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BILTHOVEN
THE NETHERLANDS**

**APPENDIX to Report no. 710401015
INTEGRATED CRITERIA DOCUMENT CHLOROBENZENES
EFFECTS**

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INTRODUCTION

Data in the present Appendix are underlying those in the chapter on "effects" (chapter 5) of the "Integrated Criteria Document Chlorobenzenes" (Slooff et al., 1991). The Criteria Document, prepared by the National Institute of Public Health and Environmental Protection in The Netherlands, comprises a systematical survey and a critical evaluation of the most important data on chlorobenzenes, as much as possible with regard to the specific situation in The Netherlands. The information in the Criteria Document will serve as a scientific basis for an "effect oriented policy" in The Netherlands, especially with regard to the general population and aquatic and terrestrial ecosystems.

The Criteria Document, including the present Appendix, has been written on behalf of the Ministry for Housing, Physical Planning and Environment, Directorate Substances and Risk-management.

The data which are considered to be necessary for a risk assessment for the general population, are described in chapter 1. Data on the impact of chlorobenzenes on aquatic and terrestrial organisms are described in chapter 2 and chapter 3, respectively. In chapter 4 data on agricultural crops and livestock are described. Chapter 5 contains the risk assessment for man and the environment.

1. HUMAN TOXICITY

1.1 KINETICS AND METABOLISM

1.1.1 Animal studies

Monochlorobenzene

Oral exposure

After oral administration of MCB ($34 \text{ mg.kg}^{-1} \text{ bw}$) to rats the urinary concentrations of 4-chlorophenyl-mercapturic acid (MA) and 4-chlorocatechol were reported to be about 13% and 5% of the administered dose, respectively (Ogata and Shimada, 1983). Following oral administration of approximately $4 \text{ g }^{14}\text{C}$ -labelled MCB to two rabbits the majority of metabolites was found in the urine and only small amounts were present in the faeces or in the tissues. The overall recovery was low (about 20%), which was probably due to loss by exhalation. The urinary metabolites were ethereal sulphates (33.9% of the dose administered), glucuronides (33.6%), mercapturic acids (23.8%), diphenols (4.2%), monophenols (2.8%) and 3,4-dihydro-3,4-dihydroxychlorobenzene (0.6%) (Smith et al., 1972). Following a single oral dose of $150 \text{ mg.kg}^{-1} \text{ bw}$ of MCB to rabbits the urine contained glucuronide (25%), ethereal sulphates (27%) and mercapturic acid (20%) (Spencer and Williams, 1950, Smith et al., 1950). Although absolute quantities and ratios may differ among species, the principal metabolites of MCB are 4-chlorophenol, 4-chlorocatechol and MA (Ware, 1988). Absorption from the gastro-intestinal tract increases in the presence of fats and oils (Deichman, 1981).

Inhalatory exposure

After male rats were exposed to 100, 400 or 700 ppm (470, 1,880 or 3,290 mg.m^{-3}) of ^{14}C -labelled MCB vapor for 1 or 5 days MCB was found in all examined tissues (blood, fat, kidneys, lungs and liver). The concentrations increased in proportion to exposure concentrations, except for adipose tissues, which increased more than 30-fold between 100 and 700 ppm. Data from rats killed 16 and 48 hrs after dosing demonstrated rapid

tissue clearance. With increasing exposure concentrations the percentage excreted in the urine decreased (from 95% to 56%) and the percentage exhaled (unchanged) increased (from 5% to 44%). The total amount excreted increased more than proportionately at 700 ppm. A dose-dependent decrease in the relative abundance of the metabolite mercapturic acid was observed. After multiple exposures the tissues contained higher amounts and more was excreted in the urine. The urinary metabolite pattern was similar (Sullivan et al., 1983).

