DRAFT

For Review Only

Public Health Goal for
CHLOROBENZENE
in Drinking Water

NOT A REGULATORY STANDARD

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PREFACE

Drinking Water Public Health Goals

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This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365), amended 1999, requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and publish PHGs for contaminants in drinking water based exclusively on public health considerations. Section 116365 specifies that the PHG is to be based exclusively on public health considerations without regard to cost impacts. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances which can cause chronic disease shall be based upon currently available data and shall be set at levels which OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of insufficient data to determine a level of no anticipated risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.

9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.

10. PHGs published by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

PHGs published by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR CHLOROBENZENE IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) proposes a Public Health Goal (PHG) of 100 µg/L (or 100 ppb) for chlorobenzene in drinking water. The principal study selected for derivation of the PHG was that of Knapp *et al*. (1979). In this 13-week study, chlorobenzene was administered via capsule to dogs at doses of 27, 54 and 272 mg/kg-day. Four of the eight dogs in the highest dose group died within 3 weeks and changes in serum enzyme chemistry as well as histopathological changes in the liver were observed in dogs at the intermediate dose level. A No-Observed-Adverse-Effect-Level (NOAEL) of 27 mg/kg-day was identified (19 mg/kg-day after adjustment for the 5 days/week dosing schedule). Calculation of the PHG incorporated an overall uncertainty factor of 1,000, considering inter-species extrapolation, exposure of potentially sensitive human sub-populations, and the relatively short exposure duration of the dog study. The exposure evaluation assumed a 70 kg body weight, an equivalent water consumption rate of 4 Leq/day, and a relative source contribution of 30 percent.

From the genotoxicity data available, it appears that chlorobenzene, at relatively high concentrations *in vitro* or high doses *in vivo,* can produce positive effects in cytogenetic indicators of genetic damage. Chlorobenzene was negative in bacterial tests of genetic toxicity.

The carcinogenic potential of chlorobenzene has been evaluated in rats and mice (Kluwe *et al*., 1985; NTP, 1985). In a two-year cancer bioassay, male and female F344/N rats and male and female $B6C3F_1$ hybrid mice (50/sex/dose) were given chlorobenzene by gavage, 5 days/week for 103 weeks. Rats and female mice were given 0 (corn oil; vehicle), 60, or 120 mg/kg-day, and male mice were given 0, 30, or 60 mg/kg-day. The only tumor type observed was neoplastic nodules of the liver in male rats of the high dose group (120 mg/kg-day). Neoplastic nodules were not malignant, and hepatocellular carcinomas were detected only in two male control animals. The tumor incidences in the male and female mice and in the female rats given chlorobenzene for two years did not exceed those in the corresponding vehicle or untreated controls.

Using a weight of evidence classification scheme, the U.S. EPA (1999a) currently designates chlorobenzene as a chemical "not classifiable as to human carcinogenicity" (Group D). The basis for this classification is the absence of human data on carcinogenicity, inadequate experimental data in animals, and mostly negative results with chlorobenzene in short-term tests (bacterial) of genetic toxicity.

The current federal Maximum Contaminant Level (MCL), of 0.1 mg/L (or 100 ppb), is also derived from the Knapp *et al*. (1979) study. OEHHA has developed a noncancer chronic reference exposure level of 1 mg/m^3 (or 300 ppb in air) for chlorobenzene (OEHHA, 2001a). OEHHA has not developed any reference values for chlorobenzene in the Proposition 65 program (OEHHA, 2001b).

INTRODUCTION

The purpose of this document is to review the information on the toxicological properties of chlorobenzene with the goal of developing a proposed PHG for chlorobenzene. California's current drinking water standard for chlorobenzene is 70 ppb. This standard, referred to as the State Maximum Contaminant Level (or State MCL), was adopted by the Department of Health Services in 1994 (California Code of Regulations, Title 22) and is similar to the federal MCL of 100 ppb. A division of the California Department of Health Services (currently OEHHA) earlier recommended a Proposed Maximum Contaminant Level (PMCL) of 30 ppb for chlorobenzene (DHS, 1988).

In developing a PHG for chlorobenzene, OEHHA staff and an outside contractor evaluated the basis for the U.S. EPA MCL. In addition, a search of the scientific literature was conducted to determine if there are any new data that would support the development of a number different than the current value. In this document, the information on the toxicity of chlorobenzene was evaluated, with the primary focus on the studies related to oral exposures, which are the most appropriate for the establishment of a PHG for drinking water. Previous reviews of this chemical (NTP, 1985; U.S. EPA, 1988a; Hellman, 1993; HSDB, 1998) provided important guides for this document.

CHEMICAL PROFILE

Chemical Identity

Chlorobenzene has no chemical isomers. The chemical formula for chlorobenzene, including pertinent identification numbers, are listed in Table 1.

Table 1. Chemical Identity of Chlorobenzene (from HSDB, 1998)

Chemical name	Chlorobenzene
Synonyms	benzene chloride; chlorobenzol; MCB; monochlorobenzene; phenyl chloride; tetrosin SP
Molecular formula	C_6H_5Cl
CAS registry number	108-90-7
RTECS registry number	NIOSH/CZ0175000

Physical and Chemical Properties

Chlorobenzene is a halogenated aromatic compound with one chlorine atom. It is a colorless liquid at room temperature. It is slightly soluble in water, but is miscible with nearly all organic solvents, including ethanol, ethyl ether, benzene, and chloroform

(HSDB, 1998). Some of the physical and chemical properties of chlorobenzene are listed in Table 2.

Table 2. Physical and Chemical Properties of Chlorobenzene[1]

Property	Value or Information
Molecular weight	112.56
Color	Colorless
Physical state	Liquid (25 °C)
Odor	Mildly aromatic, characteristically penetrating or almond and benzene-like, or like mothballs
Odor threshold in air	0.21 ppm
Odor threshold in water [2]	0.02 mg/L (ppm)
Melting point	-45.2 °C
Boiling point	131.7 °C
Flash point	29.2 °C (closed cup), 36.1 °C (open cup)
Solubility in water	502 mg/L at 25 °C
Density	1.11 g/ml at 20 °C
Log K_{ow}	2.89
Vapor pressure	12 mm Hg at 25 °C
Henry's law constant	3.77×10^{-3} atm-m^3/mole
Conversion factors	1 ppm = 4.60 mg/m^3 in air at 25 °C

[1] Sources: HSDB (1998), Leber *et al*. (1994).
[2] The "water odor threshold" is the concentration of the substance in water which will generate in the air the odor threshold (i.e. measured in the headspace of a closed container).

Production and Uses

The primary use of chlorobenzene is in production of nitrochlorobenzenes that are used as intermediates for chemicals in rubber processing, antioxidants, dye and pigments, agricultural products, and pharmaceuticals. This accounts for 65 percent of chlorobenzene use (HSDB, 1998). The production of phenol, aniline, and DDT from chlorobenzene, formerly on a large scale, has been almost entirely discontinued due to the introduction of new processes and legislation forbidding the use of DDT. Chlorobenzene is also used as a solvent, for example, in the production of bitumen and asphalt coatings for building protection. It is used as a fiber swelling agent and dye carrier in textile processing, a tar and grease remover in cleaning and degreasing

operations, a solvent in surface coating and surface coating removers, and sometimes in dry-cleaning. Chlorobenzene has use as a solvent for paints, and as a heat transfer medium (HSDB, 1998).

Chlorobenzene is produced by the chlorination of benzene in the presence of a catalyst. In 1993, production of chlorobenzene in the United States (U.S.) was estimated at 88,555,000 kg (HSDB, 1998).

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

The general population may be exposed to chlorobenzene via inhalation of ambient air, ingestion of food and drinking water, and dermal contact with vapors, food and other products containing chlorobenzene. Occupational exposure to chlorobenzene may occur through inhalation and dermal contact with this compound at workplaces where chlorobenzene is produced or used. Populations at special risk of exposure include urban residents through ambient air, people near manufacturing plants, and people near locations where products containing chlorobenzene is used (HSDB, 1998).

Air

Release of chlorobenzene to the environment is estimated to be due mostly to volatilization losses associated with its use as a solvent in pesticide formulations and in degreasing and other industrial applications (HSDB, 1998). If released into the air, its vapor pressure of 12 mm Hg at 25°C indicates that chlorobenzene will exist solely as vapor in the ambient atmosphere. Based on its Henry's Law constant of 3.77×10^{-3} atm-m^3/mole, chlorobenzene is expected to volatize rapidly from water surfaces. Additionally, it may volatize from moist and dry soil surfaces (HSDB, 1998).

The dominant source of chlorobenzene emissions is coal-fired power stations (HSDB, 1998). Chlorobenzene has been identified, but not quantified, in ambient air at 324 of 1,400 sites across the U.S. that were sampled over a 5-year period. Typical chlorobenzene concentrations in cities in the U.S. ranged from not detectable to 0.8 ppb; the maximum value measured was 12 ppb. Mean ambient air levels of chlorobenzene in California were 0.2 to 3.4 ppb in Los Angeles, 0.1 to 0.64 ppb in Oakland, 2.2 ppb in Riverside, and 0.004 ppb in Upland. Mean ambient air concentrations in 50 urban and near-source sites in the U.S. were 0.8 and 0.2 ppb, respectively. Three sites in the Netherlands, sampled over a 1-year period in 1980, contained mean ambient air concentrations of chlorobenzene ranging from 0.06 to 0.1 ppb. The ambient air concentrations of chlorobenzene in 13 study areas across the U.S. (728 samples) ranged from less than 0.02 to 2 ppb (0.09 to 9.1 $\mu g/m^3$) between the years 1989-1991. Air samples collected from 12 Canadian homes in November/December 1986 and February/March 1987 contained a mean chlorobenzene concentration of 0.5 $\mu g/m^3$ (HSDB, 1998).

Toxic Release Inventory data (U.S. EPA, 2001) indicate that 1.0 and 0.77 million lbs of chlorobenzene were released into the air in 1997 and 1998 respectively in the U.S. For

1997, approximately 0.48 million lbs of the emissions were fugitive air emissions and 0.51 million lbs were stack emissions. For 1998, 0.33 million lbs were fugitive air emissions and 0.44 million lbs were stack emissions. From 1987 to 1994, approximately 1,100 lbs of chlorobenzene were released by fugitive air emissions in California. During this same period, the stack emissions were approximately 4,100 lbs in California. Thus, the total air emissions equaled about 5,200 lbs during this period. No air emissions in California have been noted in the Toxic Release Inventory since 1994.

Soil

Chlorobenzene was not detected in sediment in an industrial river location, Lake Ontario (April to November 1980) or in sediment from Raritan Bay (lower Hudson River). Chlorobenzene was detected in bottom sediments and suspended sediments collected near an industrial outfall in Bayou d'Inde at concentrations of 1.5 and 0.22 µg/g organic carbon. Chlorobenzene was detected as a sewer overflow contaminant in sediment of the lower Passaic River, New Jersey, in concentrations ranging from 7 to 1,400 µg/kg (HSDB, 1998).

Toxic Release Inventory data (U.S. EPA, 2001) show that 1,550 lbs and 16 lbs were released onto land in 1997 and 1998 in the U.S., respectively. Underground releases of chlorobenzene were about 114,000 lbs for 1997, and 184,000 lbs for 1998 in the U.S. No land or underground releases have been noted in California.

