-tion</td><td>3 weeks

7 hours /day</td><td>0, 535 ppm
(2,500 mg/m^3)</td><td>535 ppm:
Drowsiness, suppression of body weight gain, decreases in food consumption and neutrophil ratio, hepatocellular fatty degeneration</td><td rowspan="2">U.S. NTP, 1985; Zub, 1978</td></tr>
<tr><td>Mouse
Male and Female
5 animals/ group</td><td>Inhala-tion</td><td>3 months

7 hours /day</td><td>0, 21 ppm
(100 mg/m^3)</td><td>21 ppm:
Exciting status, decrease in neutrophil ratio</td></tr>
<tr><td>Rat
SD
Male and Female
30 animals/ group
(See 7.3.5 Repro-ductive and develop-mental toxicity)</td><td>Inhala-tion</td><td>16 weeks

6 hours /day

7 days /week

10 weeks before mating to the comp-letion of lactation (females were not exposed) gestation day 20 to lactation day 4</td><td>0, 50, 150, 400 ppm
(0, 234, 702, 2,106 mg/m^3)

.</td><td>150 ppm and above:
increase in liver weight (males and females), hypertrophy of centrilobular hepatocytes (males), renal tubular dilation and interstitial nephritis (males)
450 ppm:
degeneration of the seminiferous epithelium

NOAEL : 50 ppm (234 mg/m^3) (in this assessment)</td><td>Nair et al., 1987</td></tr>
</table>

Species/ sex/number of animals	Route	Period	Dose	Results	Reference
Rat Male and Female	Inhala-tion	44 days (32 times) 7 hours /day 5 days /week	0, 200, 475, 1,000 ppm (0, 936, 2,223, 4,680 mg/m^3)	475 ppm and above: increase in liver weight, histopathological changes in the liver (details unknown) 1,000 ppm: suppression of body weight gain, histopathological changes in the lung and kidney (details unknown)	Irish, 1962
Rat SD Male 10 animals/ group	Inhala-tion	5 weeks 7 hours /day 5 days /week	0, 75, 250 ppm (0, 351, 1,170 mg/m^3)	75 ppm and above: decrease in food consumption 250 ppm: increase in kidney weight, decreases in serum AST and LDH activities	Dilley, 1977; Dilley & Lewis, 1978
Rat SD Male 10 animals/ group	Inhala-tion	11 weeks 7 hours /day 5 days /week	0, 75, 250 ppm (0, 351, 1,170 mg/m^3)	75 ppm and above: increases in food consumption and liver weight, vacuolated adrenal reticular cells, degeneration of renal cortical tubules, increases in hematocrit and platelet, decreases in reticulocytes and hematocrit 250 ppm: dcreases in leukocytes and monocyte ratio, increase in neutrophil ratio, decrease in serum AST activity	
Rat SD Male 10 animals/ group	Inhala-tion	24 weeks 7 hours /day 5 days /week	0, 75, 250 ppm (0, 351, 1,170 mg/m^3)	75 ppm and above: increase in food consumption 250 ppm: increases in liver and kidney weight, decrease in reticulocytes, decrease in serum AST activity	
Rabbit Male 10 animals/ group	Inhala-tion	5, 11 or 24 weeks 7 hours /day 5 days /week	0, 75, 250 ppm (0, 351, 1,170 mg/m^3)	<u>5-week exposure group:</u> 75 ppm and above: decrease in serum uric acid and increase in serum LDH activity (75-ppm only) 250 ppm: congestion of liver and kidney, increase in leukocytes <u>11-week exposure group:</u> 75 ppm and above: decreases in serum uric acid and AST activity <u>24-week exposure group:</u> 75 ppm: decrease in LDH activity 250 ppm: increases in lung and liver weight, increase in neutrophil ratio, decrease in serum AST activity	
Rabbit Male Guinea pigs	Inhala-tion	44 days (32 times) 7 hours /day 5 days /week	0, 200, 475, 1,000 ppm (936, 2,223, 4,678 mg/m^3)	475 ppm: increase in liver weight, histopathological changes in the liver (details unkown) 1,000 ppm: suppression of body weight gain, histopathological changes in the lung, liver and kidney (details unknown)	Irish, 1962

7.3.5 Reproductive and developmental toxicity

Studies on reproductive and developmental toxicity of chlorobenzene to experimental animals are summarized in Table 7-6.