Other routes of exposure

After a intraperitoneal injection of MCB (56, 113 and 225 mg.kg⁻¹ bw) to rats, the amounts of MA and 4-chlorocatechol excreted in the urine were about 25% and 5% of the initial dose, respectively (Ogata and Shimada, 1983). In a study in which rats were given one intraperitoneal injection of 225 mg.kg⁻¹ bw, the urinary excretion of MA and chlorophenols (including 4-chlorophenol) was reported to be about 20% and 10% of the dose, respectively (Yoshida and Hara, 1985). Male rats received intraperitoneal doses varying between 2 and 14.7 mmol.kg⁻¹ bw (245-1660 mg.kg⁻¹ bw). The plasma and liver concentrations measured 24 hours after treatment increased dose related. The fraction of the dose excreted decreased with increasing dose; at the lowest dose about 60% was excreted, whereas at the highest dose only about 20% was excreted within 24 hours (Dalich and Larson, 1985). Absorption through the skin of dermally treated rabbits appeared to be minimal or negligible (Deichman, 1981)

Dichlorobenzene (1,2-, 1,3- and 1,4-)

Oral exposure

Three hours after rats were given a single oral dose (200 or 800 mg.kg⁻¹ bw) blood, adipose tissue, kidney, liver, lung, heart and brain contained measurable levels of 1,4-DCB. After 48 hours levels in adipose tissue was still high, whereas at that time 1,4-DCB had completely disappeared from the other tissues. Two metabolites (2,5-dichlorophenyl methyl sulfoxide and 2,5-dichlorophenyl methyl sulfone) emerged in the blood, and were found for many hours even after 1,4-DCB had almost disappeared from the blood. Within 24 hours about 45% and 6% of the administered dose appeared in the urine

and faeces, respectively. The excretion of the two metabolites into the urine was much less than that of 2,5-dichlorophenol. After 96 hours the amounts excreted in urine and faeces were 48.3% and 6.5%, respectively (Kimura et al., 1979). After female rats were given oral of 250 mg.kg⁻¹ bw per day during 10 days highest concentrations were found in fat, liver, kidneys and lungs. The concentrations declined rapidly in plasma and tissues after 5 days. 97.1% of the administered dose was excreted in the urine, 1.0% was expired and 2.0% excreted in the faeces. The urine contained two main metabolites, namely a sulphate and a glucuronide of 2,5-dichlorophenol (about 50% and 33% of the excreted dose, respectively) and two minor compounds: dihydroxydichloro-benzene and a mercapturic acid of 1,4-DCB. The major compound in bile was found to be the glucuronide of 2,5-dichlorophenol (about 36%). Experiments with rats with cannulated bile ducts indicate that considerable enterohepatic circulation occurs in intact animals and that much of the material eliminated in the bile was reabsorbed to be excreted in the urine (Hawkins et al., 1980).

The metabolism of 1,2-DCB and 1,4-DCB was studied in rabbits receiving a single dose of 500 mg.kg⁻¹ bw by gavage. 1,2-DCB was mainly oxidized to 3,4-dichlorophenol (about 30% of the dose administered), which is conjugated with glucuronic and sulphuric acids. Conjugates of 2,3-dichlorocatechol and 3,4-dichloro-catechol were (excreted as) minor metabolites as well as 3,4-dichlorophenyl-mercapturic acid. The main excretion products were glucuronide (48% of the administered dose), ethereal sulphate (21%), mercapturic acid (5%), 3,4-dichlorophenol (30%) and 2,3-dichlorophenol (9%). 1,4-DCB was mainly oxidized to 2,5-dichlorophenol, which is conjugated. 2,5-Dichloroquinon occurred (about 6% of the dose), but no mercapturic acid or dichlorocatechol was found. The major excretion products were glucuronide (36%), ethereal sulphate (27%) and 2,5-dichlorophenol (35%) (Azouz et al., 1955).

