

**NIOH and NIOSH basis for an occupational  
health standard**

**Chlorobenzene**



**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES**  
Public Health Service  
Centers for Disease Control and Prevention  
National Institute for Occupational Safety and Health



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# **NIOH and NIOSH Basis for an Occupational Health Standard:**

## Chlorobenzene

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## PREFACE

A memorandum of understanding has been signed by two government agencies in the United States and Sweden—the Division of Standards Development and Technology Transfer of the National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services (DSDTT/NIOSH), and the Criteria Group of Occupational Standard Setting, National Institute of Occupational Health (NIOH) (formerly Research Department of the National Board of Occupational Safety and Health). The purpose of the memorandum is to exchange information and expertise in the area of occupational safety and health. One product of this agreement is the development of documents to provide the scientific basis for establishing occupational exposure limits. These limits will be developed separately by the two countries according to their different national policies.

This document on the health effects of occupational exposure to chlorobenzene is the fifth product of that agreement. This document was written by Dr. Björn Hellman, Department of Occupational Medicine, University Hospital, Uppsala, Sweden, and was reviewed by the Criteria Group and by DSDTT/NIOSH.

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## ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
ALAT	serum alanine aminotransferase
ASAT	serum aspartate aminotransferase
BUA	Beratergremium für umweltrelevante Altstoffe der Gesellschaft Deutscher Chemiker (Germany)
b.wt.	body weight
CNS	central nervous system
DNA	deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
GC	gas chromatography
GSH	reduced glutathione
GSSG	oxidized glutathione
HD	highest dose group
HPLC	high performance liquid chromatography
IBT	International Bio-Test Inc. (USA)
IARC	International Agency for Research on Cancer
i.p.	intraperitoneal (injection)
i.v.	intravenous (injection)
LC <sub>50</sub>	the concentration (in air or water) needed to produce death in 50 percent of exposed animals; in the present document LC <sub>50</sub> refers to inhalation concentration
LD <sub>50</sub>	the dose of chemical needed to produce death in 50 percent of dosed animals
LOEL	lowest observed effect level
m-	meta- (positions 1,3- in a disubstituted ring structure)
MD	medium dose group
NIOH	National Institute of Occupational Health (Sweden)
NIOSH	National Institute for Occupational Safety and Health (USA)

NOAEL	no observed adverse effect level
NOEL	no observed effect level
NTP	National Toxicology Program (USA)
o-	ortho- (positions 1,2- in a disubstituted ring structure)
OSHA	Occupational Safety and Health Administration (USA)
p-	para- (positions 1,4- in a disubstituted ring structure)
ppm	parts per million
RNA	ribonucleic acid
SCEs	sister chromatid exchanges
SKF 525-A	$\beta$ -diethylaminoethyl diphenylpropyl acetate
TLV	threshold limit value (relates to concentration in air at workplaces)
TWA	time-weighted average exposure

## 1 INTRODUCTION

Chlorobenzene is one of twelve possible chemical species in the group of chlorinated benzenes (36). At room temperature, the substance is a colorless volatile liquid with an odor that has been described as “not unpleasant” (63), like that of “mothballs or benzene” (25) and “almondlike” (1, 32). The compound has been used extensively in industry for several years and its main use is as a solvent and intermediate in the production of other chemicals (19, 25, 32). In occupational settings, the main exposure is from inhalation of the volatile compound.

The present document summarizes and evaluates information that has been considered most relevant for the assessment of the potential adverse health effects from occupational exposure to chlorobenzene. To achieve this objective, a literature search was performed in different biomedical and toxicological databases (e.g., in Medline; Cancerlit; Toxline; Excerpta Medica; National Technical Information Service; Healthline and Chemical Safety Newsbase) before the assessment was initiated (July, 1991).

The U.S. Environmental Protection Agency (EPA) has recently prepared a health effects criteria document (final draft) for chlorobenzene (32) as well as an updated health effects assessment (33). A similar document has also been prepared in Germany (19). These, and other reviews (e.g., 2, 6, 25, 68, 92), have been included among the references in the present document.

The various health criteria documents mentioned above have included information from several unpublished toxicity studies, mainly performed by, or on behalf of, various manufacturers of chlorobenzene. Some unpublished investigations are also cited in the present document, although the primary sources of information often were unavailable for critical examination. In such a case, this has been indicated with a short remark, provided with the citation.

## 2 PHYSICAL AND CHEMICAL PROPERTIES

If not stated otherwise, the data on the physical and chemical properties of chlorobenzene were obtained from various reference books and review articles (1, 6, 7, 15, 19, 25, 26, 32, 63). Although not always declared, it is assumed that the figures given refer to chlorobenzene of analytical quality. Only scarce information on amounts and identities of potential impurities was available. In a teratogenicity study on rats and rabbits (48) using >99.9% pure chlorobenzene, it was stated that incidental impurities found consisted of benzene (<0.005%), bromobenzene (0.018%) and water (0.0077%). Chlorobenzene of technical quality from one of the German manufacturers is at least 99.8% pure, containing at most 0.06% dichlorobenzenes and 0.08% benzene, as the major impurities (19).

Chemical name:	Chlorobenzene
CAS number:	108-90-7
Synonyms:	Monochlorobenzene; MCB; monochlorbenzene; benzene chloride; benzene monochloride; chlorbenzene; CB; chlorbenzol; phenyl chloride
Physical state at room temperature:	Colorless, neutral liquid
Molecular formula:	C <sub>6</sub> H <sub>5</sub> Cl
Structural formula:	
Molecular weight:	112.56
Boiling point [101.3 kPa]:	13-132°C
Melting point:	-44.9°C [in ref. (6): -45.6°C]

Solidification (freezing) point:	-55°C
Flash point:	28°C
Vapor pressure [25°C]:	11.8 mm Hg = 15.81 hPa (25°C) 8.8 mm Hg = 11.73 hPa (20°C)
Saturation concentration in air [25°C]:	1.55% (15,526 ppm)
Vapor density [25°C]: (air = 1)	3.88
Specific density (gravity) [20°C; 25°C]:	1.107
Refractive index [25°C]:	1.5216
Solubility in water:	Practically insoluble (0.049 g/100 ml; 20°C)
Solubility in organic solvents:	Freely soluble in alcohol, benzene, chloroform, diethyl ether
Partition coefficient n-octanol/water:	log P <sub>ow</sub> : 2.84 (58)
Other partition coefficients:	Water/air: 4.1 (79) Olive oil/air: 3,763 (79) Olive oil/water: 918 (79) Blood/air: 30.8 (71, 79)
Odor threshold: (see comments below)	In air: 0.21-0.68 ppm In water: 0.4-50 µg/l
Conversion factors [25°C; 101.3 kPa]:	1 ppm = 4.60 mg/m <sup>3</sup> 1 mg/m <sup>3</sup> = 0.217 ppm

The information on human thresholds for the detection of chlorobenzene is not uniform. In air, the recognition odor has been reported to vary between 0.21 ppm (7) and 0.68 ppm (3, 92) (i.e., between 1 and 3.1 mg/m<sup>3</sup>). However, in another source of information (91) it is stated that the almondlike odor of chlorobenzene is barely perceptible at 60 ppm (276 mg/m<sup>3</sup>). The air-dilution threshold given by Amoores and Hautala (3), 0.68 ppm, represents the geometric average of all available literature data, omitting extreme points and duplicate quotations.

The substance is practically insoluble in water, but the two liquids form an azeotrope that boils at 90°C (32). On surface water, chlorobenzene is believed to evaporate rapidly to the air. However, due to its greater density, chlorobenzene may also sink to the bottom of still volumes of water (32, 39). The reported taste/odor thresholds in water varies between 0.45–1.5 µg/l (this interval is based on the work by Tarkhova from 1965, cited in references 6 and 32) and 10–20 µg/l (based on the work by Varshavskaya from 1967, cited in references 6 and 32), respectively. However, these figures were considered difficult to interpret since none of the citations described the experimental conditions employed (32). In another, more recent work, the odor threshold for chlorobenzene in water was reported to be 50 µg/l (3). This so-called water-dilution odor threshold value was calculated from the concentration of the substance in water that would generate the air odor threshold (estimated to 0.68 ppm) in the headspace of a stoppered flask.

### 3 USES AND OCCURRENCE

The figures given below for production volumes, ambient air levels, etc., have mainly been obtained from secondary sources of information (6, 19, 32, 36, 49). Consequently, the original sources, often unpublished information, have generally not been evaluated in detail.

#### 3.1 Production and Uses

Like other chlorinated benzenes, monochlorobenzene is commercially produced by the chlorination of benzene at an elevated temperature. This is done in the presence of a chlorination catalyst such as ferric chloride (36, 39, 63). Chlorobenzene may also be produced by treating phenol with aqueous sodium hydroxide under high pressure and in the presence of chloride (39).

Chlorobenzene is one of the most widely used chlorinated benzenes and it has been the dominant commercial isomer for at least 50 years. The compound has been utilized in numerous processes. Previously, its main uses were as a chemical intermediate in the synthesis of DDT and other organochlorine pesticides, and in the production of phenol and aniline (36, 92). During the first world war, it was also used in large quantities in the production of picric acid, which was utilized as an explosive (92). Its principal use today is as a chemical intermediate in the production of chemicals such as nitrochlorobenzenes and diphenyl oxide (19, 32, 36). In 1989, 76% of the total amount of chlorobenzene manufactured in the Federal Republic of Germany was processed into nitrochlorobenzenes (19). These compounds are subsequently used as starting products for crop protection agents, dyestuffs and rubber chemicals (19). Chlorobenzene is also used as a solvent in degreasing processes (e.g., in metal cleaning operations) and in the dry cleaning industry. It serves as a solvent for paints, adhesives, waxes and polishes and has also been used as a heat transfer medium (6, 63, 92) and in the manufacture of resins, dyes, perfumes and pesticides (92).

Although the annual production rates for chlorobenzene in the United States [140,000 tons in 1975; 130,000 tons in 1981 and 116,000 tons in 1984 (6, 32, 36, 49)], show a decreasing trend, it has been estimated that the consumption of chlorobenzene would grow at an average annual rate of 1-2% in the United States (37). Large manufacturers of chlorobenzene in the United States are Monsanto Co., PPG Industries, and Standard Chlorine Chemical Co. (19). In the late 1970s, approximately 500 tons of chlorobenzene was imported into the United States (32).

In 1989, a total of 60,000 to 70,000 tons of chlorobenzene was produced in the Federal Republic of Germany by two different manufacturers, Bayer AG and Hoechst AG (19). In 1985, the total production in Western Europe was estimated to be 82,000 tons (19). In Eastern Europe, the total production of chlorobenzene in 1988 was calculated

to be 200,000–250,000 tons (19). The production volume in Japan was 28,300 tons in 1988 (19).

According to the Products Register at the National Chemicals Inspectorate [U. Rick, personal communication], monochlorobenzene occurred in ten different chemical products in Sweden in 1990. The estimated annual use of the compound that year in Sweden was 11 to 64 tons. There is no production of chlorobenzene in Sweden.

### 3.2 Occupational Exposure and Ambient Air Levels

The amount of data available with regard to the potential exposure to chlorobenzene in various types of occupational settings is limited. In Sweden, for example, the National Board of Occupational Health and Safety (an authority responsible for protecting workers who handle chemicals in the workplace from ill-health and accidents) had no information available on the present exposure levels of monochlorobenzene at Swedish workplaces.

A monitoring program of air levels in chlorobenzene- and nitrochlorobenzene-producing plants in the United States, which was performed 1978/79, showed that the chlorobenzene concentrations varied from not detectable to 18.7 mg/m<sup>3</sup> (19). Similar concentrations of chlorobenzene have been reported in various field investigations conducted by NIOSH at different types of workplaces in the United States (69c-g). In a Japanese study on the urinary levels of chlorobenzene-associated metabolites in workers at two different plants handling the compound (96), the chlorobenzene levels varied between 7.8–26.7 mg/m<sup>3</sup>, with a geometric mean of 14.5 mg/m<sup>3</sup> (3.15 ppm). A similar study from Belgium (56) showed variations in chlorobenzene concentrations ranging from 0.2–488 mg/m<sup>3</sup>, the median value being 5.5 mg/m<sup>3</sup> (1.2 ppm).

There are no natural sources for chlorobenzene and most releases result from its use as a solvent (6). The substance is delivered into the environment mainly with exhaust air and waste water from production plants, processing industries and from its use as a solvent. In the atmosphere, chlorobenzene is anticipated to degrade slowly by free radical oxidation. Due to its high volatility, chlorobenzene is expected to evaporate rapidly into air when released to surface water, but when released to the ground it has been assumed to first bind to the soil and then migrate slowly to the ground water (6). In January 1987, there was an accidental release of approximately 450 tons of monochlorobenzene to the Baltic Sea outside Kotka, Finland (39). Because the sea was calm and covered with ice, it was believed that most of the chlorobenzene sank to the bottom of the sea. The environmental consequences of this release are not known.

Chlorobenzene is resistant to biodegradation as well as to chemical and physical degeneration (6, 32). In accordance with its relatively high lipid solubility, it has been shown to bioaccumulate in, for example, fish and algae (6). In 1978 it was estimated that almost a total of 80,000 tons of chlorobenzene were released to the atmosphere each year in the United States (32). Apart from an occupational exposure, humans may be exposed to chlorobenzene from drinking water, food, ambient air and consumers' products.

Based on various national surveys, the U.S. EPA has estimated the concentrations of chlorobenzene to be less than 1-5  $\mu\text{g/l}$  in groundwater, and less than 1  $\mu\text{g/l}$  in surface water (32). The magnitude of the potential dietary intake of chlorobenzene was not estimated since the available data were considered insufficient (32). The median concentration of chlorobenzene in ambient air of urban and suburban areas has been calculated at 1.5  $\mu\text{g/m}^3$  (32). Various measurements performed in Germany and The Netherlands showed that the average outdoor air levels of chlorobenzene varied between 0.3 and 1.5  $\mu\text{g/m}^3$  (19).

Like other volatile halogenated hydrocarbons, chlorobenzene may very well be present in the indoor air of, for example, household settings in amounts exceeding those of the ambient air. When the indoor air concentrations of chlorobenzene were measured in the Bavarian city, Hof, Germany, these were found to vary between 0.1 and 4  $\mu\text{g/m}^3$ , with a geometric mean of 0.5  $\mu\text{g/m}^3$  (19). Somewhat higher indoor air concentrations were found in various cities in the USA, ranging between not detectable and 72.2  $\mu\text{g/m}^3$ , with an average of 16.5  $\mu\text{g/m}^3$  (19).

Chlorobenzene may be formed during the biotransformation of other compounds. It has, for example, been shown that chlorobenzene is a major metabolite of hexachlorocyclohexane, better known as the insecticide Lindane, at least when Lindane is incubated with rat liver microsomes under anaerobic conditions (14).

To summarize, although chlorobenzene may be present in ambient air, the levels are generally considerably lower than those that can be found in industries manufacturing or processing chlorobenzene.

### 3.3 Analytical Methods for Air Monitoring

NIOSH manual of analytical methods (69) describes a standardized method for sampling and analysis for chlorobenzene in ambient air. The method was revised in 1987 (69b). First a known volume of air is drawn through a charcoal tube to trap the organic vapors present. Then the charcoal in the tube is transferred to a stoppered sample container where the amount of chlorobenzene adsorbed to the charcoal is eluted with carbon disulfide. An aliquot of the desorbed sample is then injected into a gas chromatograph with a flame ionization detector (GC-FID). The amount of chlorobenzene present in the sample is determined by measuring and comparing the areas under the resulting peaks from the sample and those obtained from the injection of standards.

Sampling can be done either actively with adsorption tubes or passively through personal air sampling using passive diffusion techniques. It seems as if most investigators have preferred personal air sampling using a passive organic solvent sampler in the breathing zone, when they measured the occupational exposure to chlorobenzene (56, 71, 96). Using personal air sampling and GC analysis, the detection limit has been reported to be 0.05 ppm (0.23  $\text{mg/m}^3$ ) for an exposure time of 8 hr in an industrial setting (56). Alternatives to the GC-FID technique have also been used for the analysis of ambient air levels of chlorobenzene; for example, high pressure liquid chromatography (96). To confirm the identity of the compound, GC can be combined with mass spectrometry (71).

A similar technique is used when the amount of chlorobenzene is determined in water samples. The procedure used is the so-called purge-and-trap gas chromatographic procedure, a standard method for the determination of volatile organohalides in drinking water (6). An inert gas is bubbled through the sample so that chlorobenzene is trapped on an adsorbent material. The adsorbent is then heated to drive chlorobenzene onto a GC column.

### **3.4 Present Occupational Standards**

In 1989, ACGIH adopted a TLV-TWA of 10 ppm (46 mg/m<sup>3</sup>) for the occupational exposure to chlorobenzene in the United States (2). Their previous limit of 75 ppm (345 mg/m<sup>3</sup>) was considered low enough to prevent narcotic effects or chronic poisoning (92). The suggested reduction from 75 to 10 ppm was done to prevent chlorobenzene-induced injuries to the liver and kidneys. The current OSHA permissible exposure limit is 75 ppm (71b).

The maximum concentration value (the so-called MAK value) of chlorobenzene allowed in occupational settings in the Federal Republic of Germany was established as 50 ppm (230 mg/m<sup>3</sup>) in 1971 (26); a concentration that still seemed valid in 1990 (56). At present, there are no standards or recommendations for occupational exposure to monochlorobenzene in Sweden (88).

Maximum recommended concentrations in workplace air in the USSR and Czechoslovakia have been reported to be 10 and 43 ppm, respectively (92).

## 4 TOXICOKINETICS

### 4.1 Uptake

Chlorobenzene is absorbed via respiratory and dermal routes, but no quantitative experimental data was found on the pulmonary or dermal absorption rates. It is generally assumed that chlorobenzene is not readily absorbed through the skin, but prolonged contact can result in mild chemical burns (2, 91).

Consequently, in occupational settings inhalation of vapors is regarded as the major route of exposure for chlorobenzene, dermal contact being of minor importance. An unpublished simulation study by Droz, cited by ACGIH (2), suggests that the pulmonary retention at steady-state is about 60 percent.

### 4.2 Distribution

Various experimental studies have shown that, after being absorbed, chlorobenzene is distributed rapidly to various organs.

**Animals:** The toxicokinetics of inhaled chlorobenzene has been studied by Sullivan and coworkers and reported in two different papers (86, 87). The study has also been briefly reviewed in a short notice (4). Male Sprague-Dawley rats were exposed to <sup>14</sup>C-chlorobenzene (uniformly labelled) at 100, 400 or 700 ppm (460, 1,840 or 3,220 mg/m<sup>3</sup>) for 8 hr/day; either one day only or for five consecutive days. Each group consisted of six animals. Immediately after the last exposure, three rats from each group were sacrificed for determination of chlorobenzene-associated radioactivity in liver, kidneys, lungs, adipose tissue and blood. The remaining rats were kept in metabolism cages for 48 hr before they were sacrificed. The vapor concentrations of chlorobenzene were monitored with an infrared gas analyzer at 9.25 μm.

Adipose tissue was found to accumulate the largest amounts of radioactivity. The percentage of chlorobenzene-associated radioactivity in fat, presumably representing unchanged substance, was found to increase at higher exposure levels. In the other tissues investigated, the <sup>14</sup>C-levels were increased in proportion to the exposure concentration; liver and kidneys being the dominant organs. Lung and blood contained 25-50% and 10-30%, respectively, of the amounts found in the liver. When the exposure concentration was increased from 100 to 400 ppm (from 460 to 1,840 mg/m<sup>3</sup>), there was an increase of over ten-fold of the exhaled amount of radioactivity, presumably representing unchanged substance. A further increase to 700 ppm (3,220 mg/m<sup>3</sup>) caused another seven-fold increase of the exhaled amounts. The data showed that the metabolic clearance from the blood became saturated at an exposure

concentration of 400 ppm for 8 hr. At this exposure, there was also a reduced predominance of the excreted amount of mercapturic acid (the only urinary metabolite investigated) in relation to the total amount of radioactivity excreted in the urine. Consequently, the observed dose-related changes in various pharmacokinetic parameters in rats suggests that the metabolic elimination of chlorobenzene becomes saturated at high dose levels.