In two-generaion study of reproductive toxicity, male and female SD rats were exposed to chlorobenzene by inhalation at doses of 0, 50, 150 and 450 ppm (0, 234, 702 and 2,105 mg/m^3) for 6 hours/day, 7 days/week from 10 weeks before mating to the completion of lactation (F_0 females were not exposed from gestation day 20 to lactation day 4) for F_0 and from 11 weeks before mating to the completion of lactation for F_1. In the F_0 male and female rats of all dose groups, no effects on body weight, food consumption and death were found. An increase in liver weight, hypertrophy of the centrilobular hepatocytes, renal tubular dilation and interstitial nephritis were observed in the males and females at 150 ppm and above, and degeneration of the seminiferous epithelium with sufficient fertility in the males at 450 ppm. Renal pelvis dilation was also observed in the rats at 450 ppm. Degeneration of the seminiferous epithelium was found in the males at 450 ppm; however, mating, pregnancy and male fertility rates of all dose groups were simialr to those of the control group. Also in the F_1 male and female rats of all dose groups, no effects on body weight, food consumption and death were found, and an increase in liver weight (very slight increase in males at 50 ppm), degeneration of the seminiferous epithelium, hypertrophy of the centrilobular hepatocytes, renal tubular dilation and interstitial nephritis were observed in the males and females at doses of 150 ppm and above. Degeneration of the seminiferous epithelium was also observed in the F_1 males at 450 ppm and above. However, there was no difference between F_0 and F_1 in the histopathological examinaiton (Nair, et al., 1987). In this assessment, the NOAEL for parent rats is considered to be 50 ppm (234 mg/m^3), but the NOAEL for reprodective toxicity is not determined, because no effects were observed up to the highest dose of 450 ppm.

In a developmental toxicity study, female F344 rats were exposed to chlorobenzene at doses of 0, 75, 210 and 590 ppm (0, 350, 981 and 2,756 mg/m^3) from gestation day 6 to 15, decreases in body weight and food consumption and an increase in liver weight from gestation day 6 to 8 were observed in the maternal rats at 590 ppm. Slight delayed ossification was observed in the fetuses, which was considered to be a change related to maternal toxicity (John et al., 1984).

In a developmental toxicity study, NZW rabbits were exposed to chlorobenzene at doses of 0, 75, 210 and 590 ppm (0, 350, 981 and 2,756 mg/m^3) for 6 hours/day from gestation day 6 to 18, no anomaly was found in fetuses on gestation day 29 (John et al., 1984).

Based on the data summarized above, it is considered that chlorobenzene has no reproductive toxicity to rats and no develpomental toxicity including embryotixicity and teratogenicity to rats and rabbits.

Table 7-6 Reproductive and developmental toxicity of chlorobenzene

Species sex/numbers of animals	Route	Period	Dose	Results	Reference
Reproductive toxicity					
Rat SD Male and Female 30 animals/ group (2-generation reproductive toxicity test)	Inhala-tion	$F_{0:}$ 10 weeks before mating to the completion of lactation (F_0 females were not exposed from gestation day 20 to lactation day 4) $F_{1:}$ 11 weeks before mating to the completion of lactation 6 hours/day 7 day/week	0, 50, 150, 450 ppm (0, 234, 702, 2,105 mg/m^3) Test substance purity: 99.9%	F_0 and F_1: no effects on mean mating day, and pregnancy and male fertility indices. $F_{0:}$ All doses: no effects on body weight or food consumption or death was found (males and females). 150 ppm and above: increase in liver weight, hypertrophy of centrilobular hepatocytes, renal tubular dilation and interstitial nephritis (males and females) 450 ppm: no effect on degeneration of the seminiferous epithelium with sufficient fertility, renal pelvis dilation and reproductive index (fertility) All doses: similar mating, pregnancy and male fertility rate to the control $F_{1:}$ All doses: no effect on body weight or food consumption or death was found (males and females). 150 ppm and above: increase in liver weight (very slight increase in males at 50 ppm), degeneration of the seminiferous epithelium, hypertrophy of centrilobular hepatocytes, renal tubular dilation and interstitial nephritis	Nair et al., 1987
Developmental toxicity					
Rat F344 32-33 animals	Inhala-tion	Gestation day 6-15 Caesarian section: Gestation day 21 Test substance purity: 99.982%	0, 75, 210, 590 ppm (0, 350, 981, 2,756 mg/m^3) 6 hours/day	590 ppm: Dams: increase in liver weight and decreases in body weight and food consumption (from gestation day 6 to 8) fetus: slight delayed ossification No embryotoxicity or teratogenicity	John et al., 1984
Rabbit NZW 30 animals	Inhala-tion	Gestation day 6-18 Caesarian section on gestation day 29 Test substance purity: 99.982%	0, 75, 210, 590 ppm (0, 350, 981, 2,756 mg/m^3) 6 hours/day	590 ppm: Dams: increase in liver weight and decreases in body weight and food consumption No teratogenicity	John et al., 1984