Inhalatory exposure

Female rats were exposed to about 1,000 ppm ¹⁴C-labelled 1,4-DCB for 3 hrs/day during 2,4,6,8 or 10 days. The distribution was similar to that after oral administration; highest levels occurred in fat, kidneys, liver and lungs. Of the total amount of ¹⁴C excreted during 5 days 97.4% appeared in the urine, 2.5% in the faeces and 0.2% was expired. The pattern of

metabolites in the urine and in the bile was also similar (Hawkins et al., 1980). The organ distribution of 1,4-DCB was compared in male and female rats after inhalatory exposure to 500 ppm for 25 hours. Though no differences in serum levels were observed between male and female rats, the 1,4-DCB levels in the livers of females were significantly higher than those in males, whereas significantly higher levels were found in the kidney of males than of females (Umemura et al., 1990).

Other routes of exposure

After repeated subcutaneous doses of ^{14}C -MCB (250 mg.kg^{-1} per day) given to rats of distribution and excretion (metabolite) pattern was found to be very similar to that after oral or inhalatory exposure. The amount excreted in the urine was somewhat smaller (90.5%) and the amount expired greater (6.4%). After subcutaneous doses tissue concentrations declined more slowly (Hawkins et al., 1980).

Trichlorobenzene (1,2,3-, 1,2,4- and 1,3,5-)

Oral exposure

After rats were given single doses of ^{14}C -labelled 1,2,3-, 1,2,4- or 1,3,5-TCB ($10 \text{ mg.kg}^{-1} \text{ bw}$) by gavage radioactivity appeared in the blood and tissues within 30 min, indicating a rapid absorption of all three isomers. Highest radioactivity was found in liver, kidney, fat, bladder and gastrointestinal tract. Radioactivity was also relative high in adrenal tissue (for 1,2,4- and 1,3,5-) and in the epidymis (for 1,2,3-). The concentrations were generally higher after dosing with 1,3,5-TCB than after dosing with the other isomers. After 7 days tissue concentrations of 1,2,3- and 1,2,4-TCB declined to very low or background levels. In the case of 1,3,5-TCB significant levels of radioactivity were still measured in the tissues after 56 days. Excretion data were given of 1,2,3- and 1,3,5-TCB; within 24 hours 92% and 83% of the administered dose of 1,2,3- and 1,3,5-TCB, respectively, was excreted. After 48 hours an additional 4% of both compounds was excreted (Chu et al., 1987). In male rats given a single oral dose of ^{14}C -labelled 1,2,4-TCB ($50 \text{ mg.kg}^{-1} \text{ bw}$) the highest levels of the label were found in the adipose tissue and to a lesser content in skin, muscle and intestine. Other organs did not show significant increased

levels. After 3 and 7 days the adipose tissue showed only slightly higher levels than the other organs. The excretion into the urine and the faeces was about 66% and 17% of the given dose, respectively, after 7 days. The urine contained a mixture of conjugated forms (about 90%) and free (unconjugated) forms (10%). The amount of unchanged 1,2,4-TCB and halogenated derivatives (1,2-, 1,3- and 1,4-DCB's) in the breath amounted to about 2.1% of the given dose. Comparing the biliary excretion of two rats (45%) with the faecal excretion (20%), an enterohepatic circulation of TCB and its metabolites is indicated. (Tanaka et al., 1986).

Following a single oral administration $10 \text{ mg.kg}^{-1} \text{ bw}$ of ^{14}C -labelled 1,2,4-TCB to 16 rats and 2 rhesus monkeys urine was collected and metabolites determined. By 24 hours rats excreted 84% of the administered dose in the urine and monkeys 40%. The amounts in the faeces were 11% for rats and less than 1% for monkeys. Based on the urinary metabolite pattern there appeared to be a species difference between rat and monkey in the metabolism of 1,2,4-TCB. The initial formation of arene oxides is identical in both species. In rats the next step is conjugation with glutathion, whereas in monkeys hydrolysis of arene oxides occurs. The resulting dihydrodiol is excreted as a glucuronide. The slower excretion of 1,2,4-TCB by monkeys compared to rats could be (partly) explained by this (Ling et al., 1982). After oral administration of the major (urinary) metabolites of 1,2,3-TCB in the rabbit were found to be 2,3,4-trichlorophenol, 2,3,6- and 3,4,5-trichlorophenol were minor metabolites. The major metabolites of 1,2,4-TCB were 2,4,5- and 2,3,5-trichlorophenol. Three metabolites of 1,3,5-TCB were found; 2,3,5- and 2,4,6-trichlorophenol and a third metabolite, which was probably a dichlorobenzene (Kohli et al., 1976).