Maximum liver concentrations of chlorobenzene-associated radioactivity in male Sprague-Dawley rats given  $^{14}\text{C}$ -chlorobenzene as a single i.p. injection was seen 24 hr after the administration (22). The radioactivity represented both the parent compound and its metabolites.

The distribution and fate of nonvolatile radioactivity from uniformly labelled  $^{14}\text{C}$ -chlorobenzene has also been studied in female C57BL mice, using whole-body autoradiography (16). Six mice were given a single i.v. injection of the labelled compound diluted with unlabelled substance (1.2 mg/kg b.wt.; 7  $\mu\text{Ci}$  in DMSO). The survival times were 1 and 5 min; 1, 4, and 24 hr; and 4 days, respectively. Two other mice were injected i.p. and killed after 4 and 24 hr, respectively. Whole-body autoradiograms from heated tissue sections showed a selective localization of nonvolatile metabolites in the mucosa of the entire respiratory system 1 min after an i.v. injection. The labelling of the mucosa of the respiratory tract was persistent and still present 4 days after the injection. Microautoradiography showed that the chlorobenzene-associated radioactivity was bound to the epithelium of the tracheo-bronchial mucosa. Uptake of nonvolatile radioactivity was also observed in other tissues 1 and 5 min after the i.v. injection, although not to the same extent as in the respiratory tract. Relatively high amounts of nonvolatile metabolites of chlorobenzene were also 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.

**Humans:** Due to its high lipid solubility, chlorobenzene can be anticipated to accumulate in human fat, and possibly in milk. However, none of the recognized studies on chlorobenzene levels in human fat and breast milk samples from the general population included monochlorobenzene among the various isomers measured (23, 47, 64). The chlorobenzenes analyzed in the monitoring programs generally included various isomers of dichlorinated and trichlorinated benzenes and pentachlorobenzene and hexachlorobenzene.

### 4.3 Biotransformation

Like other monosubstituted halogenated benzenes, chlorobenzene is oxidized by the microsomal cytochrome P-450 system to reactive epoxide intermediates (also known as arene oxides). These have not actually been isolated and identified, but their presence has been deduced from the various metabolic end-products of chlorobenzene that have been isolated and identified, both in vitro and in vivo. Covalent binding of chlorobenzene-related epoxides to various tissue constituents has provided a convenient explanation for the cytotoxic effects observed in various organs after the administration of the otherwise unreactive chlorobenzene (for further discussion see p. 18). The epoxides are converted either nonenzymatically to various chlorophenols or enzymatically to the corresponding glutathione (GSH) conjugates

and dihydrodiol derivatives. The GSH conjugates are either eliminated as such, or transformed to even more water-soluble products and excreted in the urine as mercapturic acids. The dihydrodiol derivatives are converted to catechols and excreted as such in the urine.

**Experimental animals and cultured cells:** It has been known for a long time that chlorobenzene and other halogenobenzenes are transformed in the body into phenols and mercapturic acids. As early as 1950, Spencer and Williams (84) were able to show that Chinchilla rabbits (sex was not specified) given a single oral dose of chlorobenzene (150 mg/kg b.wt.) excreted 52 percent of the given dose as oxygen conjugates (25 percent as glucuronides and 27 percent as ethereal sulphates) and 20 percent as sulphur conjugates (mercapturic acids). The figures were based on excretion data from three rabbits. Subsequently performed experiments on rabbits showed that the first step in the metabolism of chlorobenzene was the oxidation of the aromatic nucleus, resulting in an epoxide as an intermediate precursor for the further metabolism (see, e.g., reference 59).

Lindsay Smith et al. (59) gave an emulsion of  $^{14}\text{C}$ -chlorobenzene in Cremophore E.L. and physiological saline orally to two female Dutch rabbits ( $0.5 \text{ g} \times 2/\text{day}$  for 4 days). During the four days of dosing and the three following days, urine and feces were collected separately. The amounts excreted via feces were negligible. The major metabolites identified in the urine were p-chlorophenylmercapturic acid and conjugates of 4-chlorocatechol (i.e., ethereal sulphates). Other urinary metabolites identified were quinol, 3-chlorocatechol and ortho- and m-chlorophenylmercapturate.

The hepatic metabolism of chlorobenzene in vitro was studied extensively in various experimental systems by Selander and coworkers (81). They employed both perfused rat livers from male Sprague-Dawley rats and various cell-free hepatic preparations: postmitochondrial supernatants, microsomes and reconstituted soluble hemoprotein systems. The experiments were conducted using  $^{14}\text{C}$ -labelled chlorobenzene diluted with appropriate amounts of unlabelled substance, with and without the addition of various inducers and inhibitors of cytochrome P448/P450 monooxygenases. The formation of chlorophenols and dihydrodiols was determined using HPLC-technique. Whereas the pretreatment of rats with 3-methylcholantrene, a cytochrome P448-inducer, resulted in a significant and selective increase in the formation of ortho-chlorophenol in all hepatic systems employed, a similar pretreatment with the cytochrome P450-inducer phenobarbital only resulted in a moderate increase of ortho- and para-chlorophenol. Various inhibitors of the cytochrome P450 and/or P448 systems such as carbon monoxide, metyrapone, SKF-525A and 7,8-benzoflavone, affected the biotransformation of chlorobenzene in various ways. Whereas the formation of ortho-, meta- and para-chlorophenols was inhibited by carbon monoxide and metyrapone, the addition of SKF-525A and 7,8-benzoflavone was found to inhibit the formation of ortho- and p-chlorophenol to a greater extent than that of m-chlorophenol. The addition of high concentrations of glutathione reduced the formation of all three chlorophenols.

Consequently, once absorbed, one of the first steps in the metabolic conversion of chlorobenzene is the formation of a mixture of chlorophenols. The cytochrome P450/P448 monooxygenase system is involved in the formation of ortho- and para-chlorophenol. It is during

these reactions, two different reactive epoxides are formed as intermediate species. One of the epoxides, chlorobenzene-3,4-epoxide, rearranged to p-chlorophenol. The other chlorobenzene-related epoxide, chlorobenzene-2,3-epoxide, isomerize to o-chlorophenol (50). The third chlorophenol that is formed during the metabolic biotransformation of chlorobenzene, m-chlorophenol, is probably formed by direct oxygen insertion in the parent compound (55, 81).

In a study where the *in vitro* hepatic microsomal formation of halophenols from chlorobenzene and bromobenzene was investigated in both human and mouse liver microsomes (50), important differences were observed between the metabolic pathways suggesting that humans may be more susceptible than mice to halobenzene-induced hepatotoxicity. Mouse liver microsomes were prepared from untreated male B6C3F1 mice (livers from 35 mice were pooled). Human liver microsomes were made from transplants obtained from three different donors who suffered acute head injuries in accidents. Mixtures containing microsomal proteins and various co-factors were incubated with either chlorobenzene or bromobenzene. The formation of halophenols was studied using a selective HPLC method with electrochemical detection (HPLC/ECD-technique).

The metabolism of chlorobenzene to ortho- and p-chlorophenol followed the same pattern as that of bromobenzene, both in human and mouse liver microsomes, indicating that both compounds were metabolized by the same cytochrome P450/P448-isozymes. Microsomes from the mouse liver contained approximately five times more cytochrome P450 than those taken from the livers of the three donors, but the production of p-halophenols was only two times greater in the mouse liver enzymes. When the production of p-halophenols (i.e., the metabolic pathway that has been associated with the hepatotoxicity of chlorobenzene and bromobenzene) was expressed relative to the cytochrome P450 content (i.e., nmol of halophenol produced/min/nmol of cytochrome P450), the human liver microsomes were twice as efficient as the mouse liver microsomes. Moreover, in comparison to the mouse liver microsomes, human cytochrome P450-isozymes produced less of the nonhepatotoxic o-halophenols. Whereas the ratio of para- to ortho-halophenol production was 1.3 for bromobenzene and 1.4 for chlorobenzene in the mouse microsomes, the average ratio was 4.8 for both compounds in the human microsomes. The human liver microsomes also had a slightly greater affinity for chlorobenzene and bromobenzene than the mouse microsomes. Taken together, these *in vitro* results indicate that the main metabolic pathway of chlorobenzene in human liver microsomes is through the hepatotoxic 3,4-epoxide pathway.

Studies on the metabolism of chlorobenzene have mainly been restricted to the liver. However, experiments performed *in vitro* with tissue slices prepared from pieces of nasal mucosa, lung, and liver taken from female C57BL mice, showed that chlorobenzene can also be transformed to nonextractable metabolites in extrahepatic organs (16). In these experiments, tissue slices were incubated with <sup>14</sup>C-labelled chlorobenzene (5 μM; 0.3 μCi) in a phosphate buffer containing glucose, and in the presence of oxygen, for 15, 30 or 60 min. Incubation mixtures with tissue slices heated for 10 min were used as controls. All three organs investigated were found to produce metabolites that could not be removed by extensive organic solvent treatment; nasal mucosa being the most efficient tissue. After 60 min of incubation,

the nasal mucosa had produced approximately 0.8, the lung 0.4 and the liver 0.2 pmoles <sup>14</sup>C-metabolites/mg wet weight tissue. In a second series of experiments, the effect of various mixed function oxidase inhibitors on the extrahepatic metabolism of chlorobenzene, was investigated using tissue slices from nasal mucosa and lung (16). The formation of nonextractable metabolites in vitro was decreased by metyrapone, piperonylbutoxide and SKF-525A, clearly showing that the metabolism of chlorobenzene is also cytochrome P450-dependent in these organs.

The major metabolites of chlorobenzene in man appear to be p-chlorophenol and 4-chlorocatechol (2). These are eliminated in the urine as sulphate and glucuronide conjugates in the urine. Apparently, the metabolic pathways in man differ somewhat from those in rabbits and other experimental animals. Para-chlorophenol is, for example, only a minor urinary metabolite of rabbits (12), and a major excretion product in rabbit urine, p-chlorophenyl mercapturic acid, is excreted only in min amounts in human urine.

The proposed metabolic pathways for chlorobenzene are shown in Figure 1 (p. 14). The scheme is based on in vitro findings and various experimental toxicokinetic data (2, 11, 16, 50, 55, 59, 81, 84, 86, 87), as well as human urinary excretion data (56, 70, 71, 96). The latter studies are discussed in more detail in the sections reviewing data on the elimination pattern and biological exposure indicators.

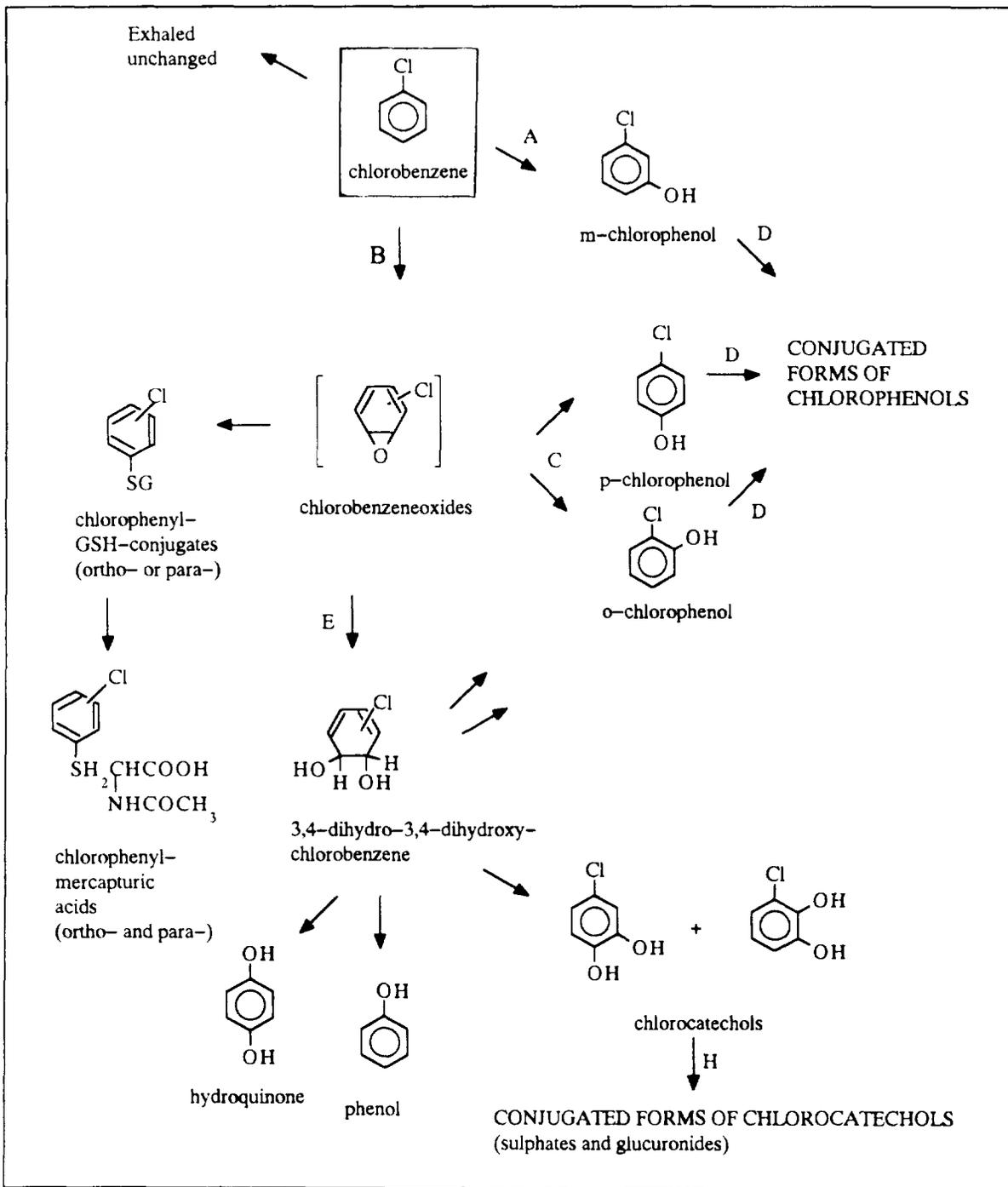
#### 4.4 Elimination

There are three potential routes of elimination for inhaled or ingested chlorobenzene, via the expired air, via the urine, and via feces. Although the eliminated amount of unchanged chlorobenzene in the expired air may be as high as 60% depending on the exposure conditions and species involved, urinary excretion of various chlorobenzene-associated metabolites is no doubt the dominant route of elimination for chlorobenzene. Excretion of unchanged substance via urine or feces is consequently unimportant. At the dose levels humans normally are exposed to, most of the chlorobenzene absorbed is believed to be metabolized and then excreted in the urine, predominantly as free and conjugated forms of 4-chlorocatechol and chlorophenols.

**Animals:** Experiments on three Chinchilla doe rabbits given a single oral dose of 500 mg chlorobenzene/kg b.wt., showed that the eliminated amount of unchanged chlorobenzene in the expired air was as high as 24-32% during the first 30 hr following the administration of the compound (11). Another experiment on Chinchilla rabbits given a single oral dose of 150 mg chlorobenzene/kg b.wt. (84), showed that 72 percent of the given dose was eliminated as various conjugates in the urine within two days after the administration.

Although the route of administration seems of minor importance for the elimination pattern of chlorobenzene, dose levels and dosing schedule may have some influence.

In the previously mentioned pharmacokinetic study of inhaled <sup>14</sup>C-chlorobenzene (86, 87), it was shown that multiple exposures of rats at doses saturating the metabolic pathways, versus a single exposure at a dose not saturating the biotransformation of chlorobenzene, resulted in



**Figure 1: Proposed metabolic pathways for chlorobenzene**

- A Hydroxylation
- B Cytochrome P450/P448-dependent microsomal oxidation
- C Rearrangement
- D Conjugation: glucuronosyl transferases and sulphotransferases
- E Epoxide hydratase

higher tissue levels of radioactivity (notably in adipose tissue), a lowered total excretion of chlorobenzene-associated radioactivity, a lesser percentage of the total amount excreted through respiration and a change in the rate of respiratory excretion. Consequently, rats exposed to 100 ppm ( $460 \text{ mg/m}^3$ ) for 8 hr, excreted only 5% of the total dose via exhalation and 95% in the urine. Repeated exposure to 700 ppm ( $3,220 \text{ mg/m}^3$ ), 8 hr/day for 4-5 days, resulted in exhalation of 32% of the total dose, the urinary excretion being 68%.

In a study of the liver toxicity of chlorobenzene in male Sprague-Dawley rats given the compound as a single i.p. injection (22), it was found that the fraction of the total dose excreted in the urine within 24 hr decreased as the dosage of chlorobenzene increased. At the lowest dose tested, 2.0 mmol/kg b.wt. (225 mg/kg), 59% of the total dose was excreted in the urine, but at the highest dose, 14.7 mmol/kg (1,655 mg/kg), the corresponding figure was only 19% (all of the excreted products represented metabolites).

In an investigation on the potential differences between various species with regard to the elimination pattern of chlorobenzene, rats, mice, and rabbits were given an i.p. injection of 0.5, 1 or 2 mmol (i.e., 56, 112 or 225 mg) monochlorobenzene/kg b.wt. (70). An additional group of rats was also given chlorobenzene orally at a dose of 0.3 mmol/kg b.wt. (34 mg/kg); the substance was administered diluted in polyethylene glycol. No information was given on the number or strains of the mice and rabbits, but the elimination pattern in the rats was established from data obtained from four females of the Wistar strain. Also included in the study was one male volunteer given chlorobenzene orally ( $3 \times 0.3 \text{ mmol/kg b.wt.}$ ) and two occupationally exposed workers with estimated inhalation exposures of  $0.84 \text{ ppm} \times 415 \text{ min}$  and  $0.5 \text{ ppm} \times 228 \text{ min}$ , respectively. Urinary samples were collected periodically and the amounts of two different chlorobenzene associated metabolites, p-chlorophenylmercapturic acid and 4-chlorocatechol were measured using HPLC. There was a dose-related increase of the excreted amounts of both metabolites in the urine from the rats, mice, and rabbits. However, whereas the mercapturic acid derivative was the dominant excretion product in the urine of the animals, it was only a fraction of the amount of 4-chlorocatechol collected in the urine from the chlorobenzene exposed humans.

In another study (55), chlorobenzene was diluted in corn oil and given to ten male Wistar rats as a single i.p. injection (500 mg/kg b.wt.). Four rats were pretreated with 80 mg phenobarbital/kg b.wt., 54 hr before the chlorobenzene injection. Twenty-four-hr urinary samples were collected over a period of seven days and analyzed for the presence of p-chlorophenylmercapturic acid, various chlorophenols and guanine adducts using different chromatographic techniques. The major urinary metabolite identified was p-chlorophenylmercapturic acid, the total amount excreted being 13.5 mg after six days. Most of the p-chlorophenylmercapturic acid was excreted during the first 24 hr (65% of the total amount). The pretreatment with phenobarbital did not significantly affect the elimination pattern of this particular metabolite. The excretion of para-, meta- and ortho-chlorophenol was significantly lower. The total amount of free chlorophenols was 1.1 mg after 6 days. The corresponding figure for free and conjugated chlorophenols was 2.55 mg. The ratio of free para- to meta- to ortho-chlorophenols was 4:3:1 and that for free and conjugated forms 3:2.3:1. Pretreatment with phenobarbital was found to have a significant effect on the elimination pattern of the various chlorophenols. The

excretion of para- and meta-chlorophenol was twice as high in rats given phenobarbital before chlorobenzene as compared to the amounts excreted by those given chlorobenzene alone. In the case of o-chlorophenol, there was a fourfold increase of the excreted amount in the phenobarbital-induced rats. A DNA-adduct, probably identical with N7-phenylguanine, was also present in the urine 1 and 2 days after the injection, and between day 4 and 6 after the administration. The total amount of adduct excreted in the urine was low (29 µg after 6 days) and was not affected by the pretreatment with phenobarbital.