7.3.6 Genotoxicity

In vitro and *in vivo* studies on genotoxicity of chlorobenzene are summarized in Table 7-7, and a summary of these results is shown in Table 7-8.

a. *in vitro* studies

In many gene mutation tests, chlorobenzene exhibited negative results for bacteria (*Salmonella*

typhimurium) with or without metabolic activation (Haworth et al., 1983; Keskinova, 1968; Lawlor and Haworth, 1979; Lyon, 1976; Monsanto, 1984; Shimizu et al., 1983; Simmon et al., 1984), and for *Aspergillus nidulans* with metabolic activation (Prasad, 1970; Prasad and Pramer, 1968). Both positive and negative results were obtained for *Actinomyces* or *Saccharomyces cerevisiae* (Keskinova, 1968; Monsanto, 1984; Simmon et al., 1984) and for mouse lymphoma cells (McGregor et al., 1988; Monsanto, 1984).

In an *in vitro* chromosomal aberration test using Chinese hamster ovary (CHO) cells, chlorobenzene showed negative results with or without metabolic activation (Loveday et al., 1989).

In DNA damage tests for bacteria of *Bacillus subtilis, Escherichia coli* and *Salmonella typhimurium* (Lawlor and Haworth, 1979; Simmon et al., 1984) and unscheduled DNA synthesis (UDS) tests for cultured rat hepatocytes (Shimada et al., 1983; Williams et al., 1989), chlorobenzene showed negative results. In an *in vitro* sister chromatid exchange (SCE) test with Chinese hamster ovary (CHO) cells, chlorobenzene showed positive results without metabolic activation and negative results with metabolic activation (Loveday et al., 1989)

Chlorobenzene transformed isolated rat hapatocytes at cytotoxic concentrations (Shimada et al., 1983).

b. *in vivo* studies

Chlorobenzene exhibited negative results in a sex-linked recessive lethal test for *Drosophila melanogaster* (Valencia, 1982) and also in a dominant lethal test for mice (Fel'dt, 1985). In micronucleus tests for mice, the results were negative in case of oral administration (Fel'dt, 1985), but positive in case of intraperitoneal injection (Mohtashamipur et al., 1987). An *in vivo* SCE test for mice showed negative results (Fel'dt, 1985).

Although chlorobenzene showed positive results in some *in vitro* and *in vivo* genotoxicity studies, it showed negative results in the majority of the studies on *in vitro* gene mutation, chromosomal aberration, DNA damage and UDS and *in vivo* SCE. From overall evaluation of these results, chlorobenzene is considered not to be genotoxic.

Table 7-7 Genotoxicity of chlorobenzene

	Test system	Species (Organisms) /Strain	Experimental condition	Concentration /Dose	Result[a)] -S9 +S9	Reference
in vitro	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	ND	0-11,243 μg/plate	- -	U.S. NTP, 1985
		Salmonella typhimurium TA92, TA98, TA100, TA1535, TA1537, TA1538	ND	ND	- -	Lawlor & Haworth, 1979
		Salmonella typhimurium TA92, TA98, TA100, TA1535, TA1537, TA1538	ND	0.1-0.5 μg/plate	- -	Simmon et al., 1984
		Salmonella typhimurium TA98, TA100	ND	ND	- ND	Lyon ,1976