Water

Chlorobenzene is relatively mobile in sandy soil and aquifer material and biodegrades slowly or not at all in these soils (U.S. EPA, 1999b). Chlorobenzene was detected in groundwater in Miami at a concentration of 1.0 mg/L; in raw water contaminated with municipal waste in Philadelphia at a concentration of 0.1 mg/L; and in raw water contaminated with industrial discharge in Cincinnati and in Lawrence, Massachusetts, at concentrations of 0.1 to 0.5 mg/L and 0.12 mg/L, respectively. Chlorobenzene was identified in surface water/groundwater samples impacted by municipal landfill leachate in Orange County, Alachua County (southwest), and Alachua County (southeast), Florida, at concentrations ranging from <0.20 to 302 µg/L. Chlorobenzene was identified as one of the 20 most abundant organic constituents in groundwater at 479 U.S. waste disposal sites; chlorobenzene was detected at 86 sites (18 percent). A study of groundwater contamination at six Superfund sites across the U.S. detected chlorobenzene in the Biscayne, Florida, aquifer study area at concentrations of 30 µg/L. Chlorobenzene was identified in groundwater samples at a former incinerator site near Amsterdam, The Netherlands, in concentrations ranging from 2 to 300 µg/L (HSDB, 1998).

Toxic Release Inventory data (U.S. EPA, 2001) indicate that releases of chlorobenzene into surface water approximated 1,200 lbs in 1997 and 912 lbs in 1998 in the U.S. No water releases have been noted in California.

According to the monitoring data collected by the California Department of Health Services (DHS, 1998) between 1984 and 1997, a total 115 water samples were reported positive for chlorobenzene. The maximum, mean, and minimum levels detected in California drinking water sources were 5.4, 2.6, and 0.6 μg/L, respectively.

Food

Chlorobenzene concentrations ranged from 4.87 to 40.1 ppb in 2 of 234 food samples analyzed, with the highest levels found in clam chowder. The concentrations of chlorobenzene in volatiles of peanut butter, garlic dressing, and flour were determined to be 1.4 to 2.1, 0.8, and 0.2 μg/kg, respectively. The occurrence of chlorobenzenes in retail vegetables in the United Kingdom was evaluated. Chlorobenzene was detected only in the inner portions of cabbages (207 μg/kg fresh weight), but not in carrots, potatoes, cauliflowers, lettuce, onions, broad beans, peas or tomatoes (Wang and Jones, 1994). The origin of the chlorobenzene in cabbage was not identified.

U.S. EPA (1999b) estimated that the bioconcentration factor of chlorobenzene in fish ranges from one to two. Two studies of chlorobenzenes in fish from the Great Lakes and Japanese coast failed to detect any chlorobenzene. Chlorobenzene was detected in catfish collected from the junction of the Calcassieu River and the Bayou d'Inde, Louisiana, in the vicinity of an industrial outfall, at a concentration of 0.05 μg/g lipid. It was also detected in Atlantic croakers, blue crabs, spotted sea trout, and blue catfish collected from the junction of the Calcassieu River and the Bayou d'Inde at concentrations of 0.10, 0.41, 0.18, and 0.05 μg/g lipid, respectively. Chlorobenzene was identified in samples of burbot (*Lota lota*) liver obtained from 68 fish collected during 1985 and 1986 at 8 sites in remote lakes and rivers of Canada (HSDB, 1998).

Human breast milk (42 samples from subjects living near manufacturing plants or industrial facilities) contained a trace to 10 ppb (0.37 ppb average) of chlorobenzene (HSDB, 1998).

METABOLISM AND PHARMACOKINETICS

Absorption

Chlorobenzene enters the systemic circulation readily after ingestion and inhalation. Chlorobenzene was orally administered to a human volunteer and the urinary metabolites, para-chlorophenylmercapturic acid and 4-chlorocatechol, were monitored. At least 31 percent of the oral dose was excreted in the urine, primarily as the 4-chlorocatechol metabolite (Ogata and Shimada, 1983; Ogata *et al*., 1991). Earlier studies in rabbit after oral dosage showed at least 75 percent of the administered dose appeared as urinary metabolites (Spencer and Williams, 1950). The relatively small molecular size and the lipophilicity of chlorobenzene, as evidenced by the octanol/water partition coefficient (log K_{OW} = 2.89), would predict that such a molecule would easily move across cell membranes. It would be reasonable to assume that an oral dose of

chlorobenzene, at environmental levels, would be completely absorbed from the gastrointestinal tract.

In studies of chlorobenzene administered by inhalation, humans exposed to ^{14}C-chlorobenzene at 0.84 ppm for 415 min, or at 0.5 ppm for 228 min, excreted 38 percent and 45 percent of the dose in the urine, respectively, primarily as the 4-chlorocatechol metabolite (Ogata *et al*., 1991). From inhalation studies in rats, it is known that exhalation of unchanged chlorobenzene is also a route of excretion (Sullivan *et al*., 1983). Azouz *et al*. (1952) showed that 24 to 32 percent of an orally administered dose is exhaled in rabbits. Thus, the major portion of absorbed chlorobenzene that is not measured as urinary metabolites is most likely accounted for by exhalation. Net uptake of inhaled chlorobenzene is likely to be about 50 percent of the available chemical in air, estimated from data on similar volatile organic chemicals (Raabe, 1986, 1988).

No published data were found on the magnitude of dermal absorption of chlorobenzene, but data on similar chemicals indicates that chlorobenzene should be readily absorbed through the skin (U.S. EPA, 1992).

Distribution

Sullivan *et al*. (1983) studied the distribution and rate of excretion of inhaled chlorobenzene. Male Sprague-Dawley rats were exposed to ^{14}C-chlorobenzene at 100, 400, or 700 ppm (460, 1,840 or 3,220 mg/m^3) for 8 hr/day. After exposure, chlorobenzene-associated radioactivity was measured in liver, kidneys, lungs, adipose tissue and blood. Adipose tissue was found to accumulate the largest amounts of radioactivity, followed by liver and kidneys. The measurements of radioactivity did not distinguish between unchanged chlorobenzene and its metabolites. Reid (1973) and Reid and Krishna (1973) reported that intraperitoneal injection of ^{14}C-chlorobenzene led to extensive covalent binding of radioactive materials to the tubules of the kidney where nephrotoxic effects are observed. The distribution and fate of nonvolatile radioactivity from ^{14}C-chlorobenzene were also studied in female C57BL mice, using whole-body autoradiography (Brittebo and Brandt, 1984). Whole-body autoradiograms from tissue sections showed a selective localization of nonvolatile metabolites in the mucosa of the respiratory system 1 minute after an intravenous injection. The labeling of the mucosa of the respiratory tract was still present 4 days after the injection. Microautoradiography showed that the chlorobenzene-associated radioactivity was bound to the epithelium of the tracheo-bronchia1 mucosa. Uptake of nonvolatile radioactivity was also observed in other tissues 1 and 5 minutes after the intravenous injection, although not to the same extent as in the respiratory tract. Relatively high amounts of nonvolatile metabolites of chlorobenzene were observed in the liver, the cortex of the kidney, the mucosa of the tongue, cheeks, and esophagus, and in the inner zone of the adrenal cortex.

Metabolism

Studies by R.T. Williams and colleagues in 1950 first showed that chlorobenzene and other halobenzenes were converted in the body into phenols and mercapturic acids

(Spencer and Williams, 1950). Chinchilla rabbits given a single oral dose of chlorobenzene (150 mg/kg) excreted 52 percent of the dose as oxygen conjugates (25 percent as glucuronides and 27 percent as ethereal sulfates) and 20 percent as sulfur conjugates (mercapturic acids). Follow-up studies on rabbits showed that metabolism of chlorobenzene proceeded by oxidation of the aromatic nucleus to form an epoxide (Azouz *et al*., 1952; Selander *et al*., 1975).

Chlorobenzene is converted to o-chlorophenol and p-chlorophenol by mono-oxygenases of the cytochrome P-450 system (reviewed by Hellman, 1993). The ethereal, glucuronide, and mercapturic conjugates of these phenols are then excreted via the urine (Spencer and Williams, 1950; Oesch *et al*, 1973; Nedelcheva *et al*., 1998). Two different reactive epoxides are formed as intermediate species. Chlorobenzene-3,4-epoxide generates p-chlorophenol and chlorobenzene-2,3-epoxide generates o-chlorophenol. A third chlorophenol, m-chlorophenol, is also found as a reaction product and may be produced by non-enzymatic direct insertion of triplet oxygen into chlorobenzene (Korzekwa *et al*., 1989). Pretreatment of rats with phenobarbital, which enhances the p-chlorophenol pathway, increases liver toxicity, whereas pretreatment of rats with 3-methylcholanthrene, which enhances the o-chlorophenol pathway, decreases liver toxicity. Thus, the enzymatic channel for the metabolic degradation of chlorobenzene can be an important determinant of toxicological activity (Hellman, 1993).

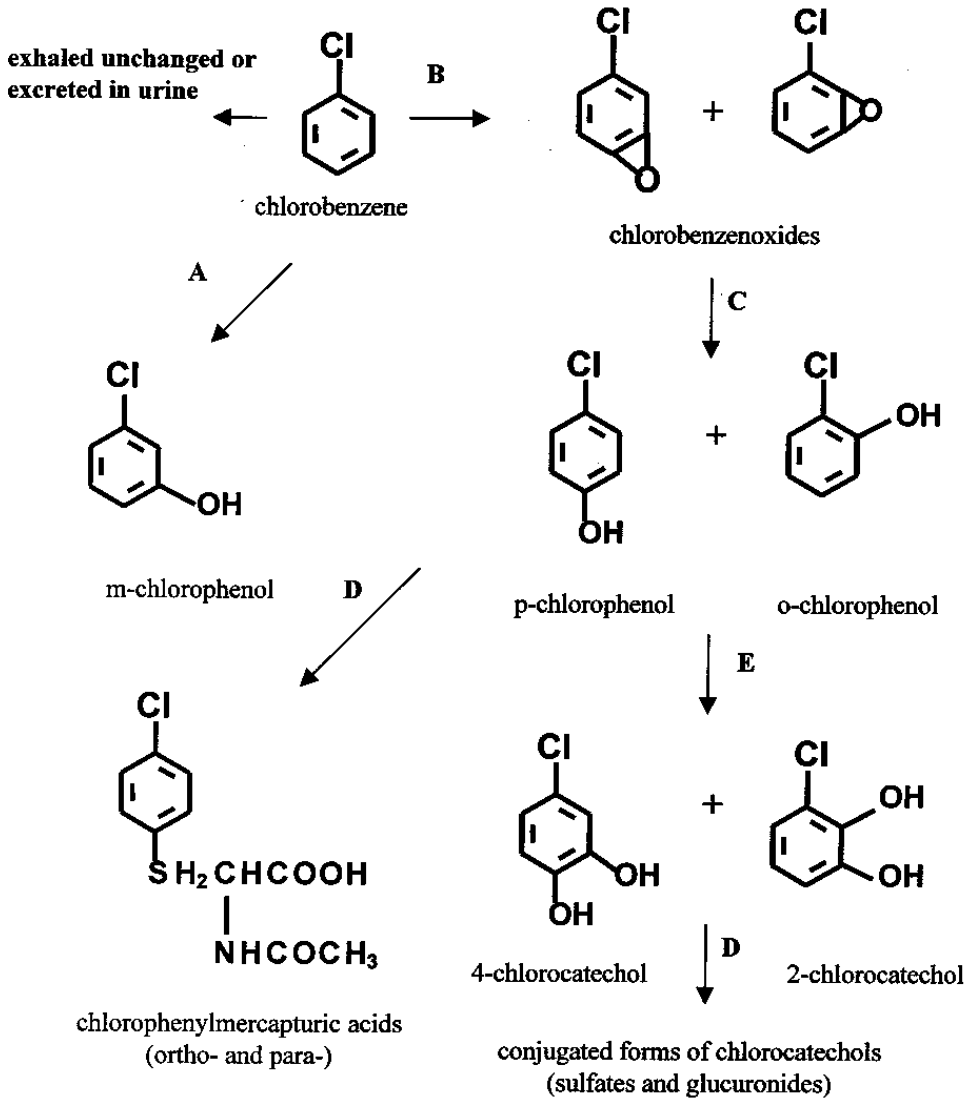
When the metabolic pathways of chlorobenzene were compared using liver microsomal preparations from humans and mice, it was noted that the human liver enzymes were about twice as active as mouse microsomes in generating the p-chlorophenols (Kerger *et al*., 1988). The unit for comparison of enzymatic activity between species was nmol of chlorophenol produced/min/nmol of cytochrome P-450. The authors suggested that, because of this metabolic difference, humans may be more susceptible than mice to chlorobenzene-induced liver toxicity.