**Humans:** As indicated above, the elimination pattern of chlorobenzene-associated metabolites in humans appears to differ from that observed in experimental animals (70). It was shown in a Japanese field study (96), for example, that 11 persons occupationally exposed to 1.7–5.8 ppm (7.8–26.7 mg/m<sup>3</sup>) chlorobenzene for 8 to 11 hr, excreted more than 75% of the urinary metabolites as 4-chlorocatechol, and more than 20% as various chlorophenols (the dominant isomer being p-chlorophenol). A main urinary metabolite of chlorobenzene in rats and rabbits, 4-chlorophenylmercapturic acid, was present only in insignificant amounts (0.4% of the total amount of the chlorobenzene-related urinary metabolites). Chlorophenylmethylsulfides were not detected at all. A similar study from Belgium on 44 chlorobenzene-exposed workers (56) showed that more than 80% of the excreted 4-chlorocatechol and p-chlorophenol in the urine was eliminated within 16 hr after the end of exposure (i.e., end of shift). Both studies are described in more detail under the section “Biological Exposure Indicators.”

In a controlled exposure chamber study (71), five male volunteers were exposed for 7 hr to either 12 or 60 ppm (55 or 276 mg/m<sup>3</sup>) monochlorobenzene. Elimination curves for major urinary metabolites were calculated using pharmacokinetic models. In the calculations, the exposure was standardized to 1 ppm chlorobenzene and it was assumed that the absorption rate for chlorobenzene in the lung was 100%. Two-compartment models gave the following estimated half-lives for 4-chlorocatechol: 2.2 hr (phase I; fast) and 17.3 hr (phase II; slow). The corresponding half-lives for p-chlorophenol were 3.0 and 12 hr, respectively. When the data were fitted to a one-compartment model, the biological half-lives of 4-chlorocatechol and p-chlorophenol were estimated to be 2.9 and 7 hr, respectively.

#### 4.5 Biological Exposure Indicators

Measurements of chlorobenzene in blood, and possibly also exhaled air, can be used for monitoring purposes (2, 71). However, the best biological exposure indicator for chlorobenzene in humans is, as previously discussed, the presence of 4-chlorocatechol and p-chlorophenol in the urine.

The urinary concentrations of 4-chlorocatechol and p-chlorophenol are determined by HPLC (56, 70, 96). Various protocols have been used, but one way is to treat the urine with perchloric acid at 95°C, and then extract the metabolites with diisopropyl ether. The ether fraction is evaporated and the residue is dissolved in acetonitrile and water. An aliquot of this fraction is then separated on a column packed with for example chrompack C<sup>18</sup>, using acetonitrile/water/hexasulphonic acid as the mobile phase (56). The detection limit using the described procedure, a flow rate of 0.8 ml/min and peak detection at 282 nm, has been reported

to 0.2 mg/ml (56). Recently, Ogata et al. (71) described a slightly modified procedure eliminating the ether extraction step. In the revised protocol, the urinary samples are first treated with various enzymes, and then the enzymatic hydrolysates are applied directly on a column for HPLC.

One of the first studies showing a good correlation between air concentrations of chlorobenzene and urinary concentrations of metabolites was the above-mentioned field study by Yoshida et al. (96). The chlorobenzene concentrations were measured in the air of two different chemical factories using personal air sampling. The total number of subjects was eleven, and the exposure time per shift varied between 8 and 11 hr. The estimated air concentrations ranged between 1.7 and 5.8 ppm, with a geometric mean of 3.15 ppm (14.5 mg/m<sup>3</sup>). The previously mentioned field study from Belgium (56), involving 44 male workers in a diphenylmethane-4-4'-diisocyanate-producing plant, confirmed the good correlation between air concentrations of chlorobenzene and urinary levels of 4-chlorocatechol and 4-chlorophenol at the end of shift. The time-weighted average exposure values in the latter study were log-normally distributed and varied from 0.05 to 106 ppm, with a median value of 1.2 ppm (5.5 mg/m<sup>3</sup>). From extrapolations performed on the data, it was calculated that 8 hr exposure to 50 ppm chlorobenzene (230 mg/m<sup>3</sup>), without any simultaneous skin contact to the compound, would give an average urinary concentration of 33 mg total chlorocatechol/g creatinine and 9 mg total p-chlorophenol/g creatinine at the end of a working day.

ACGIH recently recommended that measurements of the total amounts of 4-chlorocatechol and p-chlorophenol (i.e., both free and conjugated forms) should be used for monitoring occupational exposure (2). Based on data from studies cited above (70, 71, 96) and an unpublished simulation study by Droz (cited in reference 2), ACGIH recommended the following biological threshold limits (biological exposure indices; BEIs): 150 mg total 4-chlorocatechol/g of creatinine (= 116 mmol/mol of creatinine) and 25 mg total p-chlorophenol/g creatinine (= 22 mmol/mol creatinine) at the end of shift.

Even if 4-chlorocatechol and p-chlorophenol are assumed to be absent in the urine taken from a general population, it may be worthwhile to note that the presence of them is not exclusively linked to an occupational exposure to monochlorobenzene. Both metabolites may, for example, also be found in the urine from persons exposed to dichlorobenzenes or p-chlorophenol (2).

## 5 GENERAL TOXICITY

In this section, information from various types of general toxicity tests (i.e., tests for acute, subchronic, and chronic toxicity) on experimental animals has been gathered together with the scarce amount of data available regarding human chlorobenzene exposure. Information from various types of experimental tests measuring specific toxicological endpoints such as immunotoxicity, genotoxicity, carcinogenicity, reproductive toxicity and teratogenicity are treated separately (pp. 38–54). Moreover, following the general outline of traditional NIOH criteria documents, information on specific organ effects has been gathered in a separate section, starting on p. 28. The section “General Toxicity” begins with a discussion on toxicological mechanisms.

### 5.1 Suggested Toxicological Mechanisms

As previously discussed, chlorobenzene undergoes oxidative metabolic bioactivation to form epoxides. It is generally assumed that the toxicity of chlorobenzene is mediated by covalent binding of reactive metabolic intermediates to critical cell structures. However, the exact molecular mechanisms of action behind the various toxic effects of chlorobenzene remain unknown. Different mechanisms may be involved in the various organs that are associated with chlorobenzene-induced toxicity.

The reactive electrophilic metabolites formed in the liver are detoxified mainly by conjugation with reduced glutathione, GSH. Liver damage following exposure to chlorobenzene and other monosubstituted halogenated aromatic monocyclics has therefore been attributed to the depletion of hepatic glutathione, leaving the reactive metabolites free to bind covalently to proteins and other cellular macro-molecules (17, 76). It has been suggested that the hepatotoxic effects of chlorobenzene are mainly mediated by the 3,4-epoxide that subsequently rearranges to p-chlorophenol (50, 54). Since the hepatotoxic effects of chlorobenzene are mediated by one or several reactive metabolites, it should be possible to modulate the toxicity by affecting the enzyme systems involved. Consequently, experiments in rats have shown that the liver-damaging effect of chlorobenzene is potentiated when the cytochrome P450 enzyme system is induced with phenobarbital (17).

Impairment of the main detoxifying enzyme system, i.e., mainly the GSH conjugation pathway, could possibly also affect the hepatotoxicity of chlorobenzene. If the detoxification system is handicapped (e.g., by administration of large doses of chlorobenzene) the amount of reactive metabolites available for toxic insults would then theoretically be increased. Initial depletion of hepatic glutathione levels has been shown in both rats (22, 95) and mice (83) given chlorobenzene intraperitoneally. However, this seems to be a transient phenomenon without any obvious dose-response relationship (22, 83). It may also be pointed out that since

chlorobenzene appears to lower the cytochrome P450 levels, at least in the livers of rodents given the compound orally or intraperitoneally (9, 22), exposure to chlorobenzene seems associated with a lowered capacity of both bioactivating and detoxifying enzyme systems. The cited studies are described in more detail on pp. 29–32.

Koizumi et al. (54) showed that the bromobenzene-induced hepatotoxicity in male Wistar rats could be modified if chlorobenzene was given simultaneously. Groups of rats (6 animals/group) were given an i.p. injection of bromobenzene, alone (2 mmole/kg b.wt.) or in combination with chlorobenzene (4 mmole/kg). The rats were killed after 12, 24, 48 or 72 hr. Hepatotoxicity was assessed both biochemically and histopathologically. The injection of a mixture of bromobenzene and chlorobenzene initially suppressed the hepatotoxic effects of bromobenzene alone (24 hr after the injection). However, at a later stage there was a dramatic potentiation of the toxicity, the maximum response being observed 48 hr after the injection. This was true both with regard to the bromobenzene-induced ALAT elevation and the centrilobular necrosis. Whereas the suppression in the early phase was believed to be a result of metabolic inhibition of the 3,4-epoxidation pathway, the subsequent potentiation was most likely a result of a delayed recovery in the glutathione levels.

The causal role of protein binding to the chlorobenzene-induced hepatotoxicity has been questioned. In the previously mentioned study of male Sprague-Dawley rats given a single i.p. injection of chlorobenzene (22), little correlation was found between the histopathological and functional damages of the liver and the metabolism of the substance. A poor correlation was also found between the extent of liver damage and the degree of protein binding. The dose that produced the most extensive liver necrosis (14.7 mmol/kg b.wt., i.e., 1,655 mg/kg) gave the same degree of protein binding as the dose producing only a minimal necrosis (4.9 mmol/kg; 552 mg/kg).

Oxidative stress is one alternative mechanism of action that has been proposed to explain the hepatotoxic effects of chlorobenzene and other aryl halides (21). Evidence for this alternative, or complementary, mechanism of action was obtained from experiments on cultured rat hepatocytes. In this particular in vitro system it was shown that the toxicity of chlorobenzene, bromobenzene and iodobenzene could be manipulated in ways that modified the sensitivity of the cells to oxidative stress. Primary cultures of hepatocytes were prepared from livers taken from Sprague-Dawley rats pretreated with phenobarbital for three days (21). Chlorobenzene and the two other aryl halides were diluted in DMSO and added to the cultures for 2 hr of exposure, with and without addition of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). The latter compound inhibits glutathione reductase, an enzyme that plays an important role in the glutathione redox cycle and is responsible for the reduction of GSSG to GSH. The concentrations of chlorobenzene varied between 0.25 and 2 mM. Cell viability was determined after 4 hr. The cultured hepatocytes were not as sensitive to the toxicity of chlorobenzene as that of bromobenzene or iodobenzene. However, all compounds induced the same type of effects, although at different concentrations. At 1 mM of chlorobenzene alone, there was a 30% cell killing, but when BCNU was added to the cultures, the cell killing increased to 90%. BCNU was without toxic effects of its own. The enhanced cell killing in the presence of BCNU could be completely

prevented by SKF-525A, an inhibitor of the mixed function oxidase system. The changes of cell killing in the presence of BCNU occurred without parallel changes in the metabolism or covalent binding of [ $^{14}\text{C}$ ] bromobenzene (not investigated parameters in the case of chlorobenzene and iodobenzene).

It has also been suggested that the hepatotoxic effects of chlorobenzene are mediated through an alpha-adrenergic system (83). When phentolamine, an alpha-adrenergic antagonist, was given to male B6C3F1 mice after an i.p. injection of chlorobenzene, the chlorobenzene-induced hepatotoxicity was significantly reduced.

The kidneys are also main targets for chlorobenzene-induced toxicity (75). However, here it is the 2,3-epoxide, subsequently rearranging to o-chlorophenol, which has been suggested to be the responsible reactive species (50, 51). Pretreatment of mice and rats with piperonyl butoxide, an inhibitor of microsomal enzymes, blocks the renal toxicity of chlorobenzene. However, in contrast to the liver, pretreatment with phenobarbital did not enhance the kidney toxicity of chlorobenzene to a significant extent (75).

Covalent binding of chlorobenzene-associated metabolites has not only been observed in proteins, but also to RNA and DNA taken from various organs of mice and rats given  $^{14}\text{C}$ -labelled chlorobenzene (20, 40, 73). The reported binding of  $^{14}\text{C}$ -chlorobenzene-associated radioactivity to nucleic acids should be interpreted with some cautiousness, because of the conceivable problem of protein contamination. Reid (75), for example, stated, without giving any figures, that nucleic acids isolated from liver and kidneys of mice and rats given  $^{14}\text{C}$ -chlorobenzene did not contain any "significant amounts" of covalently bound radioactivity. To examine the reported ability of chlorobenzene to interact directly with DNA in more detail is of great interest, especially when one considers that chlorobenzene has been reported genotoxic in some short-term tests for mutagenicity and/or genotoxicity (see pp. 39-47).

The third major target for chlorobenzene-induced toxicity is the central nervous system. No studies were available with regard to the mechanism of action of the narcotic and CNS depressant effects of chlorobenzene. This is not surprising when one considers that, so far, nobody knows exactly how conventional inhalational general anesthetics act on a molecular basis. Several theories have been proposed. One theory is that general anesthetics act by inducing reversible binding directly to a particularly sensitive protein in the neuronal membrane, thereby inhibiting its normal function (37), possibly by competing with endogenous ligands (38). The current hypothesis seems to be that general anesthesia at a molecular level, either follow from changes in lipid thermotropic behavior or malfunction of neuronal proteins, or a combination of both processes (83). Since inhalation anesthetics have diverse structures and act by forming reversible bonds to the critical structure, possibly of Van der Waals type rather than irreversible covalent-ionic bonds (83), it seems likely that it is chlorobenzene itself that induces the CNS-depressant effects. Additional support for this assumption comes from the fact that the intact compound has higher lipophilicity than any of the metabolites formed. High lipophilicity seems to be a prerequisite for CNS-depressant agents.

To summarize, although the different toxic effects observed after administration of chlorobenzene usually are induced by one or more of the various metabolites formed, it cannot be excluded that the compound itself also may produce adverse effects, especially in the CNS. Exactly how chlorobenzene and/or its metabolites cause the toxic effects observed is not known in detail—not even in the liver, the organ most thoroughly studied for chlorobenzene-induced toxicity. Several possible toxicological mechanisms may be involved. Consequently, whereas the hepatotoxic and nephrotoxic action of chlorobenzene most likely are due either directly to the covalent binding of reactive metabolites to critical structures in the cells, and/or indirectly to oxidative stress, the CNS-depressant effect is probably mediated by other toxicological mechanisms, most likely provoked by the unmetabolized substance itself.

## 5.2 Acute Toxicity

The acute toxicity of chlorobenzene in experimental animals is relatively low, after oral administration, inhalation, and dermal exposure. Consistently observed chlorobenzene-induced signs of acute intoxication in various species of experimental animals include hyperemia of the visible mucous membranes, increased salivation and lacrimation, initial excitation followed by drowsiness, adynamia, ataxia, paraparesis, paraplegia, and dyspnea (i.e., mainly signs of disturbance of the central nervous system). Changes observed at gross necropsy include hypertrophy and necrosis of the liver and submucosal hemorrhages in the stomach. Histopathologically observed lesions include necrosis in the centrilobular region of the liver; the proximal convoluted tubules of the kidneys; and the bronchial epithelium of the lungs and stomach. Death is generally a result of respiratory paralysis.

**Animals:** There are many published and unpublished reports on the acute toxicity of chlorobenzene after various routes of administration (see Table 1). The information given in the table was mainly obtained from secondary sources of information. The indicated primary sources of information are in many cases either unpublished reports, or written in a language not familiar to the evaluator. It has consequently not been possible to critically examine each individual study, and the information may appear fragmentary with regard to details on strains, dose levels, methods, and observations.

Apart from the studies listed in Table 1, there are also other acute toxicity studies available in the literature. When the acute toxicity of chlorobenzene was examined in male and female F344/N rats and B6C3F1 hybrid mice (51, 68), the mice were found to be more sensitive than the rats toward the lethal effects of the compound. However, the acute toxicity after a single oral dose of chlorobenzene was also low in both species in this study. The compound was given by gavage, diluted in corn oil at the following doses: 250, 500, 1,000, 2,000 or 4,000 mg/kg b.wt. Each group consisted of 5 males and 5 females of each species. The animals were followed for 14 days and observed daily for mortality and morbidity. The animals were not subjected to necropsy and there were no records on possible effects on body weight gain. Whereas a dose of 1,000 mg/kg b.wt. was lethal to the male mice, the rats had to be given up to 4,000 mg/kg before mortality became evident. Most deaths occurred within a few days after the administration. Clinical signs of toxicity among the rats in the two highest dose groups

**Table 1.—Summary of reported LD<sub>50</sub> and LC<sub>50</sub> values in various published and unpublished acute toxicity tests in experimental animals**

Species	Strain/Sex	Route of administration	Reported LD <sub>50</sub> /LC <sub>50</sub>	Original reference	Secondary sources of information
Rat	n.g.* /n.g.	Oral	2,910 mg/kg	Irish 1962	6, 19, 26
	n.g./n.g.	Oral	2,390 mg/kg	Varshavskaya 1967	19, 32
	n.g./n.g.	Oral	2,300 mg/kg	Eitingon 1975	19
	n.g./n.g.	Oral	3,400 mg/kg	Vecerek et al. 1976	6, 19, 32
	n.g./males and females	Oral	males: 1,427 mg/kg females: 2,455 mg/kg	Bayer AG 1982	19
	n.g./n.g.	Oral	1,540 mg/kg	Monsanto Co. 1982	19
	n.g./n.g.	Inhalation	20,000 mg/m <sup>3</sup> for 2 hr = LC 100	Rozenbaum et al. 1947	19
	n.g./n.g.	Inhalation	18,016 mg/m <sup>3</sup> exposure time n.g.	Eitingon 1975	19
	n.g./males	Inhalation	13,870 mg/m <sup>3</sup> for 6 hr	Bonnet et al. 1982	19
	n.g./n.g.	i.p.	575 mg/kg	Kocsis et al. 1975	19, 25
	Sprague-Dawley/ males	i.p.	1,655 mg/kg	22	
Mouse	n.g./n.g.	Oral	1,445 mg/kg	Varshavskaya 1967	19, 32
	n.g./females	Inhalation	8,822 mg/m <sup>3</sup> for 6 hr	Bonnet et al. 1979	19, 32
Rabbit	NMRI/males	i.p.	1,355 mg/kg	66	
	n.g./n.g.	Oral	2,830 mg/kg	Irish 1962	19, 26, 32
	n.g./n.g.	Oral	2,250 mg/kg	Varshavskaya 1967	19, 32
	n.g./n.g.	Dermal	>2,212 mg/kg	AAMRL-TR (USA) 1987	19
n.g./n.g.	Dermal	>7,940 mg/kg	Monsanto Co. 1989	TSCATS database	
Guinea pig	n.g./n.g.	Oral	5,060 mg/kg	Varshavskaya 1967	19, 32
Cat	n.g./n.g.	Inhalation	17,000 mg/m <sup>3</sup> for 7 hr = lethal	Götzmann 1904	19

\* n.g. = not given in the indicated sources of information

included transient ataxia, labored breathing, and prostration. No attempt was made to evaluate the approximate LD<sub>50</sub> values.

**Humans:** The only information available on the acute effects of chlorobenzene in humans is either based on isolated case reports of poisonings or occupational exposures. However, no data on actual levels of exposures were presented in any of these reports, and in occupational exposures it was difficult to identify chlorobenzene as a causative agent since the workers were exposed to a mixture of agents.

According to various review articles (e.g., **6, 25, 32, 33, 91, 92**), citing the works of Cameron et al. from 1933, Reich from 1934, Rozenbaum et al. from 1947, Girard et al. from 1969, and Smirnova and Granik from 1970, inhalation or ingestion of chlorobenzene causes drowsiness, incoordination, and unconsciousness (i.e., signs deriving from CNS-depression—sedation and narcosis) as well as irritation of the eyes and respiratory tract. Whereas some of these reports are briefly described below, the work of Rozenbaum et al. from 1947, describing an industrial exposure situation, is described in the section discussing the chronic toxicity of chlorobenzene (p. 27).

According to the toxicology update by Whillhite and Book (**92**), citing the paper by Cameron et al. [published in *J Pathol Bacteriol* 44 (1933) 281–296], ingestion of chlorobenzene leads to pallor, cyanosis, methemoglobinemia, and collapse. These symptoms occurred after a delay in onset by several hr. No information was given on exposure levels, etc., but according to Whillhite and Book (**92**), the human probable oral acute lethal dose of chlorobenzene has been estimated at 0.5–5 g/kg b.wt.