	Test system	Species (Organisms) /Strain	Experimental condition	Concentration /Dose	Result[a)] -S9 +S9	Reference
		Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	ND	0.01-0.1 μg/plate	- -	Shimizu et al., 1983
		Salmonella typhimurium TA98, TA100, TA1535, TA1537	Preincubation method	0-3,333 μg/plate	- -	Haworth et al., 1983
		Aspergillus nidulans	ND	ND	ND -	Prasad & Pramer, 1968
		Aspergillus nidulans	ND	200 μg/ml	ND -	Prasad, 1970
		Actinomyces antibioticus 400	Vapor exposure	ND	ND +	Keskinova, 1968
		Saccharomyces cerevisiae	ND	0.01-5 μg/plate	- -	Monsanto, 1984
		Saccharomyces cerevisiae	ND	0.05-6 μg/plate	+ +	Simmon et al., 1984
		Mouse lymphoma L5178Y cells	ND	0.0001-0.1 μl/mL	- -	Monsanto, 1984
		Mouse lymphoma L5178Y tk^+/tk^- -3.7.2 cells	ND	6.25-200 μg/mL	+ +	McGregor et al., 1988
	Chromosomal aberration	CHO cells [b)]	ND	500 μg/mL	- -	Loveday et al., 1989
	DNA damage	*Bacillus subtilis rec-/rec+*	ND	10-20 μg/plate	ND -	Simmon et al., 1984
		Escherichia col polA+/ polA-	ND	10-20 μg/plate	ND -	Simmon et al., 1984
		Salmonella typhimurium TA1978 uvrB- *Escherichia coli* polA+/ polA-	ND	ND	- -	Lawlor & Haworth, 1979
	Unscheduled DNA synthesis	Rat hepatocytes	ND	0.01-10 μg/mL	ND -	Shimada et al., 1983
		Rat hepatocytes	ND	9.3×10^{-4}M (Approx. 150μg/mL)	ND -	Williams et al., 1989
	Sister chromatid exchange (SCE)	CHO cells [b)]	ND	1 mg/mL (-S9) 300μg/mL (+S9)	+ -	Loveday et al., 1989
	Cell transformation	Rat hepatocytes	ND	(at the cytotoxic concentrations)	+ ND	Shimada et al., 1983
in vivo	Sex-linked recessive lethal	*Drosophila melanogaster*	Vapor exposure 4 hours×1 time	Approx. 9000 ppm (42,100 mg/m^3)	-	Valencia, 1982
		Drosophila melanogaster	Vapor exposure 4 hours×3 times	Approx. 10,700 ppm (50,050 mg/m^3)	-	Valencia, 1982
	Dominant lethal	Mouse	Oral	3.2-400 mg/kg	-	Fel'dt, 1985

	Test system	Species (Organisms) /Strain	Experimental condition	Concentration /Dose	Result[a)] -S9 +S9	Reference
	Micronucleus	Mouse	Oral administration	3.2-400 mg/kg	-	Fel'dt, 1985
		Mouse /NMRI/ (male)	Intraperitoneal	112.5-450 mg/kg 2 times	+	Mohtashamipur, et al., 1987
	Sister chromatid exchange (SCE)	Mouse	Oral	3.2-400 mg/kg	-	Fel'dt, 1985

a) -: Negative +: Positive ND: No data available
b) CHO cells: Chinese hamster ovary cells.

Table 7-8 Genotoxicity of chlorobenzene (Summary)

	DNA damage	Mutation	Chromosal aberration
Bacteria	-	+ · -	ND
Mold / Yeast	-	+ · -	ND
Insects	ND	-	ND
Culture cells	-	+ · -	-
Mammals (*in vivo*)	-	ND	+ · -

+: Positive, -: Negative , ND: No data available

7.3.7 Carcinogenicity

Studies on carcinogenicity of chlorobenzene in experimental animals are summarized in Table 7-9.

In a 103 weeks oral (gavage) study of chlorobenzene in male and female B6C3F_1 mice at doses of 0, 30 and 60 mg/kg/day for males and 0, 60 and 120 mg/kg/day for females, mortality in male mice was increased dose-dependently, but tumor incidences in male and female mice were similar to those of the control group (Kluwe et al., 1985; U.S. NTP, 1985).

