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Unpublished experimental data from the German manufacturer Bayer AG (Suberg 1983a, 1983b), cited in the German BUA report (**19**), showed that chlorobenzene is moderately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 The same unpublished studies from Bayer AG also showed that chlorobenzene is a moderate irritant to the eyes (**19**).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 σ-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 σ-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_{10} 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 sulphobromophtalein (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 × 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 transjungular 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_1 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 ^{14}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}C-chlorobenzene (1 mmol/kg b.wt.; 10-30 μCi/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^3 (22 ppm), 7 hr/day for three months or to 2,500 mg/m^3 (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^3) 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^3) 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 prenarcotic 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 measures 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^3), 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^3) 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^3). 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^3 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^3, 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 [*S. typhimurium* 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 [*S. typhimurium* 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 [*A. nidulans*; met_3-; $pyro_4$]	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^{-4} M	-	(92)
DNA-binding	Covalent binding index in DNA from liver, kidneys, and lungs [rats and mice]	Covalent binding of ^{14}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 hematocytometer 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 1.75×10^6 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_{50} 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 ^{3}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 9.3×10^{-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, [U-^{14}C]chlorobenzene (20 mCi/mmol) was given in an amount of 127 μCi/kg b.wt. (corresponding to 8.7 μmol/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}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 μmol/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) μg/ml. Approximately 24 hr after the cultures had been initiated (1.25×10^6 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 μg/ml; in experiment two: 500 and 1,000 μg/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 μg/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 τ-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^2 was, for example, 0.67 ± 0.31 (mean ± SEM) for male rats given chlorobenzene; 0.17 ± 0.15 for male controls given tricaprylin and 1.20 ± 0.34 for male rats given 1,2,4,5-tetrachlorobenzene (judged "positive").