Following a single oral dose of $500 \text{ mg.kg}^{-1} \text{ bw}$ of 1,3,5-TCB two rabbits expired in 9 days about 10% of the dose unchanged and about 1% as MCB. In the urine from the first 3 days 2,4,6-trichlorophenol was predominant, whereas from days 4 to 9 the monochlorophenols became more important. The main bulk was found unchanged in the tissues and gut contents 8-9 days after dosing (Parke and Williams, 1960).

Other routes of exposure

After intravenous administration of 1,2,4-TCB to 16 rats and 2 rhesus monkeys the urine was collected at 24 hours and metabolites were determined. The monkey and the rats had excreted 22% and 78% of the administered dose in the urine, respectively. Rats excreted 7% in the faeces, this amount was not determined by the two monkeys. For further information on metabolite patterns and the apparent difference in metabolism between rats and monkeys, see "oral exposure" (Lingg et al., 1982).

Placental transfer

In a toxicokinetic study incorporated into a teratogenicity study it was found that none of the three TCB isomers accumulates in the fetus of rats (given oral doses up to $300 \text{ mg.kg}^{-1} \text{ bw}$ of 1,2,4-TCB and doses up to $600 \text{ mg.kg}^{-1} \text{ bw}$ of 1,2,3- and 1,3,5-TCB by gavage on days 6 through days 15 of gestation) (Black et al., 1988).

Tetrachlorobenzenes (1,2,3,4-, 1,2,3,5- and 1,2,4,5-)

Oral exposure

Male rats were given oral doses of $10 \text{ mg.kg}^{-1} \text{ bw}$ of ^{14}C -labelled 1,2,3,4-, 1,2,3,5- and 1,2,4,5-TeCB. In the case of 1,2,3,4-TeCB after 48 hours 51% of the administered dose was excreted (15% and 36% in urine and faeces, respectively). 1,2,3,5-TeCB was excreted for 25% and 21% into the urine and the faeces, respectively. The excretion of 1,2,4,5-TeCB was much slower. After 48 hours only 8% of the dose given was excreted (6% and 2% in urine and faeces, respectively). With regard to 1,2,3,4-TeCB the urinary metabolites were 2,3,4,5-tetrachlorophenol (the major one) and 2,3,4,6-tetrachlorophenol (the minor one). The major metabolite of 1,2,3,5-TeCB was 2,3,4,6-tetrachlorophenol (49% of the excreted material) and that of 1,2,4,5-TeCB was 2,3,5,6-tetrachlorophenol (61%) (Chu et al., 1984).

After single oral doses of 1,2,3,4-, 1,2,3,5- and 1,2,4,5-TeCB the urinary metabolites of rabbits were determined. The metabolites that were formed after administration of 1,2,3,4-TeCB were 2,3,4,5- and 2,3,4,6-tetrachlorophenol. The same metabolites appeared in the urine after the rabbits were given 1,2,3,5-TeCB and in addition 2,3,5,6-tetrachlorophenol

and a more polar product (which was not further determined) were formed. After dosing 1,2,4,5-TeCB only one metabolite was formed, namely 2,3,5,6-tetrachlorophenol (Kohli et al., 1976).

In the urine of squirrel monkeys, repeatedly exposed to ¹⁴C-labelled 1,2,3,4-TeCB at a dose of 100 mg.kg⁻¹ bw in corn oil by gavage, N-acetyl-s-(2,3,4,5-tetrachlorophenyl) cysteine was found to be the major metabolite. A minor metabolite was identified as 2,3,4,5-tetrachlorophenol. This is in contrast with the data on rabbits and rats, in which the tetrachlorophenols are major metabolites (Schwartz et al., 1985).