In a 103 weeks oral (gavage) study of chlorobenzene in male and female F344 rats at doses of 0, 60 and 120 mg/kg/day, an increase in neoplastic nodules was observed in the livers of male rats at 120 mg/kg/day but hepatocellular carcinoma was not induced. No significant difference in incidence of neoplastic nodules in the liver was found between the treated groups and the vehicle-control and untreated-control groups. In addition, the incidences were within the ranges of the historical control data. Furthermore, no clear dose-dependency was observed. Therefore, the authors conclude that this finding of the increase in neoplastic nodules in the liver does not indicate carcinogenicity of chlorobenzene (U.S. NTP, 1985).

Chlorobenzene is metabolized to generate epoxides in organisms. These epoxides are reported to bind to DNA, resulting in possible weak initiation activities (Grilli et al., 1985; Prodi et al., 1986). In a study to investigate the promoter activity of chlorobenzene, 51 mg/kg of diethylnitrosamine was injected intraperitoneally into F344 rats 18 to 24 hours after two thirds of the liver were removed, and 1 week and 5 weeks after the administration of diethylnitrosamine, 112.56 mg of chlorobenzene was injected intraperitoneally. Autopsy was carried out 2 weeks after the final administration. No increase in γ-GTP-positive foci were observed in the hepatocytes. From this result, it was concluded that

chlorobenzene had no promoter activity (Herren-Freund and Pereira, 1986).

As summarized above, oral (gavage) administration of chlorobenzene for 103 weeks caused no increase of tumor incidence in male and female $B6C3F_1$ mice and in male and female F344 rats. Increase of neoplastic nodules in the liver were observed in the male rats of chlorobenzene-treated groups but the incidences were comparable to those of control groups, and no hepatocellular carcinoma was observed. Therefore, chlorobenzene is considered not to be carcinogenic. In a study on promoter activity, chlorobenzene induced no increase in γ-GTP-positive foci were observed in the hepatocytes, showing that chlorobenzene has no promoter activity.

The evaluation of carcinogenicity of chlorobenzene by the international and national organizations is shown in Table 7-10.

IARC has not evaluated the carcinogenicity of chlorobenzene. ACGIH has classified chlorobenzene into A3 (a substance whose carcinogenicity was confirmed in experimental animals), based on the observation result of "neoplastic nodules in the liver" in male rats at 120 mg/kg/day in the carcinogenicity study of F344 rats conducted by the U.S. NTP (1985).

Table 7-9 Carcinogenicity of chlorobenzene

Species sex/number of animals	Route	Period	Dose	Result	Reference
Mouse $B6C3F_1$ Male and female 50 animals/ group	Oral gavage	103 weeks 5 days /week	Male: 0, 30, 60 mg/kg/day Female: 0, 60, 120 mg/kg/day (vehicle : corn oil)	In the treated groups: Male: increase in mortality Female: no toxicity symptoms Male and female: No significant differences in tumor incidence	Kluwe et al., 1985; U.S. NTP, 1985
Rat F344 Male and female 50 animals/ group	Oral gavage	103 weeks 5 days /week	0, 60, 120 mg/kg/day (vehicle: corn oil)	120 mg/kg/day Male: increases in mortality and incidence of neoplastic nodules in the liver (0 mg/kg/day: 8/100, 60 mg/kg/day: 4/49, 120 mg/kg/day: 8/49) No increase in hepatocellular carcinoma No other toxicity symptoms in males and females. Conclusion: Chlorobenzene was considered not to be carcinogenic.	U.S. NTP, 1985
Rat F344 Male and female Promotion assay	18 - 24 hours after 2/3 liver removal: intraperitoneal administration of diethylnitrosamine at 51 mg/kg 1 and 5 weeks after administration of diethylnitrosamine: intraperitoneal administration of chlorobenzene at 112.56 mg/kg Autopsy 2 weeks after the final administration			No increase in γ-GTP[1)] positive foci in hepatocytes	Herren-Freund & Pereira, 1986

[1)] γ-GTP, γ-Glutamyl transpeptidase.

