9 CARCINOGENICITY

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

9.1 Animal Studies

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

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

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

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

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

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

The tumor incidences in the male and female mice and in the female rats given chlorobenzene for 2 years did not exceed those in the corresponding vehicle or untreated controls. However, although not being a significant effect, two rare tumor types were also observed in rats given chlorobenzene: transitional-cell papillomas of the urinary bladder (one male in the low dose group, and one male in the high dose group) and a tubular-cell adenoma of the kidney (one female rat in the high dose group). The historical incidences of these tumors in Fischer F344/N rats were, at the time of the study, 0/788 for transitional cell-papilloma of the urinary bladder in corn-oil-treated males, and 0/789 for renal tubular-cell adenocarcinoma in female controls given corn oil.

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

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

9.2 Epidemiological Studies

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

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

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

10 TERATOGENICITY AND REPRODUCTIVE TOXICITY

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

10.1 Teratogenicity

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

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

Two separate experiments were performed with pregnant rabbits. Groups of adult female New Zealand White rabbits were artificially inseminated and exposed to 0, 75, 210, or 590 ppm (experiment 1) and to 0, 10, 30, 75, or 590 ppm (experiment 2) chlorobenzene, 6 hr/day from day 6 to day 18 of gestation. Each group consisted of 30 to 32 rabbits. The animals were sacrificed on day 29 of gestation. The same types of fetal observations were made as those described above for the rats. The number of pregnant animals examined varied between 28

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

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

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

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

10.2 Reproduction Toxicity

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

To satisfy the need for reproductive effects testing for chlorobenzene, Monsanto Company conducted a two-generation reproductive study on rats (**67**). Groups of 30 male and 30 female Sprague-Dawley CD rats (F0-generation) were exposed to 0, 50, 150, or 450 ppm (i.e., 0, 230, 690, or 2,070 mg/m^3) chlorobenzene vapor for 10 weeks prior to mating and through mating, gestation, and lactation. The exposure took place 6 hr/day, 7 days/week. A selected number

of the offspring from the F0-generation (30 males and 30 females/group) formed the F1-generation. These animals were exposed to the same concentrations of chlorobenzene as the F0-generation, starting 1 week post-weaning; lasting 11 weeks prior to mating; and through mating, gestation, and lactation. The progeny of the F1-generation, the F2-pups, were observed during weaning, and then they were sacrificed. A number of parameters were investigated, including body weights, food consumption, mating and fertility indices, pup and litter survival, and histopathological examinations of selected organs (liver, kidneys, pituitary gland, and male and female reproductive organs).

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

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

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

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

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

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

11 DOSE-EFFECT AND DOSE-RESPONSE RELATIONSHIPS

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

(1) The prenarcotic and irritating effects of chlorobenzene (observed in humans exposed to 60 ppm for 3-7 hr).

(2) The clear hepatotoxic effects of chlorobenzene ("lowest" LOEL value reported was 50 ppm in rats exposed for 11 weeks).

(3) The possible hematopoietic toxicity of chlorobenzene (leukopenia was reported in mice exposed to 22 ppm for 3 months).

11.1 Acute Exposure

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

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

11.2 Repeated Exposure

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

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

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

End point (effect)	Route of administration	Species	LD_{50}/LC_{50} or LOEL*	Reference
Mortality†	Oral	Rat	1,540 mg/kg	19
		Mouse	1,355 mg/kg	66
		Rabbit	2,250 mg/kg	19, 32
		Guinea pig	5,060 mg/kg	19, 32
		Man	500-5,000§ mg/kg	92
Mortality†	Inhalation	Rat	13,870 mg/m³ for 6 hr	19
		Mouse	8,822 mg/m³ for 6 hr	19, 32
CNS-depression (narcosis)	Oral	Rat	1,540 mg/kg	19, 51, 68
	Oral	Mouse	1,000 mg/kg**	51, 68
CNS effects (prenarcotic effects)††	Inhalation	Man	275 mg/m³ for 7 hr	71
Behavioral changes§§	Inhalation	Mouse	3,700 mg/m³ for 4 hr§§	24
Liver necrosis	i.p. injection	Rat	1,100 mg/kg	22
Increased serum ALAT	i.p. injection	Rat	225-1,100 mg/kg	22
Mild centrilobular changes (e.g., cloudy swelling)	i.p. injection	Rat	225 mg/kg	22
Decreased hepatic levels of glutathione	i.p. injection	Rat	225 mg/kg	22
Renal necrosis	i.p. injection	Mouse	760 mg/kg	75
Increased bile duct flow	i.p. injection	Rat	562 mg/kg	94

*LD_{50}/LC_{50} for mortality; LOEL for other effects (lowest identified values).
†Generally due to respiratory paralysis.
§Estimated probable acute lethal dose in animals.
**Animals were not subjected to necropsy.
††Acute subjective symptoms (drowsiness, headache, irritations from eyes and sore throat.
§§50% decreased immobility in a behavioral despair swimming test.

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

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

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

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

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

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

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

12 RESEARCH NEEDS

(1) The data on the genotoxic and tumor-promoting effects of chlorobenzene are not consistent. This is an area requiring further research, especially with regard to the reported ability of chlorobenzene (or more likely of some of its metabolites) to bind covalently to DNA.

(2) The structural resemblance between chlorobenzene and benzene, and the reported hematopoietic toxicity of chlorobenzene in experimental animals, call for further studies addressing the potential problem with the chlorobenzene-induced bone marrow (i.e., hematopoietic) toxicity, especially with regard to potential dose-effect and dose-response relationships.

(3) Chlorobenzene has been used in large quantities in industry for several years. However, there is still a paucity of data on actual exposure levels of chlorobenzene in occupational settings today. A survey of the potential exposure to chlorobenzene in relevant industries is therefore recommended.

(4) There are only limited epidemiological data available on the health status of workers chronically exposed to chlorobenzene. Recent data on a limited number of volunteers showed, for example, that exposure to chlorobenzene vapors at previous threshold limit values (e.g., 75 ppm in the United States and 50 ppm in Germany) can induce prenarcotic effects, and animal data show that repeated exposure to chlorobenzene at these levels may affect the liver. Information from epidemiological studies examining dose-effect and dose-response relationships, especially with regard to the prenarcotic, hepatotoxic and possibly also hematopoietic effects of chlorobenzene, would be useful.

(5) Further studies should be made to explore and assess the potential risks from the extrahepatic bioactivation of chlorobenzene (e.g., in the nasal mucosa).

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