The metabolic transformation of chlorobenzene in tissues other than liver has also been noted (Hellman, 1993). Injection of ^{14}C-labelled chlorobenzene leads to non-extractable binding of the radiolabel to tissues such as kidney, nasal mucosa, and lung. The toxicological significance of binding is not clear, as it is known that metabolism of bromobenzene, a related halobenzene, by the 2,3-arene oxide pathway also generates tissue-bound metabolites, but not liver toxicity. Dalich and Larson (1985a) did not find a correlation between the degree of chlorobenzene tissue binding and the magnitude of liver toxicity in rats, as measured by the activity of alanine aminotransferase (ALT) in the blood of the treated animals.

The pattern of metabolites generated from chlorobenzene varies among species. Humans, rats, mice, and rabbits all produce chlorophenol metabolites, but the urinary products of sulfur amino acid conjugation, such as chlorophenylmercapturic acid, are produced more abundantly in the rabbit and rodents than in humans. In a study reported by Ogata and Shimada (1983), the excretion ratios of p-chlorobenzenemercapturic acid to 4-chlorocatechol averaged 7.5 in rats, 7.2 in mice and 1.7 in rabbits. In human volunteers, the ratio was less than 0.01. The primary products of chlorobenzene metabolism in humans are 4-chlorocatechol and its conjugates.

The level of 4-chlorocatechol in human urine can be used as a marker of industrial exposure (Ogata and Shimada, 1983). Yoshida *et al*. (1986) examined the composition of urinary metabolites of workers occupationally exposed to chlorobenzene. They found that 4-chlorocatechol conjugates constituted 77 percent and 4-chlorophenol conjugates 12 percent of the urinary metabolites. The level of 4-chloromercapturic acid was less than 1 percent of the total metabolites.

The proposed metabolic pathways for chlorobenzene are summarized in Figure 1. The scheme is based on *in vitro* findings and human urinary excretion data (Hellman, 1993).



A: Hydroxylation

B: Cytochrome P450/P488-dependent microsomal oxidation
C: Rearrangement
D: Conjugation: glucuronosyl transferases and sulphotransferases
E: Epoxide hydratases

Figure 1. Proposed metabolic pathways for chlorobenzene (based on Hellman, 1993)

Excretion

Sullivan *et al*. (1983) studied the rate of excretion of inhaled chlorobenzene in male Sprague-Dawley rats. Animals were exposed to ^{14}C-chlorobenzene at 100, 400, or 700 ppm (460, 1,840 or 3,220 mg/m^3) for 8 hr/day. After exposure, animals were kept in metabolism cages so that exhaled and urinary levels of radioactivity could be quantified. Enzyme hydrolysis and extraction was used to distinguish among urinary metabolic products. Increasing the airborne exposure concentration from 100 ppm to 400 ppm, to 700 ppm magnified the exhaled amount of radioactivity, suggesting that the metabolic clearance from the blood became saturated and the route of elimination shifted from biotransformation toward more exhalation. The percentage of the total label eliminated via the respiratory route after a single 8-hr exposure was 5 percent, 21 percent, and 44 percent after 100, 400, and 700 ppm, respectively. Values of 3 percent, 13 percent, and 32 percent were found after a multiple-exposure regimen of 5 consecutive exposure days. The decreased exhalation after multiple exposures suggests the possibility of induction of metabolic enzymes, especially at 700 ppm.

The dynamics of inhaled chlorobenzene uptake and elimination have been quantified in the context of occupational exposure. Ogata *et al*. (1991) exposed 5 male volunteers to chlorobenzene at 11.8 and at 60.2 ppm in a chamber for 3 hr followed by a 1 hr break and then for another 5 hr of exposure. They then monitored blood levels of chlorobenzene and urinary levels of phenol and catechol metabolites. 4-Chlorocatechol was the principal metabolite found. The half-lives of urinary excretion of chlorobenzene metabolites were shown to be composed of two phases. For para-chlorophenol metabolites, the fast phase was 3 hr and the slow phase was 12.2 hr. For chlorocatechol metabolites, the fast phase was 2.2 hr and the slow phase was 17.3 hr.

Kumagai and Matsunaga (1995) constructed a physiologically-based pharmacokinetic model of chlorobenzene exposure in an industrial setting. Two workers were monitored for exposure and urinary metabolites of chlorobenzene were characterized. The mean chlorobenzene exposure level was 10 ppm for a 7-hr working day. This study confirmed that monitoring of urinary levels of 4-chlorocatechol is an accurate cumulative (or integrative) predictor of airborne exposures.

In a case-report of a suicide attempt by a 40-year-old man who drank 140 mL of a 90 percent chlorobenzene solution, serum chlorobenzene was monitored from day 3 to day 15 after ingestion (Babany *et al*., 1991). Analysis of the data showed a mono-exponential disappearance of chlorobenzene from blood with a half-life of 40.3 hr. This individual had impaired liver function and drank approximately 200 g of alcohol on a daily basis, so his rate of excretion of chlorobenzene may not be representative of the general population.

In summary, the toxicokinetic profile of chlorobenzene is that of a lipid soluble molecule which is readily absorbed in the gastrointestinal tract, has affinity for adipose tissue, but is not stored in tissues because of pulmonary exhalation and a relatively efficient transformation to oxidized metabolites by liver enzymes. The principal urinary

metabolites of chlorobenzene in humans are 4-chlorophenol, 4-chlorocatechol and their conjugates. One pathway of transformation, conversion of chlorobenzene to p-chlorophenol via the 3,4-arene oxide intermediate, may be associated with reactive compounds that produce toxicity to liver and kidney cells, but the precise identities of the toxic reactants are not yet known.

TOXICOLOGY

Toxicological Effects in Animals and Plants

Acute Toxicity

Median lethal doses (LD_{50}) of chlorobenzene determined in laboratory species such as the rat, mouse, rabbit and guinea pig were tabulated by Hellman (1993). The LD_{50} for orally-administered chlorobenzene in the rat ranged from 1.4 to 3.4 g/kg; other values were 1.4 g/kg for mice, 2.2 to 2.8 g/kg for rabbits, and 5.0 g/kg for guinea pigs. This potency range places chlorobenzene in the "slightly toxic" range for acute toxicity. The reported acute lethal concentration (LC_{50}) inhalation values range from 0.05 mg/L in the guinea pig to 20 mg/L for a two-hour exposure period in mice (Rozenbaum *et al*., 1947; Lecca-Radu, 1959).

In experimental animals, the manifestations of acute toxicity of chlorobenzene are consistent with irritant effects on mucous membranes (hyperemia, salivation and lacrimation, submucosal hemorrhage of the stomach lining) and anesthetic effects on the central nervous system (ataxia, decreased locomotor activity, paralysis, and labored breathing). Death in animals from ingestion or inhalation of large doses is due to severe respiratory depression (Willhite and Book, 1990; Hellman, 1993).

Chlorobenzene administered to experimental animals also produces specific organ damage, the hepatotoxic effects being the most extensively studied. Dalich and Larson (1985b) examined the temporal and dose-response relationships for chlorobenzene-induced liver toxicity in rats. A single 1.1 g/kg dose of chlorobenzene dissolved in corn oil, administered intraperitoneally, produced histological evidence of centrilobular necrosis within 48 to 72 hr. Dilated sinusoids and coagulative necrosis in liver tissues were observed, but fatty infiltration was minimal. Sulfobromophthalein (BSP) retention was increased in blood and serum ALT activity was elevated, providing confirmatory evidence of liver damage. Chlorobenzene also lowered liver glutathione levels at this dose. The elevation of serum ALT was potentiated by pretreatment of rats with phenobarbital, an inducer of certain forms of P-450 microsomal enzymes which convert chlorobenzene to reactive intermediates. The effect on serum ALT was not affected by pretreatment with diethylmaleate, a chemical which depletes liver glutathione. No consistent correlation was observed between liver glutathione levels or binding of chlorobenzene to proteins, and liver damage. The lowest-observed-effect-level (LOEL) for increased serum ALT activity after a single intraperitoneal dose of chlorobenzene to rats was estimated to be 226 mg/kg (Den Besten *et al*., 1991).

The kidney tubules are another target for chlorobenzene-induced toxicity (Reid, 1973). Male Sprague-Dawley rats and male C57BL/6J mice given a single intraperitoneal dose of chlorobenzene developed renal tubular lesions within 48 hr. For example, 80 percent of a group of mice given 760 mg/kg of chlorobenzene developed necrosis of the proximal convoluted renal tubules. Rats were less sensitive than mice to the nephrotoxic action of chlorobenzene.

In an early study by Cameron *et al*. (1937), it was reported that a single subcutaneous dose of chlorobenzene (approximately 555 mg/kg) to one rabbit produced a drop in the number of white blood cells. The potential short-term toxic effects of chlorobenzene on circulating white blood cells and on cells of the immune system are apparently not sufficient to affect host susceptibility to experimental infection. Aranyi *et al* (1986) examined the effects of 14 chemicals on host defense mechanisms against experimentally induced streptococcus aerosol infection. The ability of lung alveolar macrophages to exert bactericidal activity against inhaled *Klebsiella pneumoniae* was also determined. Single and multiple 3-hr exposures of female CD1 mice to threshold limit value (TLV) concentrations of chlorobenzene (75 ppm) did not affect susceptibility to streptococcal infection, as measured by mortality of the exposed group. The bactericidal activity of lung alveolar macrophages against *Klebsiella pneumoniae* was also not affected by chlorobenzene. At these exposure levels, chlorobenzene was apparently not detrimental to murine lung host defenses.

Subchronic Toxicity

Chlorobenzene administered to experimental animals for several weeks or months produces effects mainly on liver and kidney, an extension of its acute toxic effects (Hellman, 1993). Increased liver and kidney weights relative to body weight, changes in histology of these organs, and elevation of serum enzyme activities are typical manifestations of chlorobenzene-induced toxicity. Repeated administration of relatively large doses to experimental animals also produced histological changes in the thymus, spleen, and bone marrow (Kluwe *et al*., 1985).

Chlorobenzene was administered orally to rats 5 days/week for a total of 137 doses over 192 days, at doses of 14.4, 144 or 288 mg/kg (Irish, 1963). In the middle- and high-dose groups, there were significant increases in liver and kidney weights and some "histopathological changes" in the liver. No significant changes were observed in the low-dose group. Blood and bone marrow were normal in all animals. A NOAEL of 10.3 mg/kg-day (adjusted for the 5 days/week dosing schedule) was identified in this study.