The case-report by Reich from 1934 [published in *Samml Vergiftungsfällen* 5 (1934) 193–194], refers to a 2-year-old boy who had swallowed 5 to 10 ml of Puran, a cleaning agent containing chlorobenzene. The boy did not show any immediate signs of intoxication, but after eating lunch 2.5 hr after the ingestion of Puran, he quickly lost consciousness and suffered vascular paralysis and heart failure (possibly indicating that the absorption of chlorobenzene from the gastrointestinal tract is facilitated by ingestion of fat). The boy recovered, but the odor of Puran in his breath and urine persisted for 5 to 6 days.

Another of the cited cases of acute chlorobenzene intoxication, originally reported by Girard et al. [published in *J Med Lyon* 50 (1969) 771–773], refers to a 70-year-old woman who was exposed to a glue containing 70 percent chlorobenzene when she was manufacturing hats. Early complaints included headache and irritation of the upper respiratory tract and mucosa of the eyes.

In the previously mentioned exposure chamber study involving five volunteers exposed to up to 60 ppm chlorobenzene (276 mg/m<sup>3</sup>) for 7 hr (**71**) it was shown that this exposure was associated with acute subjective symptoms such as drowsiness, headache, irritation of the eyes, and sore throat. There is also a recently published case report (**13**) describing severe liver cell necrosis in a 40-year-old man who had ingested 140 ml of a solution containing 90% chlorobenzene in a suicide attempt. The case-report is further discussed on p. 32.

### 5.3 Subchronic Toxicity

Repeated administration of chlorobenzene to experimental animals for several weeks or months is mainly associated with liver and kidney damage. Typical evidence for the chlorobenzene-induced liver toxicity observed in the subchronic toxicity studies are increased activity of serum liver enzymes, increased liver weight, lipid accumulation, hepatic porphyria, and hepatocellular necrosis. The chlorobenzene-induced nephrotoxicity is mainly manifested as increased kidney weights, focal coagulative degeneration, and necrosis of the proximal tubules. Repeated administration of relatively large doses to experimental animals is also associated with lesions in the thymus, spleen, bone marrow, and lungs.

Although repeat-dose toxicity studies for 14 days do not fall within the category of subchronic toxicity studies, this section of the document begins with such a study (previously often referred to as “subacute” toxicity study) for practical reasons.

**Animals:** Male and female F344/N rats and B6C3F1 mice were given chlorobenzene diluted in corn oil by gavage for 14 days (51, 68). Groups of 5 animals were given daily doses varying between 0 and 2,000 mg/kg b.wt. for the rats, and between 0 and 500 mg/kg for the mice. The animals were observed daily for mortality and morbidity, weighed at the beginning and the end of the study. They were also subjected to necropsy. The 2-week daily exposure to 1,000 and 2,000 mg/kg b.wt. resulted in 100% mortality among the male and female rats. Most deaths occurred within the first few days of exposure. Clinical signs of toxicity among the rats in the highest dose groups included prostration and reduced response to stimuli. There were no toxic effects that could be related to the administration of chlorobenzene in the mice. Consequently, the NOEL for both male and female rats and mice in this 14-day study was found to be 500 mg/kg b.wt./day.

In a regular subchronic toxicity study on rats and mice (51, 68), male and female F344/N and B6C3F1 mice were given chlorobenzene by gavage, 5 days/week, for 13 weeks in the following doses: 0 (corn oil; vehicle), 60, 125, 250, 500 or 750 mg/kg b.wt./day. Each group consisted of 10 animals of each sex and species. The animals were observed daily for mortality, morbidity and clinical signs of toxicity. 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 surviving animal and analyzed for various blood parameters. Clinical biochemistry determinations were performed on blood samples obtained from cardiac puncture, taken at the time of sacrifice. All animals were subjected to a complete gross examination, and a number of organs were taken for histopathologic examination.

The mortality was increased in the two highest dose groups among the rats, and in the three highest dose groups among the mice. There were no clinical signs of toxicity reported. The body weight gain appeared to be reduced in the male rats and mice, starting from 250 mg/kg/day, and among the female rats and mice starting from 500 mg/kg/day. There were no consistent changes in the hematological parameters, and the only significant findings reported from the investigation on serum chemistry were some observations made in surviving

female rats of the two highest dose groups: slight to moderate increases in serum alkaline phosphatase and serum gamma glutamyl transpeptidase. Apart from increased urine volumes observed in some of the high-dose animals, the urinalysis showed no abnormalities.

Liver weights were slightly increased in a dose-related manner in both male and female rats and mice. Histologic examination showed chlorobenzene-induced lesions in the liver, kidney, spleen, bone marrow and thymus of both rats and mice. In the liver there was a dose-dependent centrilobular hepatocellular degeneration and necrosis (LOEL: 250 mg/kg/day). In the kidneys the lesions were characterized by vacuolar degeneration and focal coagulative necrosis of the proximal tubules (lowest LOEL: 250 mg/kg/day). The renal lesions were judged to be mild to moderate. Chlorobenzene induced moderate to severe lymphoid necrosis of the thymus in male and female mice (lowest LOEL being obtained in males: 250 mg/kg/day). However, there was no perfect dose-response relationship with regard to this effect of chlorobenzene. Lymphoid depletion of the thymus, lymphoid and myeloid depletions of the spleen, and myeloid depletion of the bone marrow, all regarded as minimal to moderate, were only seen in animals in the highest dose groups. Taken together, the results suggested a NOEL of 125 mg/kg b.wt./day. Mice appeared to be more sensitive toward the toxic effects of chlorobenzene, and males were somewhat more sensitive than females.

The subchronic toxicity of inhaled chlorobenzene has been evaluated in male rats and rabbits (28). The animals were exposed to 0, 75, or 200 ppm (0, 345, or 920 mg/m<sup>3</sup>) of chlorobenzene for 7 hr/day, 5 days/week, for up to 24 weeks. Groups of animals were killed after 5, 11, and 24 weeks and examined for hematology, clinical chemistry, and gross and histopathological changes. Chlorobenzene-related toxicity in the male rats included decreased food utilization, increased liver weights, lowered ASAT activity at all survival times, increased number of blood platelets after 11 weeks of exposure and microcytic anemia. Histopathological changes observed were occasional focal lesions in the adrenal cortex, tubular lesions in the kidneys, and congestion in both liver and kidneys. Reported toxic effects in the rabbits included increased lung weights and, after 11 weeks of exposure, decreased ASAT activity. The results were presented in a short meeting abstract that did not include any information on, for example, strains, number of animals, or nonobserved effect levels.

The subchronic toxicity of chlorobenzene has also been investigated in dogs, exposed either orally or by inhalation. The only published information from these studies available for evaluation, is a condensed meeting abstract by Knapp et al. (53) briefly reporting the results from the oral studies where chlorobenzene was administered via gelatine capsules. One of the subchronic toxicity studies on dogs, performed by Hazleton Laboratories, USA, for Monsanto Company, USA [project numbers 241-105, 1967 and 790015/DMEH ML-79-025, 1980], have been cited, and/or, evaluated by others (6, 25, 32). According to the U.S. EPA (32), the toxicity of chlorobenzene has also been tested in a 90-day inhalation study of male and female beagle dogs by Industrial Bio-Test Inc. (IBT), for the Monsanto Company [BLT-project no. 76-166, 1979; unpublished].

In the IBT-study, groups of beagle dogs (four males and four females in each group) were exposed to 0, 0.75, 1.5, or 2 mg/l air (0, 750, 1,500 or 2,000 mg/m<sup>3</sup>) of chlorobenzene vapors,

6 hr/day, 5 days/week for 90 days. Some of the animals in the two highest dose groups (HD: 5/8; MD: 2/8) became moribund and were sacrificed after approximately 30 days. According to the secondary source of information (32), the exposure to chlorobenzene resulted in lowered body weight gain (HD dogs), lower leukocyte counts and elevated levels of alkaline phosphatase, ALAT and ASAT (HD dogs), lower absolute liver weights (HD females), lower absolute heart weights (MD males only) and increased absolute pancreas weights (MD and HD females). Histopathological changes included vacuolization of hepatocytes (HD animals), aplastic bone marrow (HD dogs), cytoplasmic vacuolization of the epithelium of the collecting tubules in the kidneys (one male and three males in HD) and bilateral atrophy of the seminiferous epithelium of the testes (two males in HD). The results of the IBT-study, as reported in the secondary source of information (32), should be interpreted with some cautiousness. It is not known if this particular IBT-study has been validated. Consequently, it is not known if the study should be judged invalid, pending, supplemental, or valid.

In the inhalation study from Hazleton, six adolescent dogs per sex and group (strain not given), were exposed to various levels of chlorobenzene vapors, 6 hr/day, 5 days/week, for 6 months. The target levels of chlorobenzene were 0, 0.78, 1.57, or 2.08 mg/l air (0, 780, 1,570, or 2,080 mg/m<sup>3</sup>). Significant changes included decreased absolute adrenal weights in the male dogs of the two highest dose groups, increased relative liver weights in the female dogs of the two highest dose groups, a dose-related increased incidence of emesis in both males and females, and an increased frequency of abnormal stools in treated females. The NOAEL was determined to be 780 mg/m<sup>3</sup> (6, 32).

In one of the oral subchronic toxicity studies, male and female beagle dogs were given chlorobenzene by capsule at doses of 0, 27.25, 54.5, or 272.5 mg/kg b.wt./day, 5 days/week, for 13 weeks (93 days). Four of eight dogs in the highest dose group died within 3 weeks. At this dose level, chlorobenzene was found to produce a significant reduction of blood sugar, an increase in immature leukocytes, elevated serum ALAT and alkaline phosphatase levels and, in some dogs, increases in total bilirubin and total cholesterol (25, 32, 53). In the condensed meeting abstract it was stated that there were no consistent signs of chlorobenzene-induced toxicity at the intermediate and low dose levels (53), but according to the unpublished report, cited by, for example, the U.S. EPA (6, 32), chlorobenzene-related hepatotoxicity was observed also among the animals in the intermediate dose-group. Among the dogs in the highest dose group, histopathological changes were also observed in the kidneys, gastrointestinal mucosa, and hematopoietic tissues (53). The NOEL in dogs given chlorobenzene orally via capsules appeared to be 27.25 mg/kg b.wt./day.

According to the brief information given in the meeting abstract (53), groups of rats were also given chlorobenzene in the diet for 93 to 99 consecutive days (0, 12.5, 50, or 250 mg/kg b.wt./day). Reported effects of chlorobenzene were retarded growth (males in the highest dose group) and increased liver and kidney weights ("some rats at the high and intermediate levels"), resulting in a NOEL of 12.5 mg/kg b.wt./day.

## 5.4 Chronic Toxicity

**Animals:** Apart from a cancer study, carried out as a part of the National Toxicology Program, where chlorobenzene was given orally to F344/N rats and B6C3F1 mice, 5 days/week for 103 weeks (51, 68), there were no other chronic toxicity studies available for evaluation. Since the NTP study, as other regular cancer bioassays, was concentrated on histopathological data and consequently devoid of clinical chemistry, hematological investigation, and urinalysis, etc., the study has been evaluated under the heading “Carcinogenicity” on page 48. However, it may be useful to note that the administration of up to 120 mg/kg b.wt./day (rats and female mice) or 60 mg/kg/day (male mice) of chlorobenzene for 2 years, failed to induce the type of toxic responses (e.g., damage to the liver, kidney, and hematopoietic system) that was observed in the previously cited subchronic toxicity study in rats and mice (51).

**Humans:** Human data on the chronic toxicity of chlorobenzene are limited. Other reviews of chlorobenzene-associated toxicity (25, 32) mention a paper by Rozenbaum et al. from 1947 [published in *Gig Sanit* 12 (1947) 21–24; in Russian], reporting the results of an examination of 52 people occupationally exposed to chlorobenzene. Twenty-eight individuals in the study had been working for 1 to 2 years in a factory where chlorobenzene vapor was claimed to be the only work-related chemical exposure. Many of these individuals were reported to suffer from headache, dizziness, somnolence, and dyspeptic disorders.

There is also another Russian paper, by Lychagin et al. from 1976 [published in *Gig Tr Prof Zabol* 11 (1976) 24–26; in Russian], reporting a higher incidence of women with immunological shifts, disturbed phagocytic activity of the leukocytes, reduced absorption capacity of the neutrophils, dermal infections, occupational dermatitis, and chronic effects to respiratory organs in a glass insulating enameling department. Study design, number of workers and controls involved, exposure levels, duration of exposure, etc., were not given in the short citation (91), but the exposure situation appeared to have been complex, also involving exposure to, for example, acrolein, acetone, and glass fiber dust.

## 6 ORGAN EFFECTS

### 6.1 Skin, Eyes, and Other Mucous Membranes

Available information on the acute effects of chlorobenzene in humans (see above) shows that chlorobenzene vapors are irritating to the eyes and mucous membranes of the upper respiratory tract.

Unpublished experimental data from the German manufacturer Bayer AG (Suberg 1983a, 1983b), cited in the German BUA report (19), showed that chlorobenzene is moderately irritating to the skin. The same unpublished studies from Bayer AG also showed that chlorobenzene is a moderate irritant to the eyes (19). No detailed information was given in the short citation of these studies, but both the dermal irritancy/corrosivity study, and the eye irritation test were performed on rabbits according to the OECD guidelines for testing of chemicals (19).

### 6.2 Respiratory System

Results obtained in some of the general toxicity tests show that chlorobenzene may be toxic to the lung. Necrotic lesions in the bronchial epithelium of the lungs are one of the chlorobenzene-induced histopathological changes that have been observed in acute toxicity tests after administration of large doses. A subchronic toxicity study of inhaled chlorobenzene in rabbits (28) showed increased lung weights after up to 24 weeks of exposure to 75 or 200 ppm chlorobenzene.

Apart from the fact that inhalation of chlorobenzene vapors is irritating to the membranes of the upper respiratory tract, no other human data have been found with regard to the chlorobenzene-induced adverse effects on the lungs.

The lungs are evidently not the major targets for the chlorobenzene-induced toxicity. The reported effects from animal experiments were observed only at relatively high exposure concentrations of the compound.

### 6.3 Liver

**Animals:** As discussed above in the section "General Toxicity," the liver is one of the main targets for chlorobenzene-induced toxicity. Studies on experimental animals have shown that chlorobenzene produces various types of deleterious effects on the liver, both morphological and functional. Typical consequences of chlorobenzene exposure are increased liver weights, increased activities of serum liver enzymes, porphyria, and hepatocellular necrosis. This has,

for example, been observed in male and female rats after both acute and repeated oral administration and inhalation (22, 46, 51, 53, 67, 68), in male and female mice after acute and repeated oral exposure (51, 53), in dogs given the compound orally or by inhalation for several weeks (6, 25, 32, 53) and in pregnant rabbits after inhalation of chlorobenzene vapor during the period of gestation (48).

A carcinogenicity study on Fischer 344/N rats (51, 68) showed that there was a slight increase in the frequencies of male rats with neoplastic nodules of the liver after two years of oral exposure to 120 mg chlorobenzene/kg b.wt./day. No such changes were observed in male rats receiving a lower dose, female rats, or in male and female B6C3F1 mice (see p. 48).

In a study of chlorobenzene-induced hepatotoxicity, male Sprague-Dawley rats were injected for three consecutive days with physiological saline or phenobarbital before they were given an i.p. injection with various doses of chlorobenzene diluted in sesame oil (17). The animals were killed 24 hr after the injection with chlorobenzene. The livers were removed and examined histopathologically. The pathological changes of the hepatocytes in the centrilobular region in the non-induced rats given 0.04 ml chlorobenzene varied from glycogen loss to minimal necrosis. However, the centrilobular necrosis in the phenobarbital-pretreated rats given the same amount of chlorobenzene was found to be extensive or massive.

In another single-dose experiment on male Sprague-Dawley rats (22), the relative liver weights were found to be increased about 1.5 times those of the controls 24 hr after an i.p. injection of 9.8 mmol/kg b.wt. At this time a mild but progressive development of a hepatic lesion was observed around the central veins. The damage was manifested as a cloudy swelling and hydropic changes of the centrilobular hepatocytes. Forty-eight hr after the injection, the signs of necrosis had become even more pronounced. Rats given 9.8 or 14.7 mmol/kg (1,100 or 1,655 mg/kg), showed extensive hydropic changes throughout the liver and clear evidence of necrosis. However, signs of mild morphological alterations (cloudy swelling and hydropic changes in centrilobular regions) were also present at the lowest dose level tested (2.0 mmol/kg; 225 mg/kg). No evidence of fatty changes was observed at any dose level or survival time.

In a study on the relationship between the chemical structure of chlorinated benzenes and their effects on hepatic and serum lipid components, chlorobenzene was given to male Sprague-Dawley rats in the diet at a concentration of 500 ppm for two weeks (46). Whereas the body weight gain and kidney and spleen weights were unaffected, the liver weights were slightly increased. The level of lipid peroxide was reported to be increased in the livers of the chlorobenzene-exposed rats and this increase was accompanied by an elevated level of triglycerides and lowered levels of vitamin E and glutathione peroxidase.

In order to explore the relationship between chemical structure and liver toxicity, Ariyoshi et al. (9) gave various chlorinated benzenes suspended in 2% tragacanth gum solution orally to female Wistar rats for three consecutive days. Chlorobenzene was also given as a single oral dose of 125, 250, 500, and 1,000 mg/kg b.wt. Among the various parameters investigated in controls and exposed rats (six animals/group) were the contents of microsomal proteins,

including cytochrome P450, and phospholipids, the activities of the drug-metabolizing enzymes aminopyrine methylase and aniline hydroxylase, and the activity of  $\sigma$ -aminolevulinic acid synthetase. Oral doses of 125–1,000 mg/kg b.wt./day for three days were found to increase the hepatic heme synthesis but to decrease the microsomal cytochrome P450 content as well as the activity of aminopyrine demethylase. The activity of  $\sigma$ -aminolevulinic acid synthetase was markedly increased at all dose levels employed. Liver weights and the contents of fatty acids of phospholipids were increased in the chlorobenzene-exposed animals, but there were no compound-related effects on the contents of glycogen, triglycerides, or the total amount of microsomal proteins.

Obviously, chlorobenzene differs from many polychlorinated aromatic hydrocarbons, in not being a general inducer of the microsomal metabolism (i.e., the compound does not stimulate the activity of the cytochrome P450/P448 enzyme system). This conclusion has subsequently been confirmed in experiments performed on male Sprague-Dawley rats given a single i.p. injection of chlorobenzene (22).

One way of studying liver toxicity is to monitor the serum alanine aminotransferase (ALAT) activity. This enzyme is regarded highly specific to the liver, and its concentration in the blood is regarded directly proportional to liver damage. Several investigations have shown that chlorobenzene exposure is associated with increased serum ALAT activity. In one of these experiments (22), already mentioned above, chlorobenzene was given diluted in corn oil as a single i.p. injection of 2, 4.9, 9.8, or 14.7 mmol/kg b.wt. (225, 550, 1,100 or 1,655 mg/kg) to male Sprague-Dawley rats. Controls received vehicle only. The effects of chlorobenzene were also investigated after various intervals (3 to 72 hr) following a dose corresponding to the estimated LD<sub>10</sub> dose (1,100 mg/kg b.wt.). Each group of chlorobenzene-treated rats consisted of two to six animals. The ALAT activity was found to be significantly elevated at all intervals studied, the maximum increase being observed after 48 hr. The dose-response experiment showed elevated ALAT activities at all doses tested, but the authors considered 1,100 mg/kg b.wt. to be the LOEL with regard to this specific experimental parameter. Chlorobenzene was also found to elevate the sulphobromophthalein (BSP) retention significantly at all intervals studied. The maximum effect was already obtained 3 hr after the injection. Consequently, functional evidence of hepatotoxicity can be detected early in the time course of events induced by chlorobenzene.

In another study (83) employing male B6C3F1 mice, chlorobenzene was given as an i.p. injection at doses of 0 (corn oil) 0.01, 0.1, 0.25, 0.5, or 1 ml/kg b.wt. (higher doses than that resulted in 100% lethality within 24 hr after the injection). Each group consisted of at least nine animals. As in the above-mentioned study on the male Sprague-Dawley rats, the maximum increase of serum ALAT activity was obtained 48 hr after the injection. The LOEL with regard to increased serum ALAT activity in the male mice was established at 0.5 ml/kg b.wt.