Placental transfer

In a teratogenicity study on rats the amounts of the three TeCB-isomers were determined in maternal and fetal tissues after oral exposure. In the maternal tissues the levels of 1,2,3,4- and 1,2,3,5-TeCB were somewhat and those of 1,2,4,5-TeCB strongly (about 100-fold more than the two other isomers) increased. Perirenal fat contained in all cases the highest levels. In fetal tissues treatment with 1,2,3,4- and 1,2,3,5-TeCB had not resulted in higher levels, whereas treatment with 1,2,4,5-TeCB did increase those levels significantly (Kacew et al., 1984).

Pentachlorobenzene

Oral exposure

In male and female rats fed PeCB at dietary concentrations of 0, 125, 250, 500 or 1,000 mg.kg⁻¹ for 100 or 180 days, respectively, a dose dependent accumulation of PeCB in adipose tissue was found. The concentration in the tissue was about 2 times the dietary concentration (Linder et al., 1980). After a single oral dose of PeCB two metabolites were detected in the urine of rabbits, which were pentachlorophenol and 2,3,4,5-tetrachlorophenol (Kohli et al., 1976).

Hexachlorobenzene

Oral exposure

In female rats orally treated with ^{14}C -labelled HCB it appeared that the extent of intestinal absorption depends on the form of application. When given as a solution in oil about 80% was absorbed, regardless of the dose administered (16, 120 or 970 mg.kg^{-1} bw). When given as an aqueous suspension about 20% was absorbed of the lowest dose and about 6% of the higher doses (Koss and Koransky, 1975). Following oral administration of HCB to rats highest concentrations were reported in fat, muscle and skin tissues. Other tissues (e.g. kidneys, lungs, heart, spleen and blood) generally contained lower amounts (EPA, 1984).

After rats were treated with a single oral dose of 12 or 30 mg.kg^{-1} bw of HCB in cottonseed oil about 22% was excreted unchanged in the faeces (Albro and Thomas, 1974). HCB is metabolized slowly into other chlorinated benzenes, chlorinated phenols and other minor metabolites and forms of glucuronide and glutathion conjugates. The tissues were found to contain mainly unchanged HCB together with only small amounts of metabolites. Metabolites were mainly excreted in the urine, the faeces contained only small amounts of metabolites. The excretion of HCB from treated animals is slow and occurs mainly through the faeces. It is characterized by an initial rapid phase followed by a slow phase (EPA, 1984). The metabolism of HCB was reviewed extensively by Renner (1981), Renner et al. (1985) and Renner (1988).

After female rhesus monkeys (5) were given oral doses of 8-128 mg.kg^{-1} bw a day during 60 days, highest HCB contents were found in tissues containing high lipid amounts: body fat, bone marrow and the adrenal cortex. Adrenal medulla, liver, brain, and kidney contained smaller amounts. In one very thin monkey, with almost no adipose tissue higher HCB amounts were found in non-fat tissues and serum than in adipose tissue. This animal also had high brain levels and showed severe neurological damage. The authors noted that based on these data physically thin persons may be more susceptible to HCB poisoning (Knauf and Hobson, 1979). Male and female rhesus monkeys were given daily doses of 110 μg ^{14}C -labelled HCB (about 33 $\mu\text{g.kg}^{-1}$ bw) for 18 months. At the end of the exposure period about 6% of the administered dose was excreted in the urine and about 50% in the faeces of both sexes. The

main urinary metabolite was pentachlorophenol (more than 50% of the excreted amount). Other metabolites were pentachlorobenzene, tetrachlorobenzenes and unchanged HCB. Faecal excretion consisted of 99% of HCB (Rozman et al., 1978).