In a 13-week subchronic toxicity study on rats and mice, male and female F344/N rats and $B6C3F_1$ mice were given chlorobenzene by gavage 5 days/week for 13 weeks at 0 (corn oil; vehicle), 60, 125, 250, 500 or 750 mg/kg-day (NTP, 1985; Kluwe *et al*., 1985). Each group consisted of 10 animals of each sex and species. The animals were observed daily. Food consumption and body weights were measured weekly. Urine was collected during the last week of exposure, and at the end of the study. A blood sample was taken from the orbital venous plexus of each animal and analyzed. Clinical chemistry

measurements were performed on blood samples obtained during exposures and at the time of sacrifice. All animals were subjected to a complete gross examination. Multiple organs of the higher-dose animals and controls were taken for histopathological examination. Organs with observable changes, such as kidney, liver, and hematopoietic tissues, were then examined for histopathologic changes in lower-dose animals.

At doses of 250 and 500 mg/kg-day, body weight gain was decreased and mortality was increased in rats and mice. At 750 mg/kg-day, mortality exceeded 80 percent in rats and mice of both sexes. There were no consistent changes in hematological and urinary indices. Liver weights relative to body weight were increased in female and male rats in a dose-related manner beginning at 125 and 250 mg/kg-day, respectively. In male and female mice, the increases in relative liver weights were observed at 125 and 250 mg/kg-day, respectively. Histological examination showed chlorobenzene-induced lesions in the liver, kidney, spleen, bone marrow and thymus of both rats and mice. In the liver, hepatocellular degeneration and necrosis was detected in male rats at 250 mg/kg-day and larger doses. In the kidneys, vacuolar degeneration and focal coagulative necrosis of the proximal tubules were observed in rats and mice at 250 mg/kg-day and larger doses. Chlorobenzene produced moderate to severe depletion in lymphoid tissues and in the thymus of rats and mice of both sexes. In rats these effects were observed at 500 mg/kg-day and 750 mg/kg-day, but without a clear-cut dose-response relationship. In mice of both sexes, these effects were observed at 250 mg/kg-day and higher. Based on the results of this study, a NOAEL of 43 mg/kg-day (after adjustment of the 60 mg/kg dose for the 5 days/week dosing schedule) can be identified for both species based on the liver effects.

It should be noted that in a companion NTP (1985) two-year gavage study of chlorobenzene given to male and female F344/N rats, administration of chlorobenzene at doses of 60 and 120 mg/kg-day did not alter the body weights of the animals, and there were no overt signs of toxicity. Similarly, a two-year gavage administration of chlorobenzene to $B6C3F_1$ mice at doses of 30 mg/kg-day and 60 mg/kg-day (male) and 60 mg/kg-day and 120 mg/kg-day (female) did not produce overt signs of toxicity. Examination of the livers, kidney and hematopoietic tissues of the dosed animals at the end of 2 years did not reveal signs of organ toxicity. NTP (1985) suggested that these data indicated there was little potential for chlorobenzene to produce progressive non-neoplastic toxicity more severe than that observed in the 13-week studies in the rats and mice.

In an unpublished report cited by the U.S. EPA (1988a), groups of rats were also given chlorobenzene in the diet for 93 to 99 consecutive days at 0, 12.5, 50, 100 or 250 mg/kg-day. At the two higher doses, there were statistically significant elevations of liver and kidney weights. No significant histological changes were noted and no adverse effects were noted in the lower-dose groups. These data indicate a rat LOAEL of 100 mg/kg-day and a NOAEL of 50 mg/kg-day for subchronic administration of chlorobenzene in feed.

The subchronic toxicity of chlorobenzene, administered by oral administration or by inhalation, has also been investigated in dogs. The published information from the dog studies are limited to an abstract by Knapp *et al*. (1979), and industry reports, but the

latter have been thoroughly summarized by U.S. EPA (1988a). In an oral toxicity study, male and female beagle dogs were given chlorobenzene by gelatin capsule at 0, 27, 54, or 272 mg/kg-day 5 days/week for 13 weeks. Four of eight dogs in the highest dose group died within 3 weeks. At this dose level, histopathological changes were found in the liver, kidneys, gastrointestinal mucosa, and hematopoietic tissues. In addition, chlorobenzene produced a significant reduction of blood sugar, an increase in immature leukocytes, elevated serum ALT and alkaline phosphatase levels and, in some dogs, increases in plasma total bilirubin and total cholesterol. In the abstract, it was stated that there were no consistent signs of chlorobenzene-induced toxicity at the intermediate and low dose levels, but the U.S. EPA (1988a) concluded in their review that chlorobenzene-related hepatotoxicity, as evidenced by histopathological changes, was observed also among the animals in the intermediate dose-group (54 mg/kg-day), which was equivalent to 39 mg/kg-day after adjustment for the 5 days/week dosing schedule. Based on this study, the NOAEL for dogs given chlorobenzene via capsules was selected to be 19 mg/kg-day (adjusted).

In inhalation tests conducted by Industrial Biotest Laboratories, beagle dogs (4 males and females in each group) were reportedly exposed to 0, 750, 1,500 or 2,000 mg/m^3 of chlorobenzene vapors for 6 hr/day, 5 days/week for 90 days (U.S. EPA, 1988a). Assuming adult beagles were used with an inhalation rate of 3.5 m^3/day (U.S. EPA, 1988b), a pulmonary retention of 50 percent, and a body weight of 10 kg (Bartges *et al*., 1997; Raabe, 1986), it can be calculated that the inhalation doses in the study were 23, 46, and 62 mg/kg-day for the low-, mid-, and high-dosed groups, respectively. Some of the animals exposed to the two higher concentrations became moribund and were sacrificed after approximately 30 days. According to the U.S. EPA (1988a), exposures to chlorobenzene reduced body weight gain, lowered leukocyte counts, and elevated serum levels of alkaline phosphatase, ALT and aspartate aminotransferase (AST), and decreased the weights of the liver, heart and pancreas. At the high exposure concentration, histopathological changes were also reported to occur in the liver, bone marrow, seminiferous epithelium of the testes, and kidney tubules. It is not known if this particular Industrial Biotest Laboratories study was certified or validated, hence the reported findings must be interpreted with caution.

In another inhalation study conducted by Hazleton Laboratories for the Monsanto Company, dogs (six per sex and group) were exposed to 0, 780, 1,570, or 2,080 mg/m^3 of chlorobenzene, 6 hr/day, 5 days/week, for 6 months. At the two higher concentrations, adrenal gland weights were decreased in the male animals. There was an increased incidence of emesis in both male and female animals and an increased frequency of abnormal stools in treated females. The NOAEL in dogs, obtained by this inhalation study was 780 mg/m^3 (Hellman, 1993). Assuming an inhalation rate of 3.5 m^3/day (U.S. EPA, 1988b), a pulmonary retention of 50 percent, and a body weight of 10 kg (Bartges *et al*., 1997) for beagle dog, this is equivalent to a NOAEL of 24 mg/kg-day.

Genetic Toxicity

In a standard bacterial point mutation Ames assay using modified strains of *Salmonella typhimurium*, chlorobenzene dissolved in dimethyl sulfoxide was found not to be mutagenic with and without the presence of an activated rat liver mix (S-9 preparation). Preincubation of chlorobenzene in the test mixture of bacteria and liver enzymes also yielded negative results (Haworth *et al*., 1983; NTP, 1985). Chlorobenzene was also not mutagenic in a newer *in vitro* bioassay utilizing *Streptomyces griseus* (Bucholz *et al*., 1992).

The L5178Y mouse cell lymphoma assay is a test system for screening chemicals that produce forward mutations *in vitro*. Cultures were exposed to chlorobenzene for 4 hr and then cultured for 2 days, before plating in soft agar with or without trifluorothymidine (McGregor *et al*., 1988). Four experiments were performed without S-9 and two experiments were in the presence of S-9. The concentration of chlorobenzene ranged from 195 to 625 μg/ml without S-9 and from 70 to 190 μg/ml with S-9. The highest concentrations were toxic to the cells. Without S-9, two of the four tests yielded inconclusive results, the other two were positive, with a lowest effective concentration of 100 μg/ml). The two experiments with S-9 yielding positive responses also suggested a mutagenic effect of chlorobenzene (McGregor *et al*., 1988).

Chlorobenzene increased sister chromatid exchange in Chinese hamster ovary cells at concentrations of 300 and 500 μg/ml in the absence of S-9, but this effect was not observed in the presence of S-9 at tested concentrations up to 300 μg/ml (Loveday *et al*., 1989). When administered by intraperitoneal injection at doses of 225 to 900 mg/kg to male NMRI mice, chlorobenzene produced a dose-dependent increase in the number of micronucleated polychromatic erythrocytes as visualized from slides prepared from the femoral bone marrow (Mohtashamipur *et al*., 1987). The intraperitoneal LD_{50} of chlorobenzene in these mice was 1,355 mg/kg.

The ability of chlorobenzene to damage DNA in peripheral lymphocytes and bone marrow cells was examined after single and repeated intraperitoneal injections of 750 mg/kg to female C57BL/6 mice (Vaghef and Hellman, 1995). Cells selected under the microscope were subjected to electrophoresis and the fluorescent gel pattern quantified by computerized imaging. The appearance of "tailing" on the gel pattern indicated DNA damage. The positive control, the alkylating agent cyclophosphamide, produced clear-cut DNA damage in both peripheral lymphocytes and bone marrow cells. A single 750 mg/kg intraperitoneal dose of chlorobenzene did not affect the DNA of peripheral lymphocytes or bone marrow cells. This dose repeated for three days altered the DNA of the peripheral lymphocytes but not that of the bone marrow cells.

From these results (see Table 3), it appears that relatively high concentrations or doses of chlorobenzene, *in vitro* or *in vivo,* can produce positive effects in cytogenetic indicators of genetic damage. Chlorobenzene was negative in bacterial tests of genetic toxicity.

Table 3. Summary of Some Key Studies of the Genotoxicity of Chlorobenzene

End-point	Test system [species/strain]	Results and Comments	Reference
Gene mutations	Salmonella assay with and without S-9 [S. typhimurium strains TA1535, 1537, 1538, 98,100]	negative	NTP (1985); Haworth *et al*. (1983)
	Mouse cell lymphoma assay [L5178Y-cells] for forward mutations	positive in 2 out of 4 trials without S-9 and in 2 trials with S-9, lowest effective concentration 100 µg/ml	McGregor *et al*. (1988)
Clastogenic effects	Sister chromatid exchanges [Chinese hamster ovary cells]	positive in 1% DMSO with and without S-9 at 300 to 500 µg/ml	Loveday *et al*. (1989)
	Micronucleus tests; mouse *in vivo* [bone marrow cells]	positive at 225 to 900 mg/kg i.p.	Mohtashamipur *et al*. (1987)
	Altered DNA pattern of peripheral lymphocytes; mouse *in vivo*	positive after three 750 mg/kg i.p. injections	Vaghef and Hellman (1995)

Developmental and Reproductive Toxicity

The potential of chlorobenzene to produce embryotoxicity, teratogenicity (congenital malformations) or effects on reproduction was examined by studies in rats and rabbits (John *et al*., 1984). Female Fischer F344 rats were mated and then exposed to 0, 75, 210, or 590 ppm (0, 345, 966, or 2,714 mg/m^3) of chlorobenzene vapor for 6 hr/day from day 6 through day 15 of gestation, the period of major organogenesis. Assuming an inhalation rate of 0.26 m^3/day (U.S. EPA, 1988b), a percent retention of 50 percent, and a body weight of 0.2 kg, the inhalation doses were 56, 157, and 442 mg/kg-day for the low-, mid- and high-dosed groups, respectively. The animals were sacrificed on day 21 of gestation and the fetuses collected for examination. Among the parameters measured were: maternal body and liver weights, clinical signs of toxicity, number of live and dead fetuses, number of resorption sites; number of corpora lutea, the sex ratio of fetuses, body weight, crown-rump length of each fetus, and internal soft tissue and skeletal malformations. At the 590 ppm exposure level there was some evidence of maternal toxicity as evidenced by lowered food intake, reduced body weight gain, and increased liver weights. The incidence of malformations when considered individually or collectively was not significantly increased for any of the exposed groups when compared to the controls. Based on this study, a NOAEL of 157 mg/kg-day can be identified.