Another typical effect of chlorobenzene on the liver, is its influence on the glutathione levels. Glutathione is a tripeptide that is involved in the detoxification of electrophilic substances. The reaction between the nucleophilic groups in glutathione and electrophilic sites in reactive

molecules often leads to the formation of mercapturic acids that are excreted in the bile or, as in the case of chlorobenzene, in the urine.

In one of the studies investigating the effect of chlorobenzene on the GSH levels in the liver (95), male Wistar rats were given an i.p. injection of chlorobenzene diluted in olive oil. The compound was given either as a single dose of 2 mmol/kg b.wt. (225 mg/kg), or repeatedly four times during a 48-hr period ( $4 \times 2$  mmol/kg b.wt.). In the single-dose experiment, the rats were sacrificed after 3, 6, 24, and 30 hr, and in the repeat dose experiment, they were sacrificed either 48 hr after the first injection or 48 hr after the last injection. Each group consisted of four to five animals. Controls received olive oil only. In the single-dose experiment, chlorobenzene was found to induce a significant, but transient, decrease of the hepatic levels of total and oxidized glutathione. Six hr after the injection, the total amount of glutathione was only 24% of that in the controls. However, 24 hr after the injection, there was already a significant increase of both the total and oxidized glutathione (188% and 170% of controls, respectively), an effect that was accompanied by an increased glutathione synthesis (193% of controls) and an elevated glutathione reductase level (136% of controls). After 48 hr, all these levels were still increased, and at this survival time the liver weights, as well as the protein- and DNA-contents, were also found to be significantly increased. The repeat dose experiment confirmed the chlorobenzene-induced liver enlargement and accumulation of hepatic glutathione.

Initial depletion of glutathione levels shortly after an intraperitoneal injection of chlorobenzene to male Sprague-Dawley rats was also observed in the previously mentioned study by Dalich and Larson (22). Four hr after the injection, there was a significant depletion of GSH levels at all doses investigated (from 2.0 to 14.7 mmol/kg b.wt.). Apart from the lowest dose group, the GSH levels remained low 8 hr after the injection, the longest survival time employed for this study parameter. The experiments also included measurements on the potential effects of chlorobenzene on the microsomal cytochrome P450 levels in the liver. Four hr after the administration, these were found to be depressed between 30–50% at all dose levels tested. After 24 hr, the cytochrome P450 content was lowered to 50–80% of the control level. However, there was no obvious relationship between the dose and the observed effect on the cytochrome P450 content. With regard to the time course of the covalent binding of [ $^{14}$ C]chlorobenzene-associated radioactivity to liver proteins, measurable amounts were already present 2 hr after the injection. The amount of binding increased steadily during the first 24 hr. Again there was a poor correlation between the dose and the magnitude of the covalent binding to the liver proteins, the maximum covalent binding being obtained at 4.9 mmol/kg (550 mg/kg).

Experiments on male B6C3F1 mice showed temporal changes in hepatic glutathione concentrations following an i.p. injection of chlorobenzene (83). When groups of animals (at least 8 animals/group) were killed after 2, 4, 8, or 24 hr after an i.p. injection of either corn oil (controls) or 0.48 ml chlorobenzene/kg b.wt., the hepatic glutathione concentrations were found to be depleted maximally 4 hr after the injection of chlorobenzene (90% reduction). After 24 hr, the glutathione levels recovered back to normal. In a dose-response experiment, groups of mice (at least eight animals in each group) were given 0, 0.01, 0.1, 0.25, or 1 ml

chlorobenzene/kg b.wt. i.p. and sacrificed 3 hr later. It was reported that 0.1 ml/kg was the lowest effective dose that significantly exhausted the liver GSH. However, there was no perfect dose-response relationship for this effect (0.1 ml/kg: 23% reduction; 0.25 ml/kg: 78% reduction; 0.1 ml/kg: 76% reduction and 1.0 ml/kg: 71% reduction).

**Humans:** A recently published case report from France (13) described quite severe effects on the liver by chlorobenzene. Exposure occurred in a suicide attempt in which a 40-year-old man ingested approximately 140 ml of a 90% chlorobenzene solution. After two hr, the patient became drowsy. At that time the serum activities of ASAT and ALAT were increased approximately three times. Three days after the ingestion of chlorobenzene, the serum ASAT and ALAT activities were 345 and 201 times the upper limits of normal, respectively. The liver was not enlarged, but the patient had a diffuse erythema covering the face. A liver specimen was taken by transjugular biopsy. The histopathological examination showed centrilobular and mediolobular necrosis, but no evidence of inflammatory infiltration, hepatocyte ballooning or fibrosis. Immunoglobulin M antibodies to hepatitis A virus and to hepatitis B core antigen, as well as hepatitis B surface antigen, were absent, and the serological test results for recent infection with herpes simplex viruses were negative. The serum level of chlorobenzene was determined to be 500 µg/l 3 days after the suicide attempt, and 2 µg/l after 15 days. Although the man was described as an alcoholic (the consumption of alcohol was estimated to 200 g per day), the authors concluded that the observed liver cell necrosis was directly linked to the acute intake of chlorobenzene (there was, for example, no history of chronic liver disease, which was also confirmed by the liver biopsy). However, it cannot be ruled out that the chronic ethanol consumption might have played a role in the severity of the observed lesions. After being treated with prostaglandin E<sub>1</sub> for several days, the patient recovered.

Apart from the above-mentioned case report, no other data was found concerning hepatotoxic effects of chlorobenzene in humans. However, it may be worth noting that results obtained in vitro (50) suggest that humans may be more susceptible to the hepatotoxic effects of chlorobenzene than rodents. Liver microsomes taken from humans were reported to be more efficient in producing p-chlorophenol than mouse liver microsomes, showing that the main metabolic pathway of chlorobenzene in human livers is through the hepatotoxic 3,4-epoxide pathway (see p. 12).

## 6.4 Kidneys

**Animals:** As shown in the previously discussed general toxicity studies, but also in other toxicity tests (see p. 53), the kidney is another target for chlorobenzene-induced toxicity. This has also been shown in experiments designed to investigate the mechanisms behind the nephrotoxic action of chlorobenzene (75). Male Sprague-Dawley rats and male C57BL/6J mice given a single i.p. injection of unlabelled and/or <sup>14</sup>C-labelled chlorobenzene, developed a renal tubular lesion within 48 hr. Extensive necrosis of the proximal convoluted renal tubules was, for example, observed among 80% of the mice given 6.75 mmoles/kg b.wt. (760 mg/kg). The rats were not as sensitive as the mice to the nephrotoxic action of chlorobenzene.

The development of renal necrosis was associated with covalent binding of chlorobenzene-associated radioactivity to kidney proteins. After administration of  $^{14}\text{C}$ -chlorobenzene (1 mmol/kg b.wt.; 10–30  $\mu\text{Ci}/\text{animal}$ ), a considerable amount of chlorobenzene-associated radioactivity became covalently bound in the region with the necrotic lesions, i.e., in the proximal convoluted tubule cells. The nephrotoxic action of chlorobenzene could be reduced if the animals were pretreated with piperonyl butoxide, an inhibitor of microsomal enzymes. The pretreatment did not only block the renal toxicity, it also markedly reduced the binding of chlorobenzene-associated radioactivity to the kidney proteins. However, in contrast to the situation in the liver (see above), pretreatment with phenobarbital, an inducer of microsomal enzymes, did not significantly enhance the nephrotoxicity of chlorobenzene in the rats and mice.

**Humans:** No data was found concerning nephrotoxic effects of chlorobenzene in humans.

## 6.5 Pancreas

In a study on the effects on the pancreas of benzene and various halogenated analogues, including chlorobenzene, male Holzman rats were given an i.p. injection of 5 mmol/kg b.wt. (562.5 mg/kg) of chlorobenzene (94). Controls received an i.p. injection of the vehicle, sesame oil. Each group of animals consisted of at least 4 animals. Twenty-four hr after the injection, surgery was performed on phenobarbital anesthetized animals, cannulating the femoral vein and the common bile duct. Bile duct pancreatic fluid (BDPF) and bile were collected separately. Chlorobenzene as well as most of the other compounds investigated (e.g., bromobenzene and benzene) altered the pancreatic excretory function. In the case of chlorobenzene, this was manifested as a 10-fold increase in BDPF flow, a significant decrease in protein concentration of BDPF (70% reduction), and an increased bile flow. The mechanism(s) behind the observed effects remains unknown. Apparently these were not induced by secretin or cholinergic stimulation or secondary to liver damage.

The significance of the reported effects of chlorobenzene on the pancreatic excretory function is not known. None of the other identified studies on the toxicity of chlorobenzene in experimental animals (with the possible exception of the 90-day inhalation study on dogs from IBT, see p. 25) reported any compound-related effects in the pancreas.

There is no human data available on chlorobenzene-induced effects in the pancreas.

## 6.6 Gastrointestinal Tract

Acute toxicity studies on experimental animals have shown that exposures to high doses of chlorobenzene are associated with necrosis in the stomach as well as submucosal hemorrhages. In an oral subchronic toxicity study in dogs (53), it was noted that the animals given the highest dose of chlorobenzene (272.5 mg/kg b.wt., 5 days/week for 13 weeks) developed histopathological changes in the gastrointestinal mucosa.

No data was found concerning chlorobenzene-induced effects in the gastrointestinal tract of humans.

## 6.7 Circulatory System

Apart from an isolated case of intoxication where a 2-year-old boy was reported to suffer from vascular paralysis (an effect that could be due to CNS-depression) after having swallowed chlorobenzene (see p. 23), no other data were available on chlorobenzene-induced effects on the circulatory system.

## 6.8 Hematological System

**Animals:** It has been reported from various experiments in animals that exposure to chlorobenzene is associated with some hematopoietic toxicity. Male and female Swiss mice were exposed to chlorobenzene vapor, either to 100 mg/m<sup>3</sup> (22 ppm), 7 hr/day for three months or to 2,500 mg/m<sup>3</sup> (544 ppm), 7 hr/day for 3 weeks (97). The number of animals in each group was ten (5 males and 5 females). During the experiment, and after its termination, blood was drawn from the tail vein and examined for the leukocyte counts and blood picture. Comparisons were made with controls and mice receiving either benzene or trichlorobenzene. Chlorobenzene-induced leukopenia (characterized by neutropenia, destruction of lymphocytes and lymphocytosis) and a general bone marrow depression. Similar effects were observed in the benzene-exposed mice. However, in comparison to the latter compound, chlorobenzene was found not equally potent in inducing hematopoietic toxicity.

According to secondary sources of information (32, 92), Varhavskaya reported pathologic changes (inhibition of erythropoiesis, thrombocytosis and mitotic activity) in the bone marrow of male rats given oral doses of 0.01 or 0.1 mg chlorobenzene/day for 9 months. The results, which were presented in a paper originally published in Russian [Gig Sanit 33 (1968) 17-23], were not available for a critical examination. However, the results appear unrealistic, at least if the indicated dosages are correct. There is no evidence from any of the other available toxicity studies on rats (or other species) that chlorobenzene would be such a potent toxin to the bone marrow.

In a previously mentioned inhalation study on male rats and rabbits exposed to 75 or 250 ppm (345 or 1,150 mg/m<sup>3</sup>) chlorobenzene vapors for 11 weeks (28), both species showed unspecified pathological changes in various red cell parameters.

A dose-related increase of the number of micronucleated polychromatic erythrocytes was observed in the bone marrow of male NMRI mice given i.p. injections of 225-900 mg chlorobenzene/kg b.wt. (65, 66). No information was given on the potential general bone marrow toxicity of the substance (see p. 43).

Minimal to moderate myeloid and/or lymphoid depletions were observed in the spleen and thymus in another previously mentioned subchronic toxicity study on rats and mice given chlorobenzene by gavage for 13 weeks (51, 68). Effects on the bone marrow were only seen

in animals given the highest dose of chlorobenzene (750 mg/kg b.wt.). An increased number of blood platelets and microcytic anemia were observed in male rats and rabbits exposed to up to 200 ppm chlorobenzene vapor (920 mg/m<sup>3</sup>) for up to 24 weeks (28). Histopathological changes in hematopoietic tissues have also been reported in an oral subchronic toxicity study in dogs given 272 mg chlorobenzene/kg b.wt., 5 days/week for 13 weeks (53).

**Humans:** There are no relevant data available in the literature on chlorobenzene-induced effects on the hematological system in humans.

## 6.9 Immunological System

Subchronic toxicity studies in mice and rats showed that repeated exposure to relatively high doses of chlorobenzene produced minimal to moderate lymphoid depletion of the thymus, and lymphoid or myeloid depletions of the spleen and moderate to severe lymphoid necrosis of the mouse thymus (51, 68).

No relevant human data was available on chlorobenzene-induced toxic effects on organs and tissues constituting the immunological system.

## 6.10 Central Nervous System

Most organic solvents are to a greater or lesser extent able to induce CNS-depression when given at large doses. The neurotoxic effects of organic solvents may be divided into acute effects and chronic effects. Generally it is assumed that whereas the acute effects may be a result of the direct action of the solvent on the nerve cell membrane and energy metabolism, the chronic effects are caused by the formation of reactive intermediates (80). In the case of chlorobenzene, no information was available with regard to possible chronic neurotoxic effects of the compound. However, its potential to induce acute neurotoxic effects is well documented.

It is known that even a short duration of exposure to low concentrations of various solvents can induce moderate signs of toxicity such as mucous membrane irritation, tearing, nasal irritation, headache and nausea. At higher exposure levels, the toxic effects become more pronounced and may include overt signs of intoxication, incoordination, exhilaration, sleepiness, stupor, and beginning anesthesia (27). While the former group of symptoms combined can be viewed as preanesthetic effects, the latter symptoms are generally regarded as indicators of narcosis (27). However, it may be difficult to establish safe exposure limits with regard to the solvent-induced CNS effects following from short-term exposure. Many of the mild symptoms described above are subjective, tolerance is often developed, the estimated exposure levels are often uncertain and the recorded effects are in many cases reversible. It has therefore been argued that it would be better to use various forms of neurobehavioural tests that measure basic psycho-physiological functions such as alertness, reaction time, memory, and sensory-motor performance as indicators of mild CNS-effects, instead of reported signs of mild intoxication (27).

**Animals:** As previously discussed, acute symptoms of chlorobenzene-induced intoxication in various species of experimental animals include CNS effects such as excitation followed by drowsiness, adynamia, ataxia, paraparesis, paraplegia, and dyspnea (see p. 21).

A specific study on behavioral changes following short-term inhalation of chlorobenzene was performed in male Swiss OF1 mice (24). The animals were exposed for 4 hr to high concentrations of chlorobenzene vapor: 650, 785, 875, or 1,000 ppm (i.e., 2,990, 3,610, 4,025, or 4,600 mg/m<sup>3</sup>), respectively. Controls were exposed to clean filtered air only. The number of animals in each group was ten. Measurements were made to see whether the acute exposure affected the immobility developed in a so-called “behavioral despair” swimming test. The test is based on the fact that rodents that are forced to swim in a limited space, after a while develop a characteristic immobile posture that can be timed. Chlorobenzene, as well as other solvents included in the study, was found to reduce the total duration of immobility over a 3-min period in a dose-related manner. The exposure that would give a 50% decrease in immobility was estimated to be 804 ppm (i.e., 3,700 mg/m<sup>3</sup>) for chlorobenzene. This level was considerably higher than, for example, that calculated for benzyl chloride (15 ppm) and styrene (549 ppm), but notably lower than that calculated for other solvents such as 1,2-dichloroethylene (1,983 ppm), methyl ethyl ketone (2,056 ppm) and 1,1,1-trichloroethane (2,729 ppm).

**Humans:** As previously discussed, isolated case reports of acute poisonings have shown that inhalation or ingestion of high doses of chlorobenzene is associated with CNS-effects such as drowsiness, incoordination, and unconsciousness (see p. 23). In the previously mentioned controlled exposure chamber study (71) where five male volunteers were exposed to chlorobenzene vapors for up to 7 hr, a significant decrease in flicker-fusion values, indicating a lowered perception, was observed after 3 hr of exposure to 60 ppm (275 mg/m<sup>3</sup>). Subjective symptoms reported after 7 hr of exposure were drowsiness, headache, irritation of the eyes, and sore throat.

### 6.11 Reproductive Organs

A two-generation reproductive toxicity study on rats (67) showed that chlorobenzene induced dose-related changes in the testes. These were manifested as an increased incidence of males with a degeneration of the testicular germinal epithelium in the highest dose group (450 ppm in the diet). Despite these lesions, there were no adverse effects on the reproductive performance or fertility. The results of the reproductive toxicity study are described in more detail starting on p. 51.

Bilateral atrophy of seminiferous epithelium of the testes was also noted among some of the male beagle dogs that were exposed to 273 ppm chlorobenzene vapor for 90 days in the previously discussed IBT-study (see pp. 25-26).

### 6.12 Other Organs

Apart from the organs mentioned above, chlorobenzene has also been found to affect the adrenals of male dogs and rats in subchronic inhalation toxicity tests (28, 53). In the dogs, the

effect on the adrenals was manifested as decreased absolute adrenal weights in animals exposed to 1,570 or 2,080 mg/m<sup>3</sup> chlorobenzene vapor, 6 hr/day, 5 days/week for 6 months. In the rats, the toxicity was manifested as occasional focal lesions in the adrenal cortex (the inhalation concentrations of chlorobenzene in the latter study were 345 or 920 mg/m<sup>3</sup>, 7 hr/day, 5 days/week up to 24 weeks).

The significance of these findings is not known, and there are no other reports on chlorobenzene-induced adrenal toxicity in any of the other identified toxicity studies.

## 7 IMMUNOTOXICITY AND ALLERGY

**Animals:** Aranyi et al. (8) investigated the effects of single and multiple 3-hr exposures to TLV-concentrations of various industrial compounds (75 ppm for chlorobenzene) in female CD1 mice by monitoring their susceptibility to experimentally induced streptococcus aerosol infection and pulmonary bacterial activity to inhaled *Klebsiella pneumoniae*. The results of the study have also been presented in a short notice (5). Whereas, for example, methylene chloride, ethylene chloride and toluene affected both investigated experimental parameters, chlorobenzene apparently lacked significant effects on the murine lung host defenses.

The German BUA report on chlorobenzene (19) cited an unpublished study from Bayer AG (Mihail 1984) reporting that chlorobenzene did not induce skin sensitization (i.e., did not induce allergic contact dermatitis) in the so-called maximization test using male guinea pigs. No further information was given in the short citation.

**Humans:** No relevant reports were available with regard to immunotoxic or allergic effects of chlorobenzene in exposed humans.

## 8 GENOTOXICITY

The results from the testing of the genotoxicity of chlorobenzene in various test systems are not consistent. The overall data seem to show “limited evidence of genotoxicity” since chlorobenzene was reported “positive” in at least three different test systems measuring mutagenicity, chromosomal anomalies and DNA damage/DNA-binding (most of the other test results were reported as “negative”).

Most of the published data on the potential genotoxicity of chlorobenzene are summarized in Table 2. However, as indicated below, there are also some additional tests on the genotoxicity of chlorobenzene. Although cited, these are in general either unpublished studies performed by, or on behalf of, various chemical manufacturers, or reports written in a language not familiar to the evaluator. Consequently, it has not always been possible to judge the validity or significance of each individual result, as reported by others.

No human data are available on possible genotoxic effects following from accidental, occupational or environmental exposure to chlorobenzene.

### 8.1 Gene Mutations

The ability of chlorobenzene to induce gene mutations (point mutations) has been investigated in various strains of *Salmonella typhimurium* (43, 82), in one strain of *Aspergillus nidulans* (74), and in one mammalian cell system, the L5178Y mouse cell lymphoma assay (62). Chlorobenzene was found mutagenic in the mammalian test system, but without effects in the two reverse mutation test systems based on nonmammalian cells. The absence of a mutagenic effect in the various strains of *S. typhimurium*, and the presence of a mutagenic effect in the L5178Y cells, was not affected when a metabolic activation system was added to the test systems.