Table 7-10 Evaluations of carcinogenicity of chlorobenzene by the international and national organizations

Organization/Source	Classification	Classification criteria
IARC (2003)	-	Not evaluated for human carcinogenicity
ACGIH (2003)	A3	Confirmed animal carcinogen with unknown relevance to humans
The Japan Society for Occupational Health (2003)	-	Not evaluated for human carcinogenicity
U.S.EPA (2003b)	Group D	Not classifiable as to human carcinogenicity
U.S.NTP (2002)	-	Not evaluated for human carcinogenicity

(As of 2003)

7.4 Summary of effects on human health

Chlorobenzene is absorbed mainly through the gastrointestinal and respiratory tracts, and dermal absorption is considered low.

Chlorobenzene is lipophilic and has a tendency to accumulate in lipid-rich tissues. Chlorobenzene is metabolized to generate two kinds of epoxide by cytochrome P450, and these epoxides bind to nucleic acids and form covalent bonds with proteins in a nonspecific manner in the liver and lungs.

In the metabolic process of chlorobenzene, first, chlorobenzene is oxidized to epoxides by the cytochrome P450 system and these epoxides are metabolized into mercapturic acid derivatives. Then, these metabolites are further metabolized into chlorocatechols via dihydro-dihydroxy chlorobenzene and excreted in the urine. Chlorocatechols, epoxides and chlorophenols are metabolized and excreted in the urine as highly water-soluble glucuronoconjugates and sulfoconjugates, and some poorly water-soluble metabolites of chlorophenols and chlorocatechols are also excreted in the urine. Most chlorobenzene orally administered is excreted in the urine, and some in the feces, and as unchanged chlorobenzene excreted through the lungs.

The toxic effects of chlorobenzene in humans are debility, nausea, lethargy, headache and irritation to the upper respiratory tract and eyes. Contact of chlorobenzene with the skin induces irritation. No reports were obtained on sensitization by chlorobenzene and the sensitization potential is unknown.

The oral LD_{50} of chlorobenzene is 1,445 mg/kg in mice, 1,427 to 3,400 mg/kg in rats and 2,250 to 2,830 mg/kg in rabbits. The LC_{50} following 6-h inhalation exposure is 1,889 ppm in mice and 2,968 ppm in rats.

Slight irritation in the eyes and skin has been reported in the studies with rabbits.

The LOAEL for the repeated oral toxicity of chlorobenzene is determined to be 60 mg/kg/day with liver and kidney effects observed in the 90-day studies in mice and rats by the U.S. NTP. The NOAEL of chlorobenzene for repeated inhalation exposure is determined to be 50 ppm (234 mg/m^3) based on the results of an inhalation study in which rats were exposed to chlorobenzene from 10 weeks before mating to the completion of lactation, and the effects on the blood, liver and kidney were observed.

With the observed effects on male reproductive cells, it is considered that chlorobenzene has

reproductive toxicity, but no developmental toxicity.

Negative results were obtained in the majority of genotoxicity tests of chlorobenzene, with some positive results. The overall evaluation of the available data indicates chlorobenzene is not genotoxic.

With regard to the carcinogenicity of chlorobenzene, tumor incidence was not increased in a 103 weeks oral (gavage) study in male and female $B6C3F_1$ mice. In a 103 weeks oral (gavage) study in male and female F344 rats, the observed incidences of neoplastic nodules in the liver of males in the treated groups were comparable to those in the control groups. No carcinogenicity of chlorobenzene was detected in the studies conducted by NTP (1985). The IARC has not evaluated the carcinogenicity of chlorobenzene. ACGIH, however, classified chlorobenzene as a substance whose carcinogenicity was confirmed in experimental animals (A3) based on the results of a carcinogenicity study in F344 rats (NTP, 1985).