Additional experiments were performed by John *et al*. (1984) with pregnant rabbits. Female New Zealand White rabbits were artificially inseminated and exposed to 0, 75, 210, or 590 ppm (experiment 1) and to 0, 10, 30, 75, or 590 ppm (experiment 2) of chlorobenzene, 6 hr/day from day 6 to day 18 of gestation. Assuming an inhalation rate of 1.49 m^3/day (U.S. EPA, 1988b), a percent retention of 50 percent, and a body weight of 2 kg, the inhalation doses used in experiment 1 were 32, 90, and 252 mg/kg-day for the low-, mid-, and high-dosed groups, respectively. Similarly, the inhalation doses used in experiment 2 were 4.3, 13, 32, and 252 mg/kg-day for the 10, 30, 75, and 590 ppm groups, respectively.

Each group consisted of 30 to 32 rabbits. The animals were sacrificed on day 29 of gestation. The same types of observations on the fetuses were made as described above for rats. The only evidence of maternal toxicity detected was a significantly increased incidence of animals with enlarged livers in both groups (experiment 1 and 2) exposed to 210 ppm and 590 ppm. Based on this study, a maternal NOAEL of 32 mg/kg-day, corresponding to the exposures to 75 ppm, can be identified.

In the first experiment, the incidence of a variety of malformations in all groups, including the controls, was slightly higher than those observed in historical studies from the same laboratory. No chlorobenzene dose-related increase in malformations was evident with perhaps the exceptions of heart anomalies in the 210 ppm group and extra thoracic ribs (a skeletal malformation) in the 590 ppm group.

The second experiment was conducted to ascertain if the heart anomalies and skeletal malformations observed in the first study were, in fact, related to chlorobenzene exposure. The results did not indicate chlorobenzene-related increases for any type of malformation. There was some evidence of statistically increased incidence of percent of litters with resorption sites (indicating early embryonic deaths). The percentage of litters containing resorptions was 41 percent in the control group and 61 percent in the 590 ppm group, but this effect was not seen in the first experiment. The 61 percent incidence was considered to be within the range of historical control values for 21 similar teratology studies in the New Zealand White rabbit conducted in this laboratory. Overall, the experiments conducted on the pregnant rats and rabbits did not find evidence of teratogenic potential associated with chlorobenzene exposure. At the 590 ppm exposure conditions of 6 hr/day from day 6 to day 18 of gestation, there was some evidence of maternal toxicity and possible embryotoxic effects.

The potential of chlorobenzene to affect parameters of reproductive activity, namely, parental activities related to mating, fertility, pregnancy, lactation, and growth and development of offspring from conception through maturity, was examined in a two-generation reproductive study conducted on rats (Nair *et al*., 1987). Groups of 30 male and 30 female Sprague-Dawley rats (the F_0-generation) were exposed to 0, 50, 150, or 450 ppm (i.e. 0, 230, 690, or 2,070 mg/m^3) of chlorobenzene vapor for 10 weeks prior to mating and through mating, gestation, and lactation. The exposure took place 6 hr/day, 7 days/week. A selected number of the offspring from the F_0-generation (30 males and 30 females/group) formed the F_1-generation. These animals were then exposed to the same concentrations of chlorobenzene as the F_0-generation, starting one week post-weaning, and lasting for 11 weeks before mating, and through mating, gestation, and

lactation. The progeny of the F_1-generation, the F_2-pups, were observed during weaning, and then were sacrificed. A number of measurements were made, including body weights, food consumption, mating and fertility indices, pup and litter survival, and histopathological examinations of liver, kidneys, pituitary gland, and male and female reproductive organs.

No increased mortality was observed during the course of this study. Chlorobenzene did not affect the body weights or food consumption in any of the generations studied. Mating and fertility indices for males and females for both generations appeared unaffected by treatment. Pup and litter survival indices for all treated groups were comparable to those controls. At levels at or above 150 ppm, toxic effects of chlorobenzene were observed on liver and kidney. Histopathological examination showed dose-related changes in the livers, kidneys, and testes of F_0 and F_1 males exposed to chlorobenzene. The liver effects were manifested as hepatocellular hypertrophy and increased organ weights. The renal effects were tubular dilatation, interstitial nephritis and foci of regenerative epithelium (Table 4). The testicular effects consisted of degenerative changes of germinal epithelium, although no effects were observed on mating or fertility indices. The average daily dose in the F_0 males from a 150 ppm exposure would be 116 mg/kg-day assuming an inhalation rate of 0.027 m^3/day (U.S. EPA, 1988b), a percent vapor retention of 50 percent, and a body weight of 200 g; the 50 ppm exposure corresponds to approximately 39 mg/kg-day. Average daily doses in the F_1 rats cannot be readily estimated because of the changing physiological parameters. The effective daily dose at weaning from a 150 ppm exposure would be 129 mg/kg-day assuming an inhalation rate of 0.075 m^3/day (U.S. EPA, 1988b), a percent vapor retention of 50 percent, and a body weight of 50 g; 50 ppm corresponds to a weanling dose of 43 mg/kg-day. Average daily doses to the pups would be significantly greater, since relative breathing rates decrease during growth and development.

In summary, the studies of chlorobenzene in test animals did not give indications of teratogenic effects, potential effects on mating, fertility, pregnancy, and growth and development of the fetus and newborn. At exposure conditions toxic to the maternal organism (estimated LOAEL 90 mg/kg), there was some evidence of embryotoxicity (John *et al*., 1984). Also, at high exposure concentrations (150 and 450 ppm) in the study of Nair *et al*. (1987), there was evidence of direct toxicity to liver, kidneys and testes in males. Based on this study, a lowest-observed-adverse-effect-level (LOAEL) of 150 ppm, corresponding to a daily dose of 116 mg/kg, and a NOAEL of 50 ppm, corresponding to a daily dose of 39 mg/kg, can be identified.

Table 4. Frequencies of Liver, Kidney and Testicular Lesions in Male Rats Exposed to Chlorobenzene Vapor (data of Nair *et al*., 1987 re-tabulated by Hellman, 1993)

Organ	Lesion	Generation	Concentrations (ppm)			
			0	50	150	450
Liver	Hepatocellular hypertrophy	F_0	0/30	0/30	5/30	14/30
		F_1	2/30	0/30	3/30	7/30
Kidney	Tubular dilation-unilateral	F_0	0/30	3/30	2/30	3/30
	Tubular dilation-bilateral	F_0	0/30	1/30	4/30	15/30
	Tubular dilation-unilateral	F_1	4/30	4/30	6/30	6/30
	Tubular dilation-bilateral	F_1	4/30	3/30	8/30	16/30
	Interstitial nephritis-unilateral	F_0	0/30	0/30	0/30	1/30
	Interstitial nephritis-bilateral	F_0	1/30	2/30	7/30	9/30
	Interstitial nephritis-unilateral	F_1	1/30	2/30	1/30	0/30
	Interstitial nephritis-bilateral	F_1	0/30	1/30	6/30	11/30
Testes	Degeneration of germinal epithelium	F_0	1/30	0/30	2/30	6/30
	Degeneration of germinal epithelium	F_1	1/30	0/30	3/30	6/30

Immunotoxicity

Zub (1978) exposed mice to chlorobenzene vapor, either to 100 mg/m^3, 7 hr/day for 3 months, or to 25,500 mg/m^3, 7 hr/day for 3 weeks. Each exposure group consisted of 5 male and 5 female mice. Blood drawn from the tail vein during and at the end of the experiment was examined for white and red blood cells. Chlorobenzene was reported to induce a drop in the neutrophil count and an increase in lymphocytes. Assuming an inhalation rate of 0.05 m^3/day (U.S. EPA, 1988b) a pulmonary retention of 50 percent, and a body weight of 0.030 kg for mouse, it is estimated that the inhalation doses used in the study were 24 mg/kg-day and 6,200 mg/kg-day for the low- and high-dosed groups, respectively.

In the 13-week subchronic portion of the NTP studies, evidence of depletion of lymphoid tissues of the spleen and thymus were reported in rats and mice given chlorobenzene by gavage (NTP, 1985, Kluwe *et al*., 1985). In rats these effects were observed at the higher doses (500 and 750 mg/kg-day), and in mice at 250 mg/kg-day and higher. No consistent pattern of changes were noted in hematological values.

Neurotoxicity

Although chlorobenzene is recognized as a sedative from acute studies, no specific neurotoxicity studies were available. With repeated administration, adverse effects on liver and kidney presumably occur at doses lower than those that would cause overt neurotoxicity.

Chronic Toxicity and Carcinogenicity

Carcinogenic potential of chlorobenzene has been studied in rats and mice (Kluwe *et al*., 1985; NTP, 1985). In a two-year bioassay, male and female F344/N rats and male and female $B6C3F_1$ hybrid mice (50/sex/dose) were given chlorobenzene by gavage 5 days/week for 103 weeks. Rats and female mice were given 0 (corn oil; vehicle), 60 or 120 mg/kg-day, and male mice were given 0, 30, or 60 mg/kg-day. The study also included 50 untreated animals of each sex and species as untreated controls. The animals were weighed regularly and observed for mortality; upon sacrifice, complete necropsies were performed on all animals and tissues were taken for histopathological examination.

Chlorobenzene at these dosages for 2 years did not alter the body weights of the animals, and there were no overt clinical signs of toxicity. The survival rates were reduced for male rats at the high dose and male mice at both doses, but the survival rates of the other groups were not significantly affected.

In male mice, survival was 70 percent, 78 percent, 56 percent, and 58 percent in the untreated-control, vehicle-control, 30 and 60 mg/kg-day dose groups, respectively. Of the four low-dose male mice dying before week 52, three were moribund sacrifices without clear evidence of a toxic effect and one was severely autolyzed. Of the three high-dose male mice dying before week 52, two were found dead without evidence of toxic lesions and one was severely autolyzed. NTP (1985) concluded that these data did not indicate that chlorobenzene administration was the likely cause of the marginally reduced survival in male mice.

The livers of chlorobenzene-treated rats of both sexes showed a tendency toward lower incidences of inflammatory and cytoplasmic changes relative to control groups. The significance of this “sparing” or protective effect of chlorobenzene against age-related changes in liver histology is not known. The incidence of pituitary adenomas in both male and female rats at the high dose (120 mg/kg-day) was reduced as was the incidence of uterine stromal polyps in the low dose female rat (60 mg/kg-day). The reasons for the decreased incidences of these tumors in chlorobenzene-treated rats, and their biological significance, are not known.

The only tumor type observed at a statistically increased frequency in the chlorobenzene-exposed animals was neoplastic nodules of the liver in male high-dose rats (120 mg/kg-day). The increased incidence was significant by dose-related trend tests and by pair-wise comparisons between the combined controls and the highest dose group (Kluwe *et al*., 1985; NTP, 1985) (Table 5). The neoplastic nodules of the liver were generally considered to be late-occurring lesions. The first neoplastic nodule of the liver was

detected in a vehicle control male rat that died at week 89, and the majority were detected in all groups at study termination.

The occurrence of hepatocellular carcinomas in vehicle control male rats, 2/50 (4 percent), was greater than the program-wide recent historical rate for corn oil gavage male rats (7/789, 0.9 percent). The reason for the relatively high incidence of hepatocellular carcinomas in vehicle control male rats in this study is not known. Hepatocellular carcinomas were not diagnosed in untreated control or chlorobenzene-treated male rats in this study.