**Reverse mutations in bacteria:** One of the two recognized and published reverse mutation assays in *Salmonella* (82) was performed as a standard plate incorporation assay. The mutagenicity was tested both in the absence and presence of a liver microsomal fraction (the S9-fraction was prepared from livers from male Sprague-Dawley rats pretreated with a polychlorinated biphenyl). Five different strains of *S. typhimurium* were used: TA1537, TA1538, TA98 (for the detection of frameshift mutations), TA1535 and TA100 (for the detection of base pair substitutions). Chlorobenzene was diluted in DMSO and tested in a series of concentrations from 0.02 µl to 1.28 µl per plate (the highest concentration was clearly toxic in all strains), without being mutagenic.

Table 2.—Summary of some published studies of the potential genotoxicity of chlorobenzene

Genetic end point	Test system [species/strain]	Experimental procedure	Metabolic activation	Dose range	RESULT*	Reference
Gene mutations	Salmonella/mammalian microsome assay [ <i>S. typhimurium</i> TA1535, 1537, 1538, 98, 100]	Standard plate incorporation assay	Yes; S9 from Aroclor 1254-induced rat liver	0.02–1.28 µl per plate	-/-	(82)
	Salmonella/mammalian microsome assay [ <i>S. typhimurium</i> TA1535, 1537, 98, 100]	Suspension assay (pre-incubation procedure)	Yes; S9 from Aroclor 1254-induced rat and hamster liver	33–3,333 µg per plate	-/-	(43)
	Reverse mutations in moulds [ <i>A. nidulans</i> ; <i>met</i> <sub>3</sub> ; <i>pyro</i> <sub>4</sub> ]	Suspension assay	No	200 µg/ml (one concentration only, nontoxic)	-/n.t.	(72)
	Mouse cell lymphoma assay [L5178Y-cells]	Induction phase: 4 hr expression phase: 2 days	Yes; S9 from Aroclor 1254-induced rat liver	Without S9: 6.25–195 µg/ml; with S9: 70–190 µg/ml	+/+	(62)
Structural chromosomal aberrations	Cells arrested in metaphase [CHO-cells]	Cell suspension exposed for 8 hr (-S9) or 2 hr (+S9)	Yes; S9 from Aroclor 1254-induced rat liver	Without S9: 30–500 µg/ml; with S9: 50–500 µg/ml	-/-	(60)
	Micronucleus tests; mouse [bone marrow cells]	Two i.p. injections; survival time after first injection: 30 hr	Not applicable	225–900 mg/kg b.wt.	+	(66)
Primary DNA-damage	Hepatocyte DNA-repair test [rat hepatocytes]	Monolayer cultures exposed 5–20 hr (?)	Not necessary	Highest non-toxic concentration: 9.3 × 10 <sup>-4</sup> M	-	(92)
DNA-binding	Covalent binding index in DNA from liver, kidneys, and lungs [rats and mice]	Covalent binding of <sup>14</sup> C-labelled chlorobenzene after i.p. injection; survival time: 24 hr	Not applicable	127 µCi/kg b.wt. = 8.7 µmol/kg b.wt.	+	(40)
Other genetic effects	Sister chromatid exchanges [CHO-cells]	Cell suspension exposed for 26 hr (-S9) or 2 hr (+S9)	Yes; S9 from Aroclor 1254-induced rat liver	Without S9: 100–1,000 µg/ml; with S9: 30–300 µg/ml	+/-	(60)

\* -/-: no effect without and with metabolic activation; +/+ : effect without and with metabolic activation; +: effect; n.t.: not tested

In the other Salmonella/mammalian microsome assay (43, 68), a preincubation procedure was used instead of the standard plate protocol when testing the potential mutagenicity of chlorobenzene (and 349 other coded chemicals). Four different strains of *S. typhimurium* were used: TA1535; TA1537; TA98 and TA100. The potential mutagenicity was tested both with and without an exogenous metabolic activation system (liver S9-fractions from male Sprague-Dawley rats and Syrian hamsters induced with Aroclor 1254). Chlorobenzene was dissolved in DMSO and tested in concentrations ranging from 33.3 to 3,333.3 µg/plate. In contrast to a positive control, chlorobenzene did not increase the number of revertants in any of the strains tested.

The final draft of the health effect criteria document from EPA (32), and the BUA report (19), referred to other *Salmonella*/microsomal assays than those mentioned above. Since none of these appear to have been published [one study from Monsanto 1976 performed at Litton Bionetics; one from Dupont 1977 performed at Haskell Laboratory; one from Merck 1978 and one performed by Simmon, Riccio, and Peirce 1979], it has not been possible to evaluate them in the present document. All were reported negative, including an investigation on *E. coli* WP2. In the EPA document (32), it was noted that the statistical analysis of the data in these studies did not include information on the number of revertants per unit of survivors.

The ability of chlorobenzene to induce point mutations has apparently also been tested by Koshinova in an assay based on *Actinomyces antibioticus* 400. According to the brief details given in secondary sources of information (6, 92), chlorobenzene was reported to induce reverse mutations in the presence of an exogenous metabolic system. The original study (the information on where this article was published varies, but it appears to have been in *Genetica* 4 (1968) 121-125; presumably in Russian) was not available for evaluation and it has consequently not been possible to evaluate the significance of the reported "positive" effect in the indicated test system (not one of the most established short-term tests for genotoxicity).

**Reverse mutations in moulds:** An auxotroph strain of *Aspergillus nidulans* requiring methionine and pyridoxine was used when testing for the ability of chlorobenzene to induce reverse mutations (72). A suspension of freshly prepared spores was added to a 6% diethyl ether solution of chlorobenzene. After 1 hr of exposure, the mixture of compound, vehicle and spores was diluted and a fraction was spread over pyridoxine and methionine-supplemented minimal medium plates. The number of conidia (revertants) was estimated using a hemacytometer after 5 days of incubation at a temperature of 28°C. Chlorobenzene was tested at one concentration only (200 µg/ml). At this concentration there were no significant differences in survival or number of revertants between the controls and treated. It may be worthwhile to note that this particular test system has not been evaluated and validated to the same extent as, for example, the Salmonella/mammalian microsome assay, the L5178Y mouse lymphoma assay, or the micronucleus test, and it is not clear whether the testing conditions were optimal with regard to, for example, temperature or pH (known to be of importance at least in other types of tests involving fungi).

**Sex-linked recessive lethal mutations in *Drosophila melanogaster*:** Apparently there is at least one unpublished report (90) on the effects of chlorobenzene in the so-called *Drosophila*

sex-linked recessive lethal test (the SLRL test). The ability of chlorobenzene to induce sex-linked recessive lethal mutations in postmeiotic germ cells was evaluated in males (wild-type stock, Canton-S) that had been exposed to at least 9,000 ppm chlorobenzene for 4 hr (36,000 ppm·hr) or 10,700 ppm for 3 × 4 hr (128,400 ppm·hr) before the surviving flies were mated with three sets of virgin “Basc” females for 72 hr each. There was no indication of any mutagenic effect in any germ cell stage. The original report was not available at the time of the present evaluation and it has consequently not been possible to evaluate the experimental conditions, etc., in great detail.

**Forward mutations in mammalian cells *in vitro*:** The L5178Y mouse cell lymphoma assay is a well-established test system when screening for gene mutations *in vitro*. The test system identifies agents that can induce forward mutations in the thymidine kinase locus (TK-locus). Cultures of L5178Y, clone 3.7.2C, were exposed to chlorobenzene for 4 hr and then cultured for 2 days, before plating in soft agar with or without trifluorothymidine (62). Four experiments were performed without S9 (postmitochondrial supernatant fractions of liver homogenate from male Fischer 344 rats pretreated with Aroclor 1254), and two experiments in the presence of the metabolic activation system. Well established mutagens were included in the test as positive controls, and the solvent (DMSO) as a negative control. The dose range varied from 6.25 to 195 µg/ml (without S9) and from 70 to 190 (with S9). The highest concentrations were toxic to the cells. Without S9, two of the four tests yielded inconclusive results, the two others were positive (lowest effective concentration being 100 µg/ml). The two experiments with S9 gave significant and consistent positive responses, showing a mutagenic effect of chlorobenzene.

The final draft of the health effect criteria document from EPA (32) and the German BUA report (19), also mentioned the results of an unpublished mouse lymphoma L5178Y cell culture assay from Monsanto 1976 (testing being performed by Litton Bionetics). In contrast to the study referred to above, chlorobenzene was found to lack mutagenic effects [at 0.001 µl/ml without enzymatic activation, and at 0.001–0.01 µl/ml with activation]. The Monsanto study was not available for evaluation and it has consequently not been possible to judge the significance of the reported “negative” results.

## 8.2 Structural Chromosomal Aberrations

**Chromosomal aberrations in mammalian cells *in vitro*:** The ability of chlorobenzene to induce chromosomal aberrations has been investigated in cultured Chinese hamster ovary cells, CHO-cells, with and without the addition of a metabolic activation system (60). The S9 rat liver microsomal fraction, which was used as metabolic activation system, was prepared from Aroclor-1254-induced male Sprague-Dawley rats. DMSO was used as vehicle and cyclophosphamide (with activation) and mitomycin C (without metabolic activation) as positive controls. Chlorobenzene was tested at the following concentrations: 0, 30, 100, 300, or 500 (without activation), 0, 50, 150, or 510 (experiment one with activation) and 0, 150, 300, or 500 (experiment two with metabolic activation) µg/ml. Approximately 24 hr before the exposure, cultures were initiated at a cell density of  $1.75 \times 10^6$  cells/flask. In the experiment without activation, the cells were incubated with chlorobenzene for 8 hr, before

they were treated with colchemid for 2-2.5 hr before harvest. In the experiments with activation, the cells were incubated with the substance and S9 for 2 hr, then cultivated with medium only for another 8 hr. Colchemid was then added 2 hr before cell harvest. One hundred cells were scored for each of three concentrations (the highest test concentration containing sufficient metaphase cells, and two lower concentrations, covering a 1-log range). An increase of chromatid breaks was seen at one intermediate dose (150 µg/ml) in the first of two trials with S9. However, this effect was not reproducible in the second experiment. No aberrations were induced in the absence of a metabolic system up to a dose of 500 µg/ml, a concentration that was found to be clearly toxic to the cells.

**Chromosomal aberrations in mammalian cells *in vivo*:** The clastogenic ability of chlorobenzene *in vivo* was evaluated in a micronucleus test employing 8-week old male NMRI-mice (65, 66). The compound was given intraperitoneally in four different doses (from 225 to 900 mg/kg b.wt.); the highest amount corresponding to approximately 70% of the i.p. LD<sub>50</sub> value. The total amount of substance was divided into two equal doses (i.e., 2 × 112.5 to 2 × 450 mg/kg) and given 24 hr apart. Each group consisted of 5 exposed animals. The number of corn-oil-treated controls was 10. The frequency of micronucleated polychromatic erythrocytes (MNPCE) was recorded 30 hr after the first injection. Two smears per femur were prepared and coded, and from each bone marrow smear, 1,000 polychromatic erythrocytes were analyzed for the presence of chromosomal fragments. There was a statistically significant and dose-related increase in the number MNPCE in the mice given chlorobenzene when compared to the vehicle-treated controls. No information was given with regard to the potential bone marrow toxicity of the compound (i.e., the ratio between the number of normochromatic and polychromatic erythrocytes).

Apart from the two above-mentioned studies, there is also a Russian study available on the cytogenetic effects of chlorobenzene in bone marrow cells from mice (35). In contrast to benzene, chlorobenzene was reported to be without cytogenetic activity. No effects were seen in a micronucleus test, in a test for chromosomal aberrations in cells arrested in metaphase or in a dominant-lethal test. In each case the doses varied between 3.2 and 400 mg/kg b.wt. The article was written in Russian (apart from a short summary in English without any information on the number of animals involved, survival times, etc.) and it has consequently not been possible to judge the significance of the reported results.

Chlorobenzene has also been tested and reported negative for induction of chromosomal aberrations in CHO-cells in an EPA-sponsored, unpublished study by Loveday (cited in reference 60). In the latter study, chlorobenzene was tested at lower concentrations than those used in the more recent study presented above.

### 8.3 Numerical Chromosomal Alterations

As early as 1943, Östergren and Levan reported that chlorobenzene could induce an abnormal mitotic cell-division in a test system based on the onion *Allium cepa* (98). In a short abstract without details on experimental design, etc., it was stated that full c-mitosis disturbances were observed at a chlorobenzene concentration of 1 mM (precipitate in water); partial disturbances

at 0.3 mM (clear aqueous solution); and normal mitosis at 0.1 mM. The authors suggested that the c-mitotic property of chlorobenzene was due to the physical properties of the compound and not to its chemical properties.

With the possible exception of the “positive” finding in *Allium cepa* (the significance of this remains uncertain) no studies were available on the ability of chlorobenzene to induce aneuploidy, polyploidy or nondisjunction (i.e., numerical chromosomal aberrations). However, the reported increase in the incidence of micronuclei in bone marrow cells from chlorobenzene-exposed mice (65, 66), could apart from being interpreted as showing an ability to induce microscopically observable additions, deletions or rearrangement of parts of chromosomes, possibly also be interpreted as showing a chlorobenzene-induced aneuploidization (gain or loss of one or more intact chromosomes).

#### 8.4 Primary DNA-Damage and Binding to DNA

Chemical damage to DNA can be studied by a variety of methods. Some techniques are nonspecific, others are limited to specific types of injuries. With regard to the DNA-damaging effects of chlorobenzene, only one published study was available in the literature (93). In this study it was reported that chlorobenzene lacked effect on the unscheduled DNA synthesis in a rat hepatocyte DNA-repair test. The so-called hepatocyte/DNA-repair test is a well-established, nonspecific test for DNA damages. An increased DNA-repair synthesis, measured as an increased incorporation of tritiated thymidine in nondividing cells, seems a general response to various types of DNA damages. Another approach that has been used was to measure the DNA-binding capacity of chlorobenzene, both in vivo and in vitro (20, 40, 73). Using this procedure, chlorobenzene was reported to interact directly with DNA.

**Induction of DNA-repair in mammalian cells *in vitro*:** After hepatocytes from adult male F344 rats had been isolated, freshly prepared monolayer cultures were simultaneously exposed to chlorobenzene and <sup>3</sup>H-thymidine (93). The exposure time was not clearly stated, but was somewhere in the interval of 5–20 hr. After exposure, the cultures were fixed and the thymidine incorporation was measured autoradiographically. The criteria used for positive response were the following: at least two concentrations must have yielded net nuclear grain counts significantly greater than the concurrently run solvent controls; a positive dose-response relationship up to toxic concentrations and at least one of the increased grain counts must have been a positive value. Following these criteria, chlorobenzene did not induce DNA-repair synthesis in the primary cultures of rat hepatocytes when given in a concentration up to  $9.3 \times 10^{-4}$  M (highest nontoxic concentration tested; the dose interval was not given).

The final draft of the health effect criteria document from EPA (32) also mentioned an unpublished in vitro study [by Simmon, Riccio, and Peirce 1979] on the DNA-damaging effects of chlorobenzene in a prokaryotic test system. Chlorobenzene was reported to lack DNA-damaging effects in the so-called *pol* A-test, since it was equally toxic to repair-proficient and repair-deficient strains of *E. coli* when tested at concentrations of 10 or 20 µl/plate.

**Interaction with DNA *in vivo* and *in vitro*:** The binding of chlorobenzene and other halogenated hydrocarbons to nucleic acids and proteins was studied in various organs of mice and rats, both *in vivo* and *in vitro* (20, 40, 73). In the *in vivo* experiments, [ $^{14}\text{C}$ ]chlorobenzene (20 mCi/mmol) was given in an amount of 127  $\mu\text{Ci/kg}$  b.wt. (corresponding to 8.7  $\mu\text{mol/kg}$  b.wt.) to groups of 4 male Wistar rats and 12 adult male BALB/c mice. The animals were sacrificed 22 hr after the injection. DNA, RNA, and proteins were isolated from the livers, kidneys, and lungs. In the *in vitro* experiments, microsomal and cytosolic fractions were extracted from liver, lungs, and kidneys from male BALB/c mice and male Wistar rats, pretreated for 2 days with phenobarbital.  $^{14}\text{C}$ -labelled chlorobenzene was incubated with necessary co-factors and microsomal proteins + NADPH, or cytosolic proteins + GSH, for 60–120 min at 37°C. Similar experimental designs were employed for the other agents tested (e.g., bromobenzene, 1,2-dichlorobenzene and benzene).

Radioactivity from all compounds tested, including chlorobenzene, was found to bind covalently to the macromolecules in all organs investigated, both in rats and mice, *in vivo* as well as *in vitro*. The binding appeared to be mediated by the liver microsomes. Although there were no profound differences in DNA-binding capacity of chlorobenzene between the various organs *in vivo*, the highest value was observed in the livers from the exposed rats (0.26  $\mu\text{mol/mol}$  DNA-P), giving a covalent binding index of 38. This value has been suggested to be typical for agents with a weak oncogenic potency (78). The relative reactivity, expressed as covalent binding index to rat liver DNA *in vivo*, decreased in the following order: 1,2-dibromoethane > bromobenzene > 1,2-dichloroethane > chlorobenzene > epichlorohydrine > benzene.

Indirect evidence for the ability of chlorobenzene to interact with DNA has also been presented in a study on the elimination of urinary metabolites in rats given a single *i.p.* injection of 500 mg chlorobenzene/kg b.wt. (55). Low levels of a guanine adduct, probably identical with N7-phenylguanine, were found in the urine on days 1 and 2 and between days 4 and 6 after the injection.

## 8.5 Other Effects on the Genetic Material

In this report, data on sister chromatid exchanges has been treated separately under the heading "Other Effects on the Genetic Material." Representing rearrangements between chromatides within a chromosome (only observable with a special staining technique), SCEs do not constitute true mutations. However, there is a general agreement that there is a close correlation between an increased incidence of SCEs and various types of genotoxic effects. Consequently, sister chromatid exchanges may be looked upon as nonspecific indicators of genotoxicity. As shown in Table 2, chlorobenzene was found to induce SCEs in Chinese hamster ovary cells.

The ability of chlorobenzene to induce SCEs was investigated using cultured Chinese hamster ovary cells, both with and without the addition of a metabolic activation system (60). The activation system used was the S9 rat liver microsomal fraction prepared from Aroclor-1254-induced male Sprague-Dawley rats. Chlorobenzene was dissolved in DMSO, which also was used as the negative control. Mitomycin C was used as the positive control in the absence of,

and cyclophosphamide in the presence of, the metabolic activation system. Chlorobenzene was tested at the following concentrations: 0, 100, 300, or 999 (experiment one without activation); 0, 100, 300, 500, or 1,000 (experiment two without activation) and 0, 30, 100, and 300 (with metabolic activation)  $\mu\text{g/ml}$ . Approximately 24 hr after the cultures had been initiated ( $1.25 \times 10^6$  cells/flask), the medium was replaced and the cells were exposed to chlorobenzene or the control substances. In the experiments without metabolic activation, the cells were exposed for 2 hr before bromodeoxyuridine was added. The incubation then continued for an additional 24-hr period. The medium was removed and the cells were rinsed before new medium with bromodeoxyuridine and colchemid was added for an additional 2-hr culture period. A similar design was used in the experiment with S9, but the medium containing the test substance and the metabolic system was replaced after 2 hr of exposure. After cell harvest and fixation on slides, the cells were stained with Hoechst 33258. Selection of cells for scoring was based on well-spread chromosomes with good morphology. The total number of chromosomes analyzed for SCEs was over 1,000 for each concentration of chlorobenzene.

Chlorobenzene was found to induce a dose-related increase of SCEs in both experiments without S9. Chlorobenzene was reported to be slightly insoluble and toxic at the concentrations that gave the increased incidence of SCEs (in experiment one: 300 and 500  $\mu\text{g/ml}$ ; in experiment two: 500 and 1,000  $\mu\text{g/ml}$ ), but there were no significant decreases in the number of M2 cells. Chlorobenzene did not increase the number of SCEs in the presence of S9 up to a dose of 300  $\mu\text{g/ml}$  (a concentration that was clearly toxic to the cells).