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ABBREVIATIONS

ACGIH : American Conference of Governmental Industrial Hygienists
ADH : alcohol dehydrogenase
ALDH : aldehyde dehydrogenase
ALP : alkaline phosphatase
ALT : alanine aminotransferase
ASAT : aspartate aminotransferase
AST : aspartate aminotransferase
ATSDR : Agency for Toxic Substances and Disease Registry
BCF : Bioconcentration Factor
BHK : Syrian hamster kidney culture cells
BOD : Biological Oxygen Demand
BUN : blood urea nitrogen
CAS : Chemical Abstract Services
CAS Online : Chemical Abstract Services Online
CEPA : Commonwealth Environment Protection Agency
CERHR : Center for the Evaluation of Risks to Human Reproduction
CERI : Chemicals Evaluation and Research Institute, Japan
CHL : Chinese hamster lung cells
CHO : Chinese hamster ovary cells
CICAD : Concise International Chemical Assessment Document
Cmax : the maximum concentration of a compound in the blood, etc.
COD : Chemical Oxygen Demand
CPK : Creatinine phosphokinase
DDT : dichlorodiphenyltrichloroethane
DOC : Dissolved Organic Carbon
EA : Environment Agency of Japan
EC : European Communities
EC_{10} : Effect Concentration measured as 10% effect
EC_{50} : median Effect Concentration
ECB : European Chemicals Bureau
ECETOC : European Centre for Ecotoxicology and Toxicology of Chemicals
EEC : European Economic Communities
EHC : Environmental Health Criteria
EHI : Estimated Human Intake
EPA : Environmental Protection Agency (USA)
EU : European Union
EUSES : Europian Union System for the Evaluation of Substances
FAD : flavin adenine dinucleotide
FAO : Food and Agriculture Organisation of the United Nations
GABA : g-aminobutyric acid
GC : gas chromatography
GGT : gamma-glutamyl transpeptidase
GLP : Good Laboratory Practice
hr : hour
HSDB : Hazardous Substances Data Bank
IARC : International Agency for Research on Cancer
IC : Industrial Category
IC_{50} : median Immobilisation Concentration or median Inhibitory Concentration
ILO : International Labour Organisation
IPCS : International Programme on Chemical Safety
IRIS : Integrated Risk Information System
IUCLID : International Uniform Chemical Information Database (existing substances)
Koc : Soil adsorption coefficient Koc
Kow : octanol/water partition coefficient
LC_{50} : median Lethal Concentration

LD_{50} : median Lethal Dose
LDH : lactate dehydrogenase
LLNA : Local Lymph Node Assay
LOAEL : Lowest Observed Adverse Effect Level
LOEC : Lowest Observed Effect Concentration
LOEL : Lowest Observed Effect Level
MAO : monoamineoxydase
MATC : Maximum Acceptable Toxic Concentration
MCH : mean corpuscular hemoglobin
MCV : mean corpuscular volume
METI : Ministry of Economy, Trade and Industry, Japan
MHLW : Ministry of Health, Labour and Welfare
min : minute
MITI : Ministry of International Trade and Industry, Japan
MNLD : maximum non lethal dose
MOE : Ministry of the Environment, Japan
MOF : Ministry of Finance, Japan
MOS : Margin of Safety
MTD : maximum tolerance dose
NAT2 : N-acetyltransferase
NCI : National Cancer Institute
NICNAS : Australia's National Industrial Chemicals Notification and Assessment Scheme
NIES : National Institute for Environmental Studies, Japan
NITE : National Institute of Technology and Evaluation, Japan
NMR : nuclear magnetic resonance analysis
NOAEL : No Observed Adverse Effect Level
NOEC : No Observed Effect Concentration
NOEL : No Observed Effect Level
NTE : neurotoxic esterase
NTP : National Toxicology Program (USA)
NZW : New Zealand White
OECD : Organisation for Economic Cooperation and Development
OPIDN : Organophosphate-induced delayed neuropathy
OR : odds ratios
ppm : parts per million
$polA^-$: DNA polymerase$^-$
$polA^+$: DNA polymerase$^+$
pKa : negative log of the acid dissociation constant
PRTR : Pollutant Release and Transfer Register
RBC : Radiation Biology Center
RAR : Risk Assessment Report
RC : Risk Characterisation
RfC : Reference Concentration
RfD : Reference Dose
RTECS : Registry of Toxic Effects of Chemical Substances
SCE : Sister chromatid exchange
SDH : sorbitol dehydrogenase
SER : smooth endoplasmic reticulum
SG : Syrian golden
SIDS : Screening Information Data Set
SLRL-test : sex-linked recessive lethal test
SOD : superoxide dismutase
TDI : Tolerable Daily Intake
TE : toxic equivalent
TLV : Threshold Limit Value
Tmax : time until a concentration reaches Cmax.
TOXLINE : Toxicology Literature Online
UV : ultraviolet

v/v : volume per volume ratio
w : week
w/w : weight per weight ratio
WHO : World Health Organization
γ-GTP : γ-glutamyl transpeptidase
δALS : δ-aminolevulinic acid synthetase