The tumor incidences in the male and female mice and in the female rats given chlorobenzene for two years did not exceed those in the corresponding vehicle or untreated controls. However, although not statistically significant, two rare tumor types were also observed in rats given chlorobenzene. These were transitional-cell papillomas of the urinary bladder (one male in the low dose group and one male in the high dose group) and a tubular-cell adenoma of the kidney (one female rat in the high dose group). The historical incidences of these tumors in Fischer F344/N rats were, at the time of the study, 0/788 for transitional cell-papilloma of the urinary bladder in corn-oil-treated males, and 0/789 for renal tubular-cell adenocarcinoma in female controls given corn oil (Kluwe *et al*., 1985; NTP, 1985).

Table 5. Liver Tumors in Male Rats Given Chlorobenzene Orally for up to Two Years (NTP, 1985)

Liver neoplastic nodules	Frequency (number with tumors/number examined)		
	Combined controls	60 mg/kg-day	120 mg/kg-day
Incidence after 2 years[1]	2/73	4/32	7/26*
Overall incidence	6/100	4/49	8/49*

[1] Tumor incidence at terminal sacrifice (after 2 years of exposure)
* Statistically significant compared with the controls, $p < 0.05$

The authors of the cancer study subsequently stated that the increased incidence of neoplastic liver nodules in male rats should be considered as “equivocal” evidence of carcinogenicity, and not sufficient to conclude that chlorobenzene is a chemical carcinogen (Kluwe, 1987). The conclusion that chlorobenzene caused a significant increase in the frequencies of neoplastic nodules in the liver male rats has been questioned. The Environmental Health Committee of the Science Advisory Board that reviewed this study in 1986 considered the study to be of good quality (U.S. EPA, 1999a). The Committee noted, however, that the NTP report did not specify the number of liver sections examined or which sections of the liver were examined. The location of the liver sections could presumably cause the difference in the number and the diagnosis of the lesions formed. The Committee also noted that liver nodules were not considered

necessarily to be progressive, and consequently lethal to the host. The statistical methods used in the NTP report have also been independently questioned by Roe *et al*. (1987).

Using a weight of evidence classification scheme, the U.S. EPA (1999a) currently designates chlorobenzene as a chemical "not classifiable as to human carcinogenicity" (Group D). The basis for this classification is the absence of human data on carcinogenicity, inadequate experimental data in animals, and predominantly negative results with chlorobenzene in short-term tests (bacterial) of genetic toxicity.

Toxicological Effects in Humans

Acute Toxicity

The scientific literature on human poisoning from chlorobenzene is sparse. There appear to be no documented cases of deaths from inhalation or ingestion of chlorobenzene. Reich (1934) reported a case of a two-year old boy who swallowed 5 to 10 ml of a solution containing chlorobenzene. The boy did not show any immediate signs of intoxication, but after 2.5 hr, he lost consciousness. The boy recovered, but the odor of chlorobenzene persisted in his breath and urine for 5 to 6 days. The human probable oral acute lethal dose of chlorobenzene has been estimated at 0.5-5 g/kg (Gosselin *et al*., 1984).

Babany *et al*. (1991) presented the only carefully described case of liver toxicity from chlorobenzene poisoning. A 40-year old man weighing 58 kg, who consumed large amounts of alcohol daily, attempted to commit suicide by swallowing 140 ml of a 90 percent chlorobenzene solution (2.41 g/kg). Two hours later, the man was drowsy but there was no loss of consciousness. The only other external manifestation of poisoning was diffuse erythema, sparing the face. Serum AST and ALT were markedly elevated 1 to 5 days after ingestion, accompanied by a fall in prothrombin activity of the blood. A liver biopsy on the third day after ingestion showed centrilobular and mediolobular necrosis, without inflammatory infiltration, hepatocyte ballooning, or fibrosis. There was no serological evidence of viral hepatitis in this patient. The serum enzyme changes and the decrease in prothrombin activity, together with the histopathology seen in the biopsy specimen, are indicative of chlorobenzene-induced liver damage. The clinical chemistry values returned to normal within 10 days and the patient recovered. It was noted in this patient that serum concentrations of chlorobenzene decreased exponentially with a single regression coefficient and a half-life of 40.3 hr.

In a controlled environmental setting, Ogata *et al*. (1991) exposed five volunteers to either 11.8 ppm or 60.2 ppm of chlorobenzene in a chamber for 3 hours in the morning and 4 hours in the afternoon with a 1 hour break in between. The subjects complained of sensations of disagreeable odor and drowsiness (100 percent), a heavy feeling in the head and/or headache (75 percent), throbbing pain in the eyes (50 percent) and a sore throat (25 percent) in these experiments. Assuming an inhalation rate of 20 m^3/day, an inhalation uptake of 50 percent, and an adult body weight of 70 kg, the estimated inhalation doses in the 7 hr period are 2.3 mg/kg and 12 mg/kg for the low- and

high-dosed groups. Although no objective measure of sedation was employed, the apparent LOAEL for sedative effects could be considered to be 2.3 mg/kg in this study.

Chronic Toxicity

Girard *et al*. (1969) reported the case of a 70-year old female working at home and exposed to glue containing 70 percent chlorobenzene for a period of 6 years. She presented with symptoms of headache, eye irritation, and upper respiratory discomfort. She was diagnosed as being anemic and having "medullary aplasia." No further details were given.

There appear to be no documented studies linking chlorobenzene exposures in an occupational setting to increased risks of liver, kidney, or hematopoietic toxicity (Hellman, 1993). A search of the scientific literature did not reveal any case reports or epidemiological investigations linking chlorobenzene exposures in humans to increased risks of developmental and reproductive toxicity, genetic toxicity, or cancer.

DOSE-RESPONSE ASSESSMENT

Carcinogenic Effects

The U.S. EPA (1999a) currently designates chlorobenzene as a chemical "not classifiable as to human carcinogenicity" (Group D). The basis for this classification is the absence of human data on carcinogenicity, inadequate experimental data in animals, and mostly negative results with chlorobenzene in short-term tests (bacterial, yeast, and mammalian cells) of genetic toxicity. However, *in vivo* assays do show evidence of clastogenicity.

In the single two-year carcinogenicity study of chlorobenzene in rats and mice, the authors stated that the increased incidence of neoplastic liver nodules in male rats at the high dose (120 mg/kg-day) may be considered as "equivocal" evidence of carcinogenicity, but not sufficient to conclude that chlorobenzene is a chemical carcinogen (Kluwe *et al*., 1985; Kluwe, 1987). The Environmental Health Committee of the Science Advisory Board that reviewed this study in 1986 (U.S. EPA, 1999a) noted that liver nodules were not considered necessarily to be progressive, and consequently lethal to the host. No dose-response evaluation was performed for this endpoint, although it is considered in the risk assessment.

Noncarcinogenic Effects

Data are inadequate to establish a dose-response relationship in humans for adverse health effects from exposure to chlorobenzene. The study by Ogata *et al*. (1971) tends to indicate that the safe acute exposure level in humans should be lower than 12 ppm in air, or a dose of 2.5 mg/kg, but this study is inadequate for derivation of health protective indices for repeated exposures.

Animal studies judged to be of sufficient quality to use in establishing safe exposure levels are summarized in Table 6. Of these studies, the most appropriate for the purposes of calculating a PHG for chlorobenzene in drinking water is the oral study in beagle dogs conducted by Knapp *et al*. (1979, as described in U.S. EPA, 1988a). Male and female dogs were given chlorobenzene by gelatin capsule at doses of 0, 27, 54, or 272 mg/kg-day 5 days/week for 13 weeks. Four of eight dogs in the highest dose group died within 3 weeks and adverse effects such as reduction of blood sugar, an increase in immature leukocytes, elevated serum ALT and alkaline phosphatase levels were observed. The U.S. EPA review of this study concluded that chlorobenzene-related hepatotoxicity was observed among the animals in the intermediate dose-group. For the purpose of this evaluation, a NOAEL of 27 mg/kg-day (19 mg/kg-day after adjustment for the 5 days a week dosing schedule) was selected for the derivation of a PHG.

The rat study reported by Irish (1963), which has the lowest NOAEL, was not selected as the basis for the development of a PHG for chlorobenzene because its LOAEL of 103 mg/kg-day was higher than the LOAELs of 89 mg/kg-day in a subchronic rat study and 86 mg/kg in a chronic rat study (NTP, 1985). The lower NOAEL of 10.3 mg/kg-day of the Irish (1963) study is related to the larger spacing between doses.

It should be noted that the NOAELs and LOAELs identified in two inhalation studies, one in dogs (Monsanto, as cited by Hellman, 1993) and one in rabbits (John *et al*., 1984) (Table 6) are only slightly higher than those identified in the oral dog studies. This evidence provides additional support for the selection of the 19 mg/kg-day NOAEL for the derivation of a PHG. Several studies in rats also show liver changes at less than five times the lowest relevant NOAEL.

The NOAEL of 19 mg/kg-day in the dog study of Knapp *et al* (1979) was also selected by U.S. EPA (1988a) for the calculation of safe drinking water levels.

Table 6. Summary of Candidate Studies for Derivation of a Chlorobenzene PHG

End point (effect)	Route/duration of administration	Species	LOAEL mg/kg-d	NOAEL mg/kg-d	Reference
Increased liver and kidney weights; hepatotoxicity	gavage, 5 days per week for 27 weeks	rat	103 §	10.3 §	Irish, 1963
Increased liver weight	gavage, 5 days per week for 13 weeks	female rat	89 §	43 §	NTP, 1985
Increased liver weight	gavage, 5 days per week for 13 weeks	male mouse	89 §	43 §	NTP, 1985
Increased liver and kidney weights	diet, 13 to 14 weeks	rat	100	50	U.S. EPA, 1988a
Liver toxicity	oral capsule, 5 days per week for 13 weeks	male and female dog	39 §	19 §	Knapp *et al*., 1979
Adrenal weight increased; emesis	inhalation, 6 hr/day 5 d/wk for 6 months	male and female dog	48	24	Monsanto, as cited by Hellman, 1993
Increased liver weight	inhalation, 6 hr/day, from GD 6 to GD 18	pregnant rabbit	90 (210 ppm)	32 (75 ppm)	John *et al*., 1984
Changes in liver, kidney, and testes morphology	inhalation, 6 hr/day, 7 days/wk prenatally and through mating, gestation, and lactation	male rat, F_0 and F_1	116 (150 ppm)	39 (50 ppm)	Nair *et al*., 1987
Increased neoplastic nodules in liver	oral, 5 days per week for 2 years	male rat	86 §	43 §	NTP, 1985

§ Dose adjusted for the 5 days/week dosing schedule.

CALCULATION OF PHG

Calculations of concentrations of chemical contaminants in drinking water associated with negligible risks for carcinogens or noncarcinogens must take into account the toxicity of the chemical itself, as well as the potential exposure of individuals using the water. Tap water is used directly as drinking water, for preparing foods and beverages. It is also used and for bathing or showering, and in washing, flushing toilets and other household uses resulting in potential dermal and inhalation exposures.

Carcinogenic Effects

In a series of two-year cancer bioassay of chlorobenzene in rats and mice (NTP, 1985), the tumor incidences observed in the treated male and female mice and in the treated female rats did not exceed those in the corresponding vehicle or untreated controls. The only tumor observed at a statistically increased frequency in the treated animals was neoplastic nodules of the liver in male high-dose rats.