In a review of the genotoxicity of hexachlorobenzene and other chlorinated benzenes (18), it was stated that monochlorobenzene failed to actively induce SCEs in a cultivated human cell line. Since no reference was given to the original paper, it has been impossible to evaluate and judge the validity of this information. Chlorobenzene has also been tested and reported negative for induction of SCEs in an earlier study on CHO-cells than that reported above. In this EPA sponsored, unpublished study by Loveday (cited in reference 60), chlorobenzene was tested at lower concentrations than those employed in the more recent study presented above.

Chlorobenzene was reported to induce reciprocal recombination in the yeast *S. cerevisiae*, strain D3. The number of recombinants/ $10^5$  survivors was increased when chlorobenzene was tested at concentrations of 0.05 or 0.06% in the presence of a metabolic activation system (32). However, since these findings originate from an unpublished report from 1979 by Simmon, Riccio, and Peirce, it has not been possible to evaluate the significance of the reported "positive" finding. No studies were available on the ability of chlorobenzene to induce other types of genetic effects such as reciprocal exchanges between homologous chromosomes, gene conversion, gene amplification, insertional mutations, etc.

## 8.6 Cell Transformation and Tumor Promotion

Cell transformation tests may provide some information of the ability of chemicals to induce neoplastic transformation of cultured somatic cells. These tests do not generally provide any

direct information on the molecular mechanisms of action that could be either genotoxic or epigenetic. Chlorobenzene has apparently been tested in such an assay (29). Cultured adult rat liver cells were exposed to various concentrations of chlorobenzene: 0, 0.001, 0.005, 0.05, or 0.01%. The cells were exposed 12 times. Each exposure lasted 16 hr with sufficient time to recover from toxicity between each exposure. It was reported that chlorobenzene induced a low, but definitive, anchorage independency in the cells, indicating an ability of the substance to induce cell transformation in vitro. This study, originating from the American Health Foundation, has apparently not been published. The information was obtained from a condensed abstract in the TSCATS database and it has consequently not been possible to evaluate the data in great detail.

The ability of chlorobenzene and other halogenated benzenes to promote hepatocarcinogenesis has also been evaluated in a rat liver foci bioassay (45). The end point of the assay (i.e., the occurrence of altered foci of hepatocytes in vivo) is considered to show putative preneoplastic lesions. Male and female Sprague-Dawley rats were subjected to a partial hepatectomy before being given an oral dose of the liver tumor initiator diethylnitrosamine (0.5 mmole/kg b.wt.). One and five weeks after the injection of the carcinogen, groups of rats (5-7 animals) were given an i.p. injection of 0.5 mmole chlorobenzene/kg b.wt. (the total amount corresponding to 112 mg/kg). Two weeks after the final injection, the rats were sacrificed. Pieces of the liver were removed and stained for the presence of  $\tau$ -glutamyltranspeptidase activity (GGT-foci). In contrast to 1,2,4,5-tetrachlorobenzene and hexachlorobenzene, monochlorobenzene was reported to be without tumor promoting activity in male and female rats. However, since the data were presented in a summarized form only, without any indication of having been subjected to statistical analysis, it is difficult to judge the significance of the reported findings. The GGT foci/cm<sup>2</sup> was, for example,  $0.67 \pm 0.31$  (mean  $\pm$  SEM) for male rats given chlorobenzene;  $0.17 \pm 0.15$  for male controls given tricaprylin and  $1.20 \pm 0.34$  for male rats given 1,2,4,5-tetrachlorobenzene (judged "positive").

## 9 CARCINOGENICITY

The potential carcinogenicity of chlorobenzene has been tested in rats and mice but no epidemiological data were available with regard to its carcinogenic effects in humans.

### 9.1 Animal Studies

In a 2-year cancer bioassay on male and female F344/N rats and B6C3F1 hybrid mice, groups of 50 males and 50 females were given chlorobenzene by gavage, 5 days/week for 103 weeks (51, 68). Rats and female mice were given 0 (corn oil; vehicle), 60 or 120 mg/kg b.wt./day; and male mice were given 0, 30, or 60 mg/kg/day. The highest doses used differed by factors of 2-4 from those required to produce severe tissue injury in the previously mentioned subchronic toxicity studies (see p. 24). Also included in the study were 50 untreated animals of each sex and species (untreated controls). The animals were observed daily for mortality, and those animals appearing moribund were sacrificed. Complete necropsies were performed on all animals and a number of tissues were taken for histopathological examination.

The administration of chlorobenzene for 2 years did not significantly affect the body weights of the animals, and there were no overt clinical signs of toxicity. Although the survival rates were slightly reduced in some chlorobenzene-treated groups, a closer analysis showed that this was not due to the compound. The only tumor type found to occur at a statistically significant increased frequency in the chlorobenzene-exposed animals was neoplastic nodules

Table 3.—Frequencies of liver tumors in male rats given chlorobenzene orally up to 2 years (51, 52, 68)

Type of tumor	Frequency (numbers with tumors/numbers examined)			
	Untreated control	Corn oil control	Chlorobenzene exposed	
			60 mg/kg/day	120 mg/kg/day
Neoplastic nodules				
overall incidence	4/50	2/50	4/49	8/49
incidence after 2 years*	2/34	0/39	4/32	7/26
Neoplastic nodules + carcinoma				
overall incidence	4/50	4/50	4/49	8/49
incidence after 2 years*	2/34	2/39	4/32	7/26

\*Tumor incidence at terminal sacrifice (after 2 years of exposure)

in the livers of male rats in the highest dose group. The increased incidence was significant by dose-related trend tests, and by pair-wise comparisons between the vehicle controls and the highest dose group. Neoplastic nodules are of a benign nature, and the only hepatocellular carcinomas diagnosed among the male rats affected two control animals.

The tumor incidences in the male and female mice and in the female rats given chlorobenzene for 2 years did not exceed those in the corresponding vehicle or untreated controls. However, although not being a significant effect, two rare tumor types were also observed in rats given chlorobenzene: 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.

The conclusion that chlorobenzene caused a slight increase in the frequencies of male rats with neoplastic nodules of the liver has been challenged, mainly for statistical reasons (77). The authors of the cancer study disagreed with most of the criticisms, but said that the increased incidence of benign liver tumors in male rats should be considered only as equivocal evidence of carcinogenicity, not sufficient to conclude that chlorobenzene is a chemical carcinogen (52). Using their weight of evidence classification scheme (30), the U.S. EPA rated chlorobenzene in Group D: inadequate evidence of carcinogenicity (6, 33).

In a summary of the results from 86 different 2-year carcinogenicity studies conducted by NTP, Haseman et al. (41) divided the various studies into four different categories: studies showing carcinogenic effects (43/86), studies with equivocal evidence of carcinogenicity (5/86), studies showing no carcinogenic effects (36/86), and inadequate studies (2/86). The increased incidence of neoplastic nodules in the livers of male rats was regarded as evidence showing carcinogenic effects of chlorobenzene. However, no attempt was made to distinguish between “clear” and “some” evidence of carcinogenicity (these agents were pooled into one group).

## 9.2 Epidemiological Studies

Two different surveys of cancer mortality rates for U.S. counties revealed an increased mortality rate from bladder cancer in some northwestern counties in Illinois during the periods 1950-69 and 1970-79; these surveys resulted in a bladder cancer incidence study in eight of the counties incorporated in that region (61). Eligible cases were those diagnosed with bladder cancer during the period of 1978 and 1985. Age-adjusted standardized incidence ratios (SIRs) were calculated for each county and for the 97 zip codes within these counties. When the data were analyzed, only two zip codes were found to have an elevated risk level, and one of these, with a total population of 13,000 inhabitants in 1980, had a significantly increased risk for bladder cancer in both males (number of cases: 21; SIR: 2.6; 95% confidence interval: 1.1-2.6) and females (number of cases: 10; SIR: 2.6; CI: 1.2-4.7).

Since it was revealed that there could have been a potential environmental exposure to trichloroethylene, tetrachloroethylene, benzene, and other organic solvents from the drinking water wells used by that community, a follow-up cross-sectional etiologic study was initiated (74). No risk factors unique to the reported cluster, such as smoking and occupation, could be identified, and the only factor that stood out was the fact that most of the cases had lived in the community for twenty years or more [K. Mallin, personal communication]. With regard to the potential environmental exposure to chlorobenzene, this was probably insignificant, since no trace of the compound was ever found in the community wells themselves, even though it was found in the landfill close to the wells.

There are no case reports or epidemiological studies available concerning the potential carcinogenicity of chlorobenzene in humans.

## 10 TERATOGENICITY AND REPRODUCTIVE TOXICITY

Data on the potential teratogenicity and reproductive toxicity of chlorobenzene are limited to findings obtained in experimental animals. From these experiments there were no indications of any teratogenic effects. However, there was some evidence of embryotoxicity, but only at doses of chlorobenzene that also affected the adult animal. The biological consequences of these effects are difficult to interpret. No adverse effects on reproductive performance or fertility have been observed in animals exposed to chlorobenzene.

### 10.1 Teratogenicity

Until now, there have been no reports on chlorobenzene-induced adverse effects on human fetal development available in the literature. The experimental data on the potential embryotoxicity and teratogenicity of chlorobenzene derives from an inhalation teratology study in rats and rabbits (48). The study, which was performed by Dow Chemicals, also exists in an unpublished version (44). The results have been reported elsewhere in, for example, a review of teratological data on several industrial chemicals (49).

Adult virgin female Fischer F344 rats were mated with adult males of the same strain (48). Groups of 32 to 33 bred females were then exposed to 0, 75, 210, or 590 ppm (0, 345, 966, or 2,714 mg/m<sup>3</sup>) chlorobenzene vapor for 6 hr/day, from day 6 through day 15 of gestation. The animals were sacrificed on day 21 of gestation. The number of dams examined varied between 27 and 29. Among the parameters investigated were: maternal body and liver weights, clinical signs of toxicity, number and position of fetuses in utero; number of live and dead fetuses; number and position of resorption sites; number of corpora lutea; fetal sex ratio, body weight, and crown-rump length of each fetus; gross external alterations of the fetuses; and internal soft tissue malformations and skeletal alterations. Some evidence of chlorobenzene-induced maternal toxicity was observed among the females in the highest dose group: lowered food intake, reduced body weight gain, and increased absolute and relative liver weights. However, the inhalation of chlorobenzene vapor did not induce any teratogenic effects. The only compound-related fetal effect registered was a slight delay in skeletal development of fetuses in the highest dose group.

Two separate experiments were performed with pregnant rabbits. Groups of adult 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) chlorobenzene, 6 hr/day from day 6 to day 18 of gestation. Each group consisted of 30 to 32 rabbits. The animals were sacrificed on day 29 of gestation. The same types of fetal observations were made as those described above for the rats. The number of pregnant animals examined varied between 28

and 31. The only evidence of maternal toxicity observed among the rabbits was a significantly increased incidence of animals with enlarged livers in the two highest dose groups.

In the first experiment, there was a slightly increased incidence of a variety of malformations in all groups examined. Among those were several cases of external and visceral malformations scattered among the chlorobenzene exposed groups. There was no apparent trend for a dose-related increase in any of the single malformations that occurred, with the possible exception of a low incidence of heart anomalies in the highest dose groups (*controls*: 0/117; 75 ppm: 0/110; 210 ppm: 1/193; and 590 ppm: 2/122). With regard to skeletal anomalies, there was a significantly increased incidence of fetuses with an extra thoracic rib in the highest dose group.

In the second experiment, there was a significantly increased incidence of implantations undergoing resorption (showing early embryonic death) in the highest dose group. The percentage of litters containing resorptions was 41% in the control group, 48% in the group exposed to 10 ppm, 50% in the 30 and 75 ppm groups and 61% in the 590 ppm group. The second experiment in rabbits did not show any compound-related increases of any type of malformation. Taken together, the experiments performed on the pregnant rats and rabbits showed some evidence of embryotoxic effects of chlorobenzene at the highest exposure concentration. LOEL with regard to embryotoxicity (delayed skeletal development in rats, an extra rib, and possibly also an increased incidence of early embryonic deaths in rabbits) was 590 ppm (2,714 mg/m<sup>3</sup>), an exposure concentration that was found to induce toxic effects in the adult animal.

John et al. (49) considered that the absence of significant adverse fetal effects in the pregnant experimental animals was evidence enough to suggest that the TLV (at that time, 75 ppm in the United States) afforded an adequate margin of safety for the unborn human child. In 1986, a similar attempt to evaluate the prenatal risks following from occupational exposure to various industrial chemicals was made in Germany (85). Chlorobenzene was one of eighteen agents considered safe at the occupational exposure limit (at that time, 50 ppm in Germany).

## 10.2 Reproduction Toxicity

In a final test rule for chlorobenzene, released in July 1986 (31), the U.S. EPA required manufacturers and processors of chlorobenzene to conduct reproductive effects testing of chlorobenzene to elucidate the potential reproductive hazard of the compound. At that time, EPA believed that the information available from general toxicity tests (probably those deriving from IBT, see p. 25) on testicular effects in dogs exposed to chlorobenzene, suggested a potential reproductive hazard in humans.

To satisfy the need for reproductive effects testing for chlorobenzene, Monsanto Company conducted a two-generation reproductive study on rats (67). Groups of 30 male and 30 female Sprague-Dawley CD rats (F0-generation) were exposed to 0, 50, 150, or 450 ppm (i.e., 0, 230, 690, or 2,070 mg/m<sup>3</sup>) 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 F0-generation (30 males and 30 females/group) formed the F1-generation. These animals were exposed to the same concentrations of chlorobenzene as the F0-generation, starting 1 week post-weaning; lasting 11 weeks prior to mating; and through mating, gestation, and lactation. The progeny of the F1-generation, the F2-pups, were observed during weaning, and then they were sacrificed. A number of parameters were investigated, including body weights, food consumption, mating and fertility indices, pup and litter survival, and histopathological examinations of selected organs (liver, kidneys, pituitary gland, and male and female reproductive organs).

**Table 4.—Frequencies of liver, kidney and testicular lesions in male rats exposed to chlorobenzene vapor up to eleven weeks (67)**

Organ	Lesion	Generation	Doses (ppm)			
			0	50	150	450
Liver	Hepatocellular hypertrophy (minimal to mild)	F0	0/30	0/30	5/30	14/30
		F1	2/30	0/30	3/30	7/30
Kidney	Tubular dilation (unilateral + bilateral)	F0	0/30	4/30	6/30	18/30
		F1	8/30	7/30	14/30	22/30
	Interstitial nephritis (unilateral + bilateral)	F0	1/30	2/30	7/30	10/30
		F1	1/30	3/30	7/30	11/30
	Foci of regenerative epithelium (unilateral + bilateral)	F0	0/30	1/30	5/30	8/30
		F1	1/30	0/30	5/30	11/30
Testes	Degeneration of germinal epithelium (unilateral + bilateral)	F0	1/30	0/30	2/30	6/30
		F1	1/30	0/30	3/30	6/30

Chlorobenzene did not significantly affect the body weights or food consumption in any of the generations studied. However, the histopathological examination showed dose-related changes in the livers, kidneys, and testes of F0 and F1 males. The hepatotoxicity was manifested both as hepatocellular hypertrophy and significantly increased mean and absolute liver weights. The lowest LOEL for the latter effect was 50 ppm (i.e., 230 mg/m<sup>3</sup>), the F0-males being most sensitive.

The renal changes appeared as an increased incidence of animals with tubular dilation with eosinophilic material, interstitial nephritis, and foci of regenerative epithelium. There was an

increased incidence of animals with a degeneration of the testicular germinal epithelium among the F0-males in the highest dose group (bilateral changes), and F1-males in the two highest dose groups (unilateral changes only).

Despite the testicular lesions observed in the male rats of the highest dose groups, there were no chlorobenzene-induced adverse effects on the reproductive performance or fertility of the adult animals. The maternal body weights during the gestation and lactation were comparable with those of the controls, mating and fertility indices were unaffected in both the F0 and F1-generation, and the pup and litter survival indices for all exposed groups were comparable with those of the corresponding controls.

## 11 DOSE-EFFECT AND DOSE-RESPONSE RELATIONSHIPS

Tables 5-8 on the following pages summarize the various toxic effects of chlorobenzene. It is suggested that the following effects and dose levels should be taken into consideration when establishing permissible levels of occupational exposure:

- (1) The prenarctic and irritating effects of chlorobenzene (observed in humans exposed to 60 ppm for 3-7 hr).
- (2) The clear hepatotoxic effects of chlorobenzene ("lowest" LOEL value reported was 50 ppm in rats exposed for 11 weeks).
- (3) The possible hematopoietic toxicity of chlorobenzene (leukopenia was reported in mice exposed to 22 ppm for 3 months).

### 11.1 Acute Exposure

Table 5 summarizes some data obtained in various acute toxicity studies on experimental animals. The table also includes some information on the acute toxicity of chlorobenzene in humans. However, our knowledge of the acute toxicity of chlorobenzene in humans derives almost exclusively from isolated case reports of poisonings or accidental occupational exposures, showing that chlorobenzene may induce significant CNS-depression (i.e., narcotic effects such as drowsiness, incoordination, and unconsciousness) at high acute dose exposures. Unfortunately, these reports cannot be used for the establishment of dose-effect relationships, mainly because they do not include any information on the actual levels of exposures.

The critical effect of acute exposure to chlorobenzene vapors appears to be the prenarctic effects of the substance. An exposure chamber study on five male volunteers exposed to 60 ppm (275 mg/m<sup>3</sup>) for 7 hr (71) showed that these concentrations induced acute subjective symptoms such as drowsiness, headache, irritation to the eyes, and sore throat. A significant decrease in flicker-fusion values, indicating a lowered perception, was observed after 3 hr of exposure to the same concentration of chlorobenzene vapor (71). The information on the human recognition odor threshold for chlorobenzene varies, but is probably about 0.68 ppm (i.e., 3.1 mg/m<sup>3</sup>) (3).

### 11.2 Repeated Exposure

The various effects following repeated exposures of chlorobenzene have been summarized in Tables 6-8. Some previously cited studies in this report, are not included in these tables. The main reason for this is that they were considered insufficient with regard to information on

dose-effect and dose-response relationships, thereby preventing a meaningful evaluation of NOEL and LOEL values. To get a complete picture of the toxicity of chlorobenzene after repeated exposure, the reader is referred to earlier sections of this document.

Table 5.—Acute effects of chlorobenzene in experimental animals and humans

End point (effect)	Route of administration	Species	LD <sub>50</sub> /LC <sub>50</sub> or LOEL <sup>*</sup>	Reference
Mortality <sup>†</sup>	Oral	Rat	1,540 mg/kg	19
		Mouse	1,355 mg/kg	66
		Rabbit	2,250 mg/kg	19, 32
		Guinea pig	5,060 mg/kg	19, 32
		Man	500–5,000 <sup>§</sup> mg/kg	92
Mortality <sup>†</sup>	Inhalation	Rat	13,870 mg/m <sup>3</sup> for 6 hr	19
		Mouse	8,822 mg/m <sup>3</sup> for 6 hr	19, 32
CNS-depression (narcosis)	Oral	Rat	1,540 mg/kg	19, 51, 68
	Oral	Mouse	1,000 mg/kg <sup>**</sup>	51, 68
CNS effects (prenarcotic effects) <sup>††</sup>	Inhalation	Man	275 mg/m <sup>3</sup> for 7 hr	71
Behavioral changes <sup>§§</sup>	Inhalation	Mouse	3,700 mg/m <sup>3</sup> for 4 hr <sup>§§</sup>	24
Liver necrosis	i.p. injection	Rat	1,100 mg/kg	22
Increased serum ALAT	i.p. injection	Rat	225–1,100 mg/kg	22
Mild centrilobular changes (e.g., cloudy swelling)	i.p. injection	Rat	225 mg/kg	22
Decreased hepatic levels of glutathione	i.p. injection	Rat	225 mg/kg	22
Renal necrosis	i.p. injection	Mouse	760 mg/kg	75
Increased bile duct flow	i.p. injection	Rat	562 mg/kg	94

\*LD<sub>50</sub>/LC<sub>50</sub> for mortality; LOEL for other effects (lowest identified values).

†Generally due to respiratory paralysis.

§Estimated probable acute lethal dose in animals.

\*\*Animals were not subjected to necropsy.

