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 An unpublished simulation study by Droz, cited by ACGIH (**2**), suggests that the pulmonary retention at steady-state is about 60 percent.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 ^{14}C-chlorobenzene (uniformly labelled) at 100, 400 or 700 ppm (460, 1,840 or 3,220 mg/m^3) 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 ^{14}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^3), 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^3) 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}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}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 μCi 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 etheral 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}C-chlorobenzene in Cremophore E.L. and physiological saline orally to two female Dutch rabbits (0.5 g × 2/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., etheral 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}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 ^{14}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 ^{14}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 ^{14}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

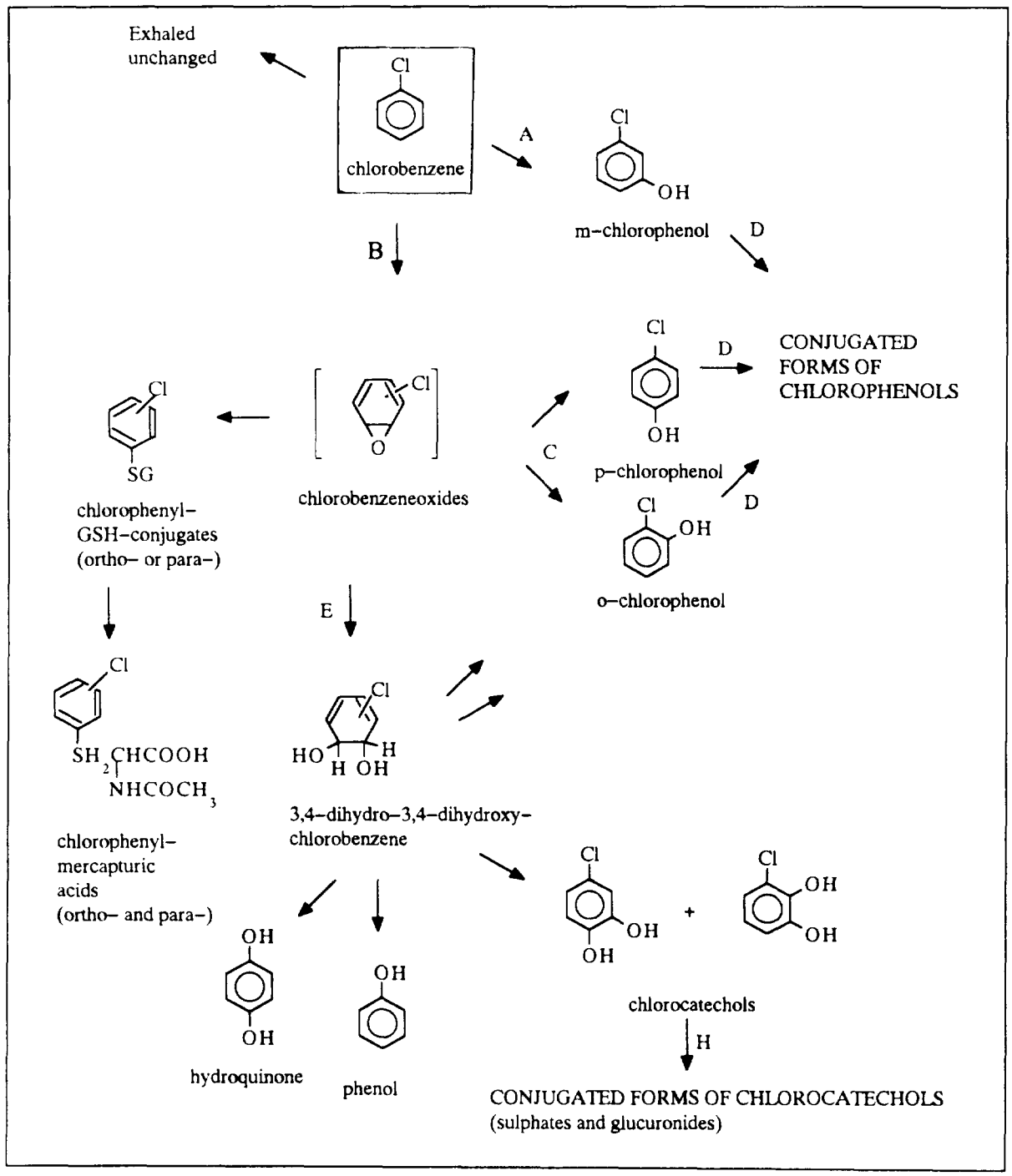

Figure 1: Proposed metabolic pathways for chlorobenzene

A Hydroxylation
B Cytochrome P450/P448-dependent microsomal oxidation
C Rearrangement
D Conjgation: 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 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 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 × 0.3 mmol/kg b.wt.) and two occupationally exposed workers with estimated inhalation exposures of 0.84 ppm × 415 min and 0.5 ppm × 228 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^3) 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^3) 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^{18}, 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^3). 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^3). From extrapolations performed on the data, it was calculated that 8 hr exposure to 50 ppm chlorobenzene (230 mg/m^3), 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}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}C-labelled chlorobenzene (**20, 40, 73**). The reported binding of ^{14}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}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_{50} and LC_{50} values in various published and unpublished acute toxicity tests in experimental animals

Species	Strain/Sex	Route of administration	Reported LD_{50}/LC_{50}	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^3 for 2 hr = LC 100	Rozenbaum et al. 1947	19
	n.g./n.g.	Inhalation	18,016 mg/m^3 exposure time n.g.	Eitingon 1975	19
	n.g./males	Inhalation	13,870 mg/m^3 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^3 for 6 hr	Bonnet et al. 1979	19, 32
	NMRI/males	i.p.	1,355 mg/kg	66	
Rabbit	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^3 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_{50} 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^3) 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^3) 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^3) 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), cytoplasmatic 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^3). 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^3 (**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 capsulae 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.