The conclusion that chlorobenzene caused a significant increase in the frequencies of neoplastic nodules in the liver male rats has been questioned. The Environmental Health Committee of the Science Advisory Board that reviewed this study in 1986 considered the study to be of good quality (U.S. EPA, 1999a). The Committee noted, however, that the NTP report did not specify the number of liver sections examined or which sections of the liver were examined. The location of the liver sections could presumably cause the difference in the number and the diagnosis of the lesions formed. The Committee also noted that liver nodules were not considered necessarily to be progressive, and consequently lethal to the host.

Using a weight of evidence classification scheme, the U.S. EPA (1999a) currently designates chlorobenzene as a chemical "not classifiable as to human carcinogenicity" (Group D). The basis for this classification is the absence of human data on carcinogenicity, inadequate experimental data in animals, and predominantly negative results with chlorobenzene in short-term tests (bacterial) of genetic toxicity. OEHHA concurs with this assessment.

Noncarcinogenic Effects

Calculation of a public health-protective concentration (C, in mg/L) for chemicals in drinking water for noncarcinogenic endpoints follows the general equation:

$$C = \text{LOAEL or } \frac{\text{NOAEL} \times \text{BW} \times \text{RSC}}{\text{UF} \times \text{DWC}}$$

where,

NOAEL = no-observed-adverse-effect-level;

BW = adult body weight (a default of 70 kg);

RSC = relative source contribution (RSC) for the chemical in drinking water, usually in the range of 20 to 80 percent (0.2 to 0.8);

UF = uncertainty factor (which might include factors of 10 for inter-species extrapolation and human variability, 10 for extrapolating from a LOAEL to a NOAEL or from a subchronic study to chronic exposures, and other factors to acknowledge the potential for dire effects such as cancer, and database deficiencies); and

DWC = volume of drinking water consumed (2 L/day default, or a higher value if inhalation exposure from other uses of water such as bathing, showering, and washing dishes would be significant).

The critical toxicity value risk assessment in this case will be derived from the liver weight changes and hepatotoxicity. Chlorobenzene causes liver toxicity in multiple species in subchronic exposures at LOAELs of about 40 to 100 mg/kg-day. Other effects, including most commonly kidney changes, may also be observed at this or higher doses. NOAELs for the liver effects range from 10 to 50 mg/kg in both subchronic and chronic studies. The most appropriate study for extrapolation to humans appears to be the 13-week dog oral study of Knapp *et al*. (1979), which reported a LOAEL of 39 mg/kg-day and a NOAEL of 19 mg/kg-day. Because of the observation of an increased incidence of neoplastic nodules of the liver in the male high-dose rats following a 2-year dosing period, an uncertainty factor of 10 is used to account for the relatively short exposure in the dog study reported by Knapp *et al*. (1979).

Relative source contribution is assumed to be 30 percent. Chlorobenzene has been detected in both air and water in California. Mean ambient air levels of chlorobenzene in urban areas of California were found to range from 0.1 ppb (0.46 $\mu g/m^3$) to 3.4 ppb (15.6 $\mu g/m^3$) (HSDB, 1998). Assuming an average concentration of 0.5 ppb (2.3 $\mu g/m^3$), an inhalation rate of 20 m^3/day, and 50 percent uptake and retention, the dose resulting from the air is estimated to be 23 µg/day. Only trace levels of chlorobenzene levels have been detected in a small number of food samples; dietary intake of chlorobenzene is believed to be insignificant compared to inhalation and water consumption. Assuming an equivalent water consumption rate of 4 Leq/day and a contaminant level of 3 ppb (average concentration measured in California drinking water sources) (DHS, 1998), the dose associated with water consumption is estimated to be approximately 12 µg/day, which is about 30 percent of the total exposure.

An uncertainty factor of 10 was used to account for inter-species extrapolation, 10 for intra-species variability, and 10 for the relatively short exposure duration.

A health-protective concentration (C) of chlorobenzene in drinking water is therefore calculated as:

$$C = \frac{19 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.3}{1{,}000 \times 4 \text{ Leq/day}} = 0.1 \text{ mg/L or } 100 \text{ ppb}$$

OEHHA therefore proposes a PHG of 100 µg/L (100 ppb) for chlorobenzene in drinking water based on liver toxicity, with the specific value derived from a non-cancer toxicity study reported by Knapp *et al*. (1979).

RISK CHARACTERIZATION

The general population may be exposed to chlorobenzene via inhalation of ambient air, ingestion of food and drinking water, and dermal contact with consumer products containing chlorobenzene. Typical chlorobenzene concentrations in cities in the U.S. ranged from not detectable to 0.8 ppb; the maximum value measured was 12 ppb. Mean ambient air levels of chlorobenzene in California cities ranged from 0.1 to 3.4 ppb. According to the monitoring data collected by Department of Health Services of California (DHS, 1998) between 1984 and 1997, a total 115 water samples were reported positive for chlorobenzene. The maximum, mean, and minimum levels detected in drinking water sources of California were 5.4, 2.6, and 0.6 μg/L, respectively

Chlorobenzene has been detected at low concentrations (ppb level) in a number of food products such as clam chowder, fish, crab, peanut butter, garlic dressing, and flour. Traces of chlorobenzene (0.37 ppb average) have also been detected in mother's milk.

The acute toxicity of chlorobenzene, as determined by the LD_{50}, categorizes this chemical as "slightly toxic" with an estimated single oral lethal dose in adult humans of 1 pint (250 g). The amount of human data on the toxicity of chlorobenzene is, however, limited, despite the long-standing use of this chemical in the occupational environment. No reports of fatalities from chlorobenzene were found, but liver toxicity has been documented in a poisoning case.

Inhalation of chlorobenzene vapors is apparently irritating to the eyes and the mucous membranes of the upper respiratory tract. In a study of 5 male volunteers exposed to 12 ppm or 60 ppm (55 mg/m^3 or 275 mg/m^3) for 7 hr, these concentrations of chlorobenzene induced symptoms such as drowsiness, headache, irritation to the eyes, and sore throat (Ogata *et al*., 1991). Assuming an inhalation rate of 20 m^3/day and an adult body weight of 70 kg, the estimated inhalation doses in the 7-hr period are 2.3 and 12 mg/kg for the low- and high-dosed groups, respectively. Due to the small number of subjects in the study and the short duration of the exposure, results of this study were not used as the basis for PHG determination.

Single dose or repeated administration of chlorobenzene to experimental animals for several weeks or months produces adverse effects mainly on the liver and kidneys. These organs are the primary targets for chlorobenzene-induced toxicity and characterization of these risks are the main basis for setting guidelines. The hepatotoxicity of chlorobenzene is revealed as increased activities of serum enzymes such as ALT and AST, altered liver histology, and increased liver weight. Toxic effects seen in other organs after chronic dosage include lesions of the thymus (lymphoid depletion and necrosis), spleen (lymphoid or myeloid depletion), leukopenia, increased lung weights, and degeneration of the germinal epithelium of the testes. These effects, however, appear to occur at dosages higher than those required to produce hepatotoxicity.

The pathways of metabolic transformation and activation of chlorobenzene to a hepatotoxin have been characterized in experimental animals. Chlorobenzene is converted to electrophilic epoxides by cytochrome P-450-dependent microsomal enzymes. The 2,3-chlorobenzeneoxide pathway is considered to be the source of the

toxic reactant. Similar reactive intermediates have been associated with carcinogenicity for other substituted benzenes.

There is some ambiguity in the assessment of the genotoxic properties of chlorobenzene. In the Ames *Salmonella typhimurium* assay, chlorobenzene is clearly inactive. However, in high concentrations *in vitro*, chlorobenzene has been shown to be positive in the L5178Y mouse cell lymphoma assay, and in the sister chromatid exchange assay. Chlorobenzene, administered at large doses (750 mg/kg) *in vivo*, is positive in the micronucleus test and alters DNA in peripheral lymphocytes but not in bone marrow cells. Overall, chlorobenzene may be characterized as having marginal genotoxic potential.

Animal experiments in rats and rabbits did not reveal any significant teratogenic activity from chlorobenzene. A two-generation reproductive toxicity study in rats did not show any chlorobenzene-induced adverse effects on reproductive performance or fertility. In a series of two-year cancer bioassays (NTP, 1985) chlorobenzene induced benign liver tumors in male rats, but was without tumorigenic effects in female rats and in male and female mice. The limited and equivocal evidence of carcinogenicity in experimental animals, combined with marginal evidence of genotoxicity and the absence of evidence of increased cancer risks in occupational exposures, implies that chlorobenzene, at present, should be regarded as an agent not classifiable as to human carcinogenicity (U.S. EPA classification of Group D).

Choice of the subchronic dog study with a NOAEL of 19 mg/kg rather than the chronic rat study results in the use of a lower toxicity value, but requires an additional safety factor of 10 for the uncertainty associated with the short-term study. Another 10-fold factor is used for interspecies extrapolation from dogs to humans, and 10-fold for variation in sensitivity among humans. Basing the calculation on the potentially most-exposed population and combined uncertainty factors of 1,000 should be adequate to protect against adverse effects in infants, children, and any other potential sensitive subgroups.

Based on a non-cancer toxicity study reported by Knapp *et al*. (1979), OEHHA proposes a PHG of 100 µg/L or (100 ppb) for chlorobenzene in drinking water.

OTHER REGULATORY STANDARDS

U.S. EPA has classified chlorobenzene as a group D carcinogen, based on inadequate or no human and animal evidence of carcinogenicity (HSDB, 1998).

For occupational exposures, the Occupational Safety and Health Administration (OSHA) Standard for Permissible Exposure Limit, 8-hr Time Weighted Average, is 75 ppm (345 mg/m^3). The American Conference of Governmental Industrial Hygienists (ACGIH, 1998) Threshold Limit Values (TLV) for Chemical Substances and Physical Agents Biological Exposure Indices for 1998 set an 8-hr Time Weighted Average (TWA) of 10 ppm (46 mg/m^3) for chlorobenzene (ACGIH, 1998).

The U.S. EPA has established a Maximum Contaminant Level Goal (MCLG) and a Maximum Contaminant Level (MCL) of 0.1 mg/L (100 ppb) for chlorobenzene in

drinking water (U.S. EPA, 1999b). This value is based on liver pathology observed in dogs as reported by Knapp *et al*. (1979). From this study, a NOAEL of 19 mg/kg-day (adjusted for 5 days per week dosing schedule) was identified. U.S. EPA applied an uncertainty factor of 1,000 (to account for sensitive human subpopulations, extrapolation from an animal study, and for use of a study which was less than lifetime) and derived a reference dose (RfD) of 0.02 mg/kg-day. WHO (1984) recommended a guideline for chlorobenzene of 3 ppb based upon avoidance of taste and odor problems.

California developed a Proposed Maximum Contaminant Level (PMCL) of 30 ppb for chlorobenzene (DHS, 1988). The PMCL was based on a LOAEL of 43 mg/kg-day based on the neoplastic formation observed in male F344 rats (NTP, 1985), a relative source contribution of 20 percent, an overall uncertainty factor of 10,000, an adult body weight of 70 kg, and a drinking water consumption rate of 2 L/day. The overall uncertainty factor includes a factor of 10 for LOAEL to NOAEL extrapolation, a factor of 100 to account for inter- and intra-species variations, and a factor of 10 to account for the potential carcinogenicity of the chemical.

California, Arizona, Maine, Minnesota, and New Jersey have state drinking water standards for chlorobenzene of 70, 50, 47, 60, and 100 ppb, respectively (HSDB, 1998).

REFERENCES

ACGIH (1998). Threshold limit values for chemical substances and physical agents, pocket version. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

Aranyi C, O'Shea WJ, Graham JA, Miller FJ (1986). The effects of inhalation of organic chemical air contaminants on murine lung host defenses. Fundam Appl Toxicol 6:713-720.