††Acute subjective symptoms (drowsiness, headache, irritations from eyes and sore throat).

§§50% decreased immobility in a behavioral despair swimming test.

**Table 6.—Effects of chlorobenzene after repeated exposure: hepatotoxic effects**

End point (effect)	Route of administration	Duration	Species	LOEL	NOEL	Reference
Increased serum levels of liver enzymes	Oral	13 weeks	Rat	500 mg/kg	250 mg/kg	51, 68
		13 weeks	Dog	54-272 mg/kg	27-54 mg/kg	32, 53
Increased total liver porphyrin	Oral	13 weeks	Rat	500 mg/kg	250 mg/kg	51, 68
Increased liver weights (relative)	Oral	2 weeks	Rat	2,300 mg/kg	not determined	46
		13 weeks	Rat	125 mg/kg	60 mg/kg	51, 68
		13 weeks	Mouse	250 mg/kg	125 mg/kg	51, 68
	Inhalation	11 weeks	Rat	230 mg/m <sup>3</sup>	not determined	67
		6 months	Dog	1,570 mg/m <sup>3</sup>	780 mg/m <sup>3</sup>	32, 53
Hepatocellular degeneration/hypertrophy and/or necrosis	Oral	13 weeks	Rat	250 mg/kg	125 mg/kg	51, 68
		13 weeks	Mouse	250 mg/kg	125 mg/kg	51, 68
	Inhalation	11 weeks	Rat	690 mg/m <sup>3</sup>	230 mg/m <sup>3</sup>	67
Neoplastic nodules	Oral	2 years	Male rats	120 mg/kg	60 mg/kg	51, 68

**Table 7.—Effects of chlorobenzene after repeated exposure: additional toxic effects**

Organ/Effect	Route of administration	Duration	Species	LOEL	NOEL	Reference
Kidneys Necrosis or degeneration (proximal tubules)	Oral	13 weeks	Rat	500 mg/kg	250 mg/kg	51, 68
		13 weeks	Mouse	250 mg/kg	125 mg/kg	51, 68
Tubular dilation, interstitial nephritis and/or foci of regenerative epithelium	Inhalation	11 weeks	Rat	690 mg/m <sup>3</sup>	125 mg/m <sup>3</sup>	51, 68
Lungs Increased weight	Inhalation	24 weeks	Rabbit	345 mg/m <sup>3</sup>	not determined	28
Testes Degeneration of germinal epithelium	Inhalation	11 weeks	Rat	690 mg/m <sup>3</sup>	230 mg/m <sup>3</sup>	67
Thymus Lymphoid necrosis	Oral	13 weeks	Mouse	250 mg/kg	125 mg/kg	51, 68
Spleen Lymphoid or myeloid depletion	Oral	13 weeks	Rat	750 mg/kg	500 mg/kg	51, 68
		13 weeks	Mouse	250 mg/kg	125 mg/kg	51, 68
Bone marrow* Myeloid depletion	Oral	13 weeks	Rat	500 mg/kg	250 mg/kg	51, 68
Embryotoxicity	Inhalation	During pregnancy	Rat	2,714 mg/m <sup>3</sup>	966 mg/m <sup>3</sup>	48
		During pregnancy	Rabbit	2,714 mg/m <sup>3</sup>	966 mg/m <sup>3</sup>	48

\*Zub (97) showed that male and female Swiss mice developed leukopenia after having been exposed to 22 ppm (100 mg/m<sup>3</sup>) chlorobenzene 7 hr/day for 3 months and it has been reported in secondary sources of information (32, 92) that Varhavskaya observed various types of pathological changes in the bone marrow of male rats given oral doses of 0.01 mg chlorobenzene/kg b.wt./day for 9 months (the significance of the latter study is questionable; such low doses have not induced hematopoietic toxicity in any other study).

**Table 8.—Effects of chlorobenzene after repeated exposure: mortality and body weight gain**

End point (effect)	Route of administration	Duration	Species	LOEL	NOEL	Reference
Mortality	Oral	14 days	Rat	1,000 mg/kg	500 mg/kg	51, 68
		13 weeks		500 mg/kg	125 mg/kg	51, 68
		14 days	Mouse	1,000 mg/m	500 mg/kg	51, 68
		13 weeks		125 mg/kg	60 mg/kg	51, 68
	Inhalation	13 weeks	Dog	272 mg/m <sup>3</sup>	54 mg/m <sup>3</sup>	32, 53
Decreased body weight gain	Oral	13 weeks	Rat	250 mg/kg	125 mg/kg	51, 68
		99 days	Rat	250 mg/kg	50 mg/kg	53
		13 weeks	Mouse	250 mg/kg	125 mg/kg	51, 68

## 12 RESEARCH NEEDS

- (1) The data on the genotoxic and tumor-promoting effects of chlorobenzene are not consistent. This is an area requiring further research, especially with regard to the reported ability of chlorobenzene (or more likely of some of its metabolites) to bind covalently to DNA.
- (2) The structural resemblance between chlorobenzene and benzene, and the reported hematopoietic toxicity of chlorobenzene in experimental animals, call for further studies addressing the potential problem with the chlorobenzene-induced bone marrow (i.e., hematopoietic) toxicity, especially with regard to potential dose-effect and dose-response relationships.
- (3) Chlorobenzene has been used in large quantities in industry for several years. However, there is still a paucity of data on actual exposure levels of chlorobenzene in occupational settings today. A survey of the potential exposure to chlorobenzene in relevant industries is therefore recommended.
- (4) There are only limited epidemiological data available on the health status of workers chronically exposed to chlorobenzene. Recent data on a limited number of volunteers showed, for example, that exposure to chlorobenzene vapors at previous threshold limit values (e.g., 75 ppm in the United States and 50 ppm in Germany) can induce preneoplastic effects, and animal data show that repeated exposure to chlorobenzene at these levels may affect the liver. Information from epidemiological studies examining dose-effect and dose-response relationships, especially with regard to the preneoplastic, hepatotoxic and possibly also hematopoietic effects of chlorobenzene, would be useful.
- (5) Further studies should be made to explore and assess the potential risks from the extrahepatic bioactivation of chlorobenzene (e.g., in the nasal mucosa).

According to EXICHEM (34), an OECD database on projects on existing chemicals, there are ongoing or planned activities in several countries with regard to the evaluation and assessment of the potential adverse health and environmental effects of chlorobenzene. Most of these activities seem to involve gathering of scientific data on toxicological and ecotoxicological effects, monitoring of environmental levels, and health and/or environmental hazard evaluations. It may be worthwhile to note that chlorobenzene has been designated "future high priority" by IARC (34).

## 13 DISCUSSION AND EVALUATION OF HEALTH EFFECTS

Chlorobenzene (also known as monochlorobenzene or benzene chloride) is 1 of 12 possible chemical species in the group of chlorinated benzenes. At room temperature, chlorobenzene is a colorless, volatile liquid with an odor that has been described as almondlike, or like that of mothballs and benzene. Chlorobenzene is hardly soluble in water, but is freely soluble in lipids and various organic solvents.

Chlorobenzene has been used extensively in industry for many years, and its main use is as a solvent and intermediate in the production of other chemicals. In occupational settings, the main exposure is that following inhalation of chlorobenzene vapors.

Once absorbed, chlorobenzene is rapidly distributed to various organs in the body. Highest levels are found in fat, liver, lungs, and kidneys. Chlorobenzene is metabolically activated to two different intermediate electrophilic epoxides by cytochrome P450/P448-dependent microsomal enzymes. Chlorobenzene is not only bioactivated in the liver, but also in other organs and tissues such as the lungs and nasal mucosa. The reactive metabolites of chlorobenzene are converted either nonenzymatically to various chlorophenols, or enzymatically to the corresponding glutathione conjugates and dihydrodiol derivatives. The glutathione conjugates are then either eliminated as such, or transferred to even more water-soluble products and excreted in the urine as mercapturic acids. The dihydrodiol derivatives are converted to catechols and excreted as such in the urine. The absolute quantities and ratios between the various metabolites formed differs among various species.

The major human urinary metabolites of chlorobenzene are the free and conjugated forms of 4-chlorocatechol and p-chlorophenol. It has been recommended that measurements of these should be used as biological exposure indicators for monitoring occupational exposure. Recommended biological threshold limits at the end of a working shift are 116 mmol of total chlorocatechol and 22 mmol total p-chlorophenol per mol creatinine (2).

The toxic effects of chlorobenzene in experimental animals are relatively well documented, although many toxicity studies were unpublished reports or written in a language not familiar to the evaluator. No major data gaps could be identified, and the majority of the identified studies appeared to be of acceptable quality, permitting a meaningful risk identification. The amount of human data on the toxicity of chlorobenzene is, however, limited.

The acute toxicity of chlorobenzene in experimental animals is relatively low. The lowest acute inhalation LC<sub>50</sub> value identified was 8,800 mg/m<sup>3</sup> (female mice exposed for 6 hr). The acute exposure to high concentrations of chlorobenzene is mainly associated with various CNS-effects. These are generally manifested as initial excitation followed by drowsiness,

adynamia, ataxia, paraparesis, paraplegia and dyspnea. Death is generally a result of respiratory paralysis. CNS-depressant effects (drowsiness, incoordination and unconsciousness) have also been observed in humans after acute poisoning or occupational exposure to high concentrations of chlorobenzene. The human probable oral acute lethal dose of chlorobenzene has been estimated at 0.5–5 g/kg b.wt.

An exposure chamber study of five male volunteers exposed to 60 ppm (275 mg/m<sup>3</sup>) for 7 hr showed that this concentration of chlorobenzene induced acute subjective symptoms such as drowsiness, headache, irritation to the eyes, and sore throat. A significant decrease in flicker-fusion values, indicating lowered perception, was observed after 3 hr of exposure to the same concentration of chlorobenzene vapor. Inhalation of chlorobenzene vapor is irritating to the eyes and the mucous membranes of the upper respiratory tract. Prolonged skin contact may lead to mild chemical burns.

Repeated administration of chlorobenzene to experimental animals for several weeks or months, is mainly associated with various effects in the liver and kidneys. These organs are, with the CNS, the primary targets for chlorobenzene-induced toxicity. The hepatotoxicity of chlorobenzene is manifested as increased activities of serum liver enzymes, increased liver weight, hepatic porphyria and hepatocellular necrosis. Similar effects have also been observed in a man who ingested 140 ml of a 90% chlorobenzene solution in a suicide attempt. It may be useful to note that there is some evidence from in vitro studies, showing that humans, due to metabolic differences, may be more susceptible to the hepatotoxic effects of chlorobenzene than rodents. The nephrotoxic action is mainly manifested as increased kidney weight, focal coagulative degeneration, and necrosis of the proximal tubules.

Chlorobenzene differs from many polychlorinated aromatic hydrocarbons in not being a general inducer of the cytochrome P450/P448 enzyme system. Instead, chlorobenzene appears to lower the cytochrome P450 levels. Since administration of chlorobenzene induces an initial, but transient, depletion of the glutathione levels in the liver, exposure to this compound seems associated with a lowered capacity of both bioactivating and detoxifying enzyme systems.

Repeated administration of chlorobenzene to experimental animals is also associated with lesions of the thymus (lymphoid depletion and necrosis), spleen (lymphoid or myeloid depletion), bone marrow (leukopenia, myeloid depletion, general bone marrow depression), lungs (increased lung weights, necrotic lesions in the bronchial epithelium), and testes (bilateral or unilateral degeneration of the germinal epithelium). Of these effects, the hematopoietic toxicity is of special interest. When male and female mice were exposed to 100 mg/m<sup>3</sup> (22 ppm) of chlorobenzene, 7 hr/day for 3 months, they were reported to develop leukopenia and a general bone marrow depression.

It is generally assumed that the toxic effects of chlorobenzene are mediated by covalent binding of reactive metabolites to critical cell structures in the target organs. However, the exact molecular mechanisms of action behind the various toxic effects of chlorobenzene are still unknown. Several possible toxicological mechanisms may be involved. Whereas, for example, the hepatotoxic and nephrotoxic action of chlorobenzene may be a direct result of covalent

binding to critical structures and/or an indirect effect of oxidative stress, the CNS-depressant effect is most likely mediated by other toxicological mechanisms, probably induced by the unmetabolized substance itself.

It appears as if halogenated aromatic monocyclics form a complex group when it comes to the interpretation of their genotoxicity. In the case of chlorobenzene there is no problem with lack of information. At least 12 different published investigations representing various types of genetic endpoints and/or test systems were identified. Apart from the published information, there are also several unpublished studies mentioned in the present document. Even if some results only are presented as a figure or symbol in a summarizing table, the conceivable problem with condensed presentations of study designs, protocols and results do not seem of major importance in the case of chlorobenzene. There were no obvious differences in study qualities between those reporting absence of genotoxic effects and those showing effects.

The major problem in interpreting the existing genotoxicity data for monochlorobenzene relates to the fact that the compound was reported "negative" in some test systems, and "positive" in others. The interpretation becomes even more complex when one also has to consider that whereas some authors reported that chlorobenzene was genotoxic/mutagenic in a given test system, other investigators reported a "negative" result. In the case of chlorobenzene, this seems to be the situation in the L5178Y mouse cell lymphoma assay, the micronucleus test, and when measuring SCEs in vitro (at least when one includes unpublished information).

The combination of being positive in an L5178Y gene mutation assay and in a SCE assay and simultaneously being negative in the Ames test and in an assay for chromosomal aberrations in CHO cells, is not unique for chlorobenzene (1, 89). However, the mutagenic effect of chlorobenzene observed in the L5178Y cells, with and without exogenous metabolic activation, and its ability to induce sister chromatid exchanges in cultivated Chinese hamster cells in the absence of metabolic activation, are not isolated positive responses. Consequently, chlorobenzene has also been shown to increase the incidence of micronuclei in bone marrow cells of exposed mice in a dose-dependent manner. Although chlorobenzene apparently lacked DNA-damaging effects in a rat hepatocyte DNA-repair test, radioactivity from  $^{14}\text{C}$ -chlorobenzene was reported to bind covalently directly to DNA in various organs, including the liver. This was shown in both mice and rats, in vivo as well as in vitro. The latter findings suggest that chlorobenzene, or more likely, some of its metabolites, can interfere directly with the DNA-molecule.

The data on DNA-binding should be interpreted with some care because it cannot be excluded that the relatively low levels of DNA-binding is an artifact resulting from protein contamination. The reported binding of [ $^{14}\text{C}$ ]chlorobenzene-associated radioactivity to nucleic acids deserves particular attention and should be further examined. At present, it is suggested that chlorobenzene should be regarded as an agent capable of inducing a certain degree of DNA-binding after administration of large doses.

Beside the above-mentioned “positive” results from various short-term tests, chlorobenzene has also been reported to induce point mutations in *Actinomyces antibioticus* 400, abnormal mitotic cell-division in *Allium cepa*, and reciprocal recombination in *Saccharomyces cerevisiae*. However, the significance of these results remains unclear for various reasons.

Previously, when the number of available genotoxicity studies was limited, it was suggested that chlorinated benzenes, including chlorobenzene, appeared to lack significant genotoxic properties (18). However, paying attention to more recent findings it may be wise to reconsider such a conclusion, or at least to initiate more careful and exhaustive reevaluation of the potential genotoxicity of chlorobenzene. Although not always consistent and clear, the overall data are judged to show “limited evidence of genotoxicity” of chlorobenzene. This judgment is based on the fact that chlorobenzene has been reported “positive” in at least three different test systems measuring mutagenicity, chromosomal anomalies, and DNA damage/DNA-binding, at the same time as the majority of test results were reported as “negative.” With regard to the question of how potent genotoxic agent chlorobenzene might be, the available “positive” studies showed that its genotoxic potential is low. The effects were generally observed only after administration of relatively high concentrations of chlorobenzene.

The ability of chlorobenzene to induce neoplastic transformation has also been tested with conflicting results. Whereas the compound was found to induce a low, but definite, anchorage-independency in cultured rat liver cells, it was without activity in a rat liver foci bioassay. The significance of these results remains unclear.

Chlorobenzene induced benign liver tumors in male rats, but was without tumorigenic effects in female rats and male and female mice given the compound by gavage, 5 days/week for 103 weeks (60 or 120 mg/kg b.wt./day). The inadequate/equivocal evidence of carcinogenicity in experimental animals, in combination with the limited evidence of genotoxicity from short-term tests and the absence of epidemiological data, implies that chlorobenzene, at present, should be regarded as an agent not classifiable as to human carcinogenicity.

Animal experiments on the potential teratogenicity and reproductive toxicity of chlorobenzene did not show any significant teratogenic potential of the compound. However, there was some evidence of embryotoxic effects in both rabbits (skeletal anomalies and an increased incidence of early embryonic deaths) and rats (delayed skeletal development), but these effects were only seen at doses found toxic to the adult animal (LOEL with regard to embryotoxicity was established to 590 ppm (i.e., 2,714 mg/m<sup>3</sup>). A two-generation reproductive toxicity study in rats did not show any chlorobenzene-induced adverse effects on the reproductive performance or fertility.

Apparently, chlorobenzene is without immunotoxic effects in mice after multiple exposures at 75 ppm (345 mg/m<sup>3</sup>), and the compound was reported not to induce skin sensitization in a maximization test on male guinea pigs.

CNS effects (i.e., prenarcoctic effects) are judged to be the most critical effects following acute exposure to chlorobenzene vapors. An exposure chamber study involving five male volunteers

exposed to 60 ppm (276 mg/m<sup>3</sup>) for up to 7 hr, showed that this relatively low concentration of chlorobenzene vapor resulted in acute subjective symptoms such as drowsiness, headache, irritation of the eyes, and sore throat.

Based on what is presently known about the various toxic effects of chlorobenzene, the hepatotoxic and nephrotoxic (LOEL in the most sensitive species after 11 weeks of inhalation was 50 ppm), and possibly also the hematopoietic effects (leukopenia was observed in mice after 3 months of exposure to 22 ppm), are judged to be the most critical effects observed after exposure to chlorobenzene. Consequently, it is on these effects that various threshold limit values should be based.

So far, there is no reliable scientific data showing that oral doses and/or inhalation of air concentrations below the indicated LOEL values would induce other types of significant adverse effects in experimental animals.

## 14 SUMMARY

B. Hellman: NIOH and NIOSH Basis for an Occupational Health Standard: Chlorobenzene. Expert Group for Documentation of Occupational Exposure Limits. *Arbete och Hälsa* 1992:31, pp. 1-73.

In the present document, relevant data are summarized and evaluated for the purpose of establishing permissible levels of occupational exposure to chlorobenzene. Of the various effects described, the effects on the central nervous system (prenarcotic effects) of chlorobenzene, together with its hepatotoxic effects, should be considered in setting occupational exposure limits. At present, there is "limited evidence" indicating that chlorobenzene is genotoxic and that it may induce hematopoietic toxicity at relatively moderate doses. It is presently not classifiable as to human carcinogenicity. 105 references.

**Key-words:** Chlorobenzene; occupational exposure limits; CNS effects; hepatotoxicity; genotoxicity, hematopoietic toxicity.

## 15 SAMMANFATTNING PÅ SVENSKA

B. Hellman: NIOH and NIOSH Basis for an Occupational Health Standard: Chlorobenzene. Expertgruppen för Gränsvärdesdokumentation. Arbete och Hälsa 1992:31, sid 1-73.

I det aktuella dokumentet görs en genomgång och utvärdering av den litteratur som befunnits vara relevant som underlag för fastställande av ett hygieniskt gränsvärde för yrkesmässig klorbensenexponering. Av de effekter som beskrivs, bör hänsyn tas till klorbensens påverkan på det centrala nervsystemet (prenarkotiska effekter), samt dess levertoxiska effekter, när de hygieniska gränsvärdena fastställs. För närvarande föreligger även misstanke om att klorbensen är en lågpotent genotoxisk substans, och att man inte kan utesluta en påverkan på blodbilden vid relativt måttliga exponeringsnivåer. För närvarande kan man inte med säkerhet uttala sig om huruvida klorbensen är att betrakta som varande en kemisk carcinogen eller ej. 105 referenser.

**Nyckelord:** Klorbensen; hygieniska gränsvärden; centralnervösa effekter; levertoxicitet; genotoxicitet, benmärgstoxicitet.

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