Azouz WM, Parke DV, Williams RT (1952). Studies in detoxication. 42. Fluorobenzene. Spectrophotometric determination of the elimination of unchanged halogenobenzenes by rabbit. A comparison of the oxidation *in vivo* of fluorobenzene and of benzene. Biochem J 50:702-706.

Babany G, Bernuau J, Cailleux A, Cadranel JF, Degott C, Erlinger S, Benhamou JP (1991). Severe monochlorobenzene-induced liver cell necrosis. Gastroenterology 101:1734-1736.

Bartges JW, Osborne CA, Felice LJ, Koehler LA, Ulrich LK, Bird KA, Chen M (1997). Influence of two diets on pharmacokinetic parameters of allopurinol and oxypurinol in healthy beagles. Am J Vet Res 58(5):511-515.

Brittebo E, Brandt I (1984). Metabolism of chlorobenzene in the mucosa of the murine respiratory tract. Lung 162:79-88.

Buchholz SE, Omer CA, Viitanen PV, Sariaslani FS, Stahl RGJ (1992). Activation and detection of (pro)mutagenic chemicals using recombinant strains of Streptomyces griseus. Appl Biochem Biotechnol 32:149-158.

Cameron GR, Thomas JC, Ashomore SA, Buchan JL, Warren EH (1937). The toxicity of certain chlorine derivatives of benzene, with special reference to o-dichlorobenzene. J Pathol Bacteriol 44:281-296.

Dalich GM, Larson RE (1985a). A comparative study of the hepatotoxicity of monochlorobenzene in the rainbow trout (Salmo gairdnerii) and the Sprague-Dawley rat. Comp Biochem Physiol C 80:115-122.

Dalich GM, Larson RE (1985b). Temporal and dose-response features of monochlorobenzene hepatotoxicity in rats. Fundam Appl Toxicol 5:105-116.

Den Besten C, Vet JJRM, Besselink HT, Kiel GS, van Berkel BJM, Beems R, van Bladeren PJ (1991). The liver, kidney and thyroid toxicity of chlorinated benzenes. Toxicol Appl Pharmacol 111: 69-81.

DHS (1998). Personal communication with Dr. David Storm, California Department of Health Services. Sacramento CA. December 1998.

DHS (1988). Proposed maximum contaminant level: monochlorobenzene. Community Toxicology Unit (currently Office of Environmental Health Hazard Assessment,

Cal/EPA, Oakland/Sacramento), Department of Health Services, State of California, Berkeley.

Girard, R, Martin P, Bourret J (1969). Haemopathies graves et exposition a des derives chlores du benzene (a propos de 7 cas). J Med Lyon 50:771-3 (in French).

Gosselin RE, Smith RP, Hodge HC (1984). Clinical Toxicology of Commercial Products, 5th ed. Williams and Wilkins, Baltimore, pp. 11-170.

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983). Salmonella mutagenicity test results for 250 chemicals. Environ Mutagen [Suppl] 1:3-142.

HSDB (1998). Chlorobenzene. Hazardous Substances Databank, National Library of Medicine's Toxicology Data Network. Accessed May, 1999.

Hellman B (1993). Basis for an Occupational Health Standard: Chlorobenzene. National Institute for Occupational Safety and Health. DHHS (NIOSH) Publication No. 93-102.

Irish DD (1963). Halogenated hydrocarbons. II. Cyclic. In: Patty's Industrial Hygiene and Toxicology, Vol. 2, DW Fassett and DD Irish, eds. Interscience Publishers, New York, NY. pp. 1333-1362.

John JA, Hayes WC, Hanley TRJ, Johnson KA, Gushow TS, Rao KS (1984). Inhalation teratology study on monochlorobenzene in rats and rabbits. Toxicol Appl Pharmacol 76:365-373.

Kerger BD, Roberts SM, James RC (1988). Comparison of human and mouse liver microsomal metabolism of bromobenzene and chlorobenzene to 2- and 4-halophenols. Drug Metab Dispos 16:672-677.

Kluwe WM (1987). Effect of monochlorobenzene on rat liver reconsidered [letter]. J Toxicol Environ Health 21:536-538.

Kluwe WM, Dill G, Persing R, Peters A (1985). Toxic responses to acute, subchronic, and chronic oral administrations of monochlorobenzene to rodents. J Toxicol Environ Health 15:745-767.

Knapp WK, Jr., Busey WM, Kundzins W (1979). Subacute oral toxicity of monochlorobenzene in dogs and rats. Toxicol Appl Pharmacol 19:393 [Abstract]

Korzekwa KR, Swinney DC, Trager WF (1989). Isotopically labeled chlorobenzenes as probes for the mechanism of cytochrome P-450 catalyzed aromatic hydroxylation. Biochemistry 28:9019-9027.

Kumagai S, Matsunaga I (1995). Effect of variation of exposure to airborne chlorobenzene on internal exposure and concentrations of urinary metabolite. Occup Environ Med 52:65-70.

Leber, AP, Benya, TJ (1994). Halogenated benzenes. Chap. 22, Patty's Industrial Hygiene and Toxicology, 4th Edition, Vol. 2, Part B., GD Clayton and FE Clayton, eds. pp. 1443-1501.

Lecca-Radu R (1959). Modifications of blood carbonic anhydrase and leucocytic indophenol oxidase in chronic benzene and monochlorobenzene intoxication. Igiena 8:231-240.

Loveday KS, Lugo MH, Resnick MA, Anderson BE, Zeiger E (1989). Chromosome aberration and sister chromatid exchange test in Chinese hamster ovary cells *in vitro*: II. Results with 20 chemicals. Environ Mol Mutagen 13:60-94.

McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Riach C, Caspary WJ (1988). Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals [erratum: Environ Mol Mutagen 1988;12(3):345]. Environ Mol Mutagen 12:85-154.

Mohtashamipur E, Triebel R, Straeter H, Norpoth K (1987). The bone marrow clastogenicity of eight halogenated benzenes in male NMRI mice. Mutagenesis 2:111-113.

Nair RS, Barter JA, Schroeder RE, Knezevich A, Stack CR (1987). A two-generation reproduction study with monochlorobenzene vapor in rats. Fundam Appl Toxicol 9:678-686.

Nedelcheva V, Gut I, Soucek P, Frantik E (1998). Cytochrome p450 catalyzed oxidation of monochlorobenzene, 1,2-and 1,4-dichlorobenzene in rat, mouse, and human liver microsomes. Chem-Biol Interact 115:53-70.

NTP (1985). Technical Report on the Toxicology and Carcinogenesis Studies of Chlorobenzene (CAS No. 108-90-7) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). National Toxicology Program, U.S. Dept. Health and Human Services. NTP TR 261, NIH Publication No. 86-2517, October 1985.

OEHHA (2001a). Determination of noncancer chronic reference exposure levels. Batch 2A, December 2000. Chlorobenzene. Office of Environmental Health Hazard Assessment, Oakland, California (www.oehha.ca.gov/air/chronic_rels/AllChrels.html).

OEHHA (2001b). Non-Significant Risk Levels. Office of Environmental Health Hazard Assessment, Oakland, California (www.oehha.ca.gov/prop65/getNSRLs.html).

Oesch F, Jerina DM, Daly JW, Rice JM (1973). Induction, activation and inhibition of epoxide hydrase: an anomalous prevention of chlorobenzene-induced hepatotoxicity by an inhibitor of epoxide hydrase. Chem Biol Interact 6:189-202.

Ogata M, Shimada Y (1983). Differences in urinary monochlorobenzene metabolites between rats and humans. Int Arch Occup Environ Health 53:51-57.

Ogata M, Taguchi T, Hirota N, Shimada Y, Nakae S (1991). Quantitation of urinary chlorobenzene metabolites by HPLC: concentrations of 4-chlorocatechol and chlorophenols in urine and of chlorobenzene in biological specimens of subjects exposed to chlorobenzene. Int Arch Occup Environ Health 63:121-128.

Raabe OG (1986). Inhalation uptake of selected chemical vapors at trace levels. Final Report to the California Air Resources Board, Contract No. A3-132-33.

Raabe OG (1988). Retention and metabolism of toxics. Inhalation uptake of xenobiotic vapors by people. Final report to the California Air Resources Board, Contract No. A5-155-33.

Reich H (1934). Puran (Monochlorbenzene) poisoning in a 2-yr old child. Samml. Von Vergiftungsfallen 5:193-194.

Reid WD (1973). Mechanism of renal necrosis induced by bromobenzene or chlorobenzene. Exp Mol Pathol 19:197-214.

Reid WD, Krishna G (1973). Centrolobular hepatic necrosis related to covalent binding of metabolites of halogenated aromatic hydrocarbons. Exp Mol Pathol 18:80-99.

Roe FJ, Lee PN, Major IR (1987). Effect of monochlorobenzene on rat liver [letter]. J Toxicol Environ Health 21:535-538.

Rozenbaum ND, Block RS, Kremneva SN, Ginzburg SL, Pozhatiskii IV (1947). Use of chlorobenzene as a solvent from the standpoint of industrial hygiene. Gig Sanit 12(1):21-24.

Selander HG, Jerina DM, Daly JW (1975). Metabolism of chlorobenzene with hepatic microsomes and solubilized cytochrome P-450 systems. Arch Biochem Biophys 168:309-321.

Spencer B, Williams RT (1950). Studies in detoxication. 33. The metabolism of halogenobenzene. A comparison of the glucuronic acid, etheral sulphate and mercapturic acid conjugations of chloro-, bromo- and iodo-benzenes and of the o-, m- and p-chlorophenols. Biosynthesis of o-, m- and p-chlorophenylglucuronides. Biochem J 47:279-284.

Sullivan TM, Born GS, Carlson GP, Kessler WV (1983). The pharmacokinetics of inhaled chlorobenzene in the rat. Toxicol Appl Pharmacol 71:194-203.

U.S. EPA (1988a). Drinking Water Health Document on Monochlorobenzene. U.S. Environmental Protection Agency, Washington, D.C. National Technical Information Service PB89-192116.

U.S. EPA (1988b). Recommendations for and documentation of biological values for use in risk assessment. Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, Cincinnati, OH. EPA 600/6-87/008.

U.S. EPA (1992). Dermal exposure assessment: principles and applications. Interim Report. Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, Cincinnati, OH. EPA 600/8-91/011B.

U.S. EPA (1999a). Chlorobenzene. Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency (www.epa.gov/ngispgm3/iris).

U.S. EPA (1999b). Drinking Water and Health. National Primary Drinking Water Regulations – Technical fact sheet on: Chlorobenzene. U.S. Environmental Protection Agency (www.epa.gov/ogwdw000/dwh/t-voc/chlorobe.html).

U.S. EPA (2001). Toxics Release Inventory (TRI). Chlorobenzene. U.S. Environmental Protection Agency (www.epa.gov/triexplorer/explorer.htm).

Vaghef H, Hellman B (1995). Demonstration of chlorobenzene-induced DNA damage in mouse lymphocytes using the single cell gel electrophoresis assay. Toxicology 96:19-28.

Wang MJ, Jones KC (1994). Occurrence of chlorobenzenes in nine United Kingdom retail vegetables. J Agric Food Chem 42:2322-2328.

Willhite CC, Book SA (1990). Toxicology update: chlorobenzene. J Appl Toxicol 10:307-310.

WHO (1984). Guidelines for drinking water quality, Vol. 1. Recommendations. World Health Organization, Geneva.

Yoshida M, Sunaga M, Hara I (1986). Urinary metabolite levels in workers exposed to chlorobenzene. Ind Health 24:255-258.

Zub M (1978). Reactivity of the white blood cell system to toxic action of benzene and its derivatives. Acta Biol Cracoviensia Ser: Zoologia 21: 163-174.