Other routes of exposure

After rats were given single intraperitoneal doses of ^{14}C -labelled HCB (4 mg.kg $^{-1}$ bw) radioactivity was highest in fat and in the skin. Liver, brain, kidney, blood and muscle contained amounts about 50-fold lower than fat (Koss and Koransky, 1975). After rats were given 2 or 3 intraperitoneal doses of ^{14}C -labelled HCB (total dose 260 or 390 mg.kg $^{-1}$ bw) the major urinary metabolites were pentachlorophenol, tetrachlorohydroquinone and pentachlorothiophenol, together accounting for more than 90% of the radioactivity. A minor metabolite was tetrachlorothiophenol. The faeces contained pentachlorophenol and pentachlorothiophenol. After 4 weeks the 2/3 of the administered dose was retained in the body and about 1/3 was excreted (for 50% unchanged HCB) (Koss et al., 1976).

After rats were given a single intraperitoneal dose of 4 mg.kg $^{-1}$ bw of ^{14}C -labelled HCB (in oil solution) within 2 weeks 34% of the administered dose was excreted in the urine and 5% in the faeces. The percentage unchanged HCB in the urine and faeces were 4% and 80% of the excreted amounts, respectively (Koss and Koransky, 1975).

Placental transfer

Placental transfer of HCB has been demonstrated in rats, mice, hamsters, monkeys and guinea pigs. After pregnant rats were administered orally doses of 5 to 120 mg.kg $^{-1}$ bw of HCB during days 6-16 of pregnancy, HCB residues were determined in maternal and fetal livers, fetal brain and whole fetus. The compound crossed the placenta and accumulated in the fetus in a dose-dependent way. The maternal liver contained the highest amounts of HCB (up to 86 mg.kg $^{-1}$), followed by the fetal liver (up to 36 mg.kg $^{-1}$), whole fetus and fetal brain (18 mg.kg $^{-1}$) (Villeneuve and Hierlihy, 1975). The tissue distribution of HCB was studied in pregnant hamsters and guinea pigs given 0, 1.0, 10.0 or 50.0 mg.kg $^{-1}$ bw (in corn oil) a day by gavage from day 5 to 10 of gestation for hamsters and from day 14 to 19 for guinea pigs. Samples of fat, thymus, skin, liver, lung, brain, spleen, urinary bladder, muscle,

plasma and blood were analyzed as well as fetuses placentas and yolk sacs. In maternal hamsters fat had the highest accumulation of HCB (up to 2,260 mg.kg⁻¹ wet weight at the highest dose) and followed by thymus and the skin. For all organs, including fetus and placenta, there was a dose-response relationship. The concentrations in the fetus (up to 13 mg.kg⁻¹ wet weight at the highest dose) were lower than in the placenta. Most tissues of the guinea pig also showed a dose-dependent increase in levels, with highest amounts in fat (1,460 mg.kg⁻¹), thymus, skin and liver. The fetus contained also less HCB (4 mg.kg⁻¹) than the placenta (Courtney et al., 1985).

The tissue distribution of HCB administered orally to pregnant and nonpregnant mice was found to be similar. Highest concentrations were found in fat, thymus, skin and urinary bladder (Courtney et al., 1976).

The transfer of HCB to nursing infant rhesus monkeys from lactating mothers receiving 64 mg.kg⁻¹bw a day for 60 days was studied. The study included three mother-infant pairs. Milk levels were about 12 times higher than the maternal serum levels. Serum levels were higher in the infants than in their mothers. In the fetus fat, bone marrow, lymph nodes and adrenals contained highest HCB levels (Bailey et al., 1980).

1.1.2 Human studies

Monochlorobenzene

Oral exposure

MCB was administered orally to a male volunteer three times at a dosage of 0.3 mmol.kg⁻¹bw (34 mg.kg⁻¹bw) and the excretion of two metabolites (MA and 4-chlorocatechol) was determined. It appeared that 4-chlorocatechol was the main metabolite (Ogata and Shimada, 1983).

Inhalatory exposure

In the urine of 11 workers exposed to about 3.15 ppm MCB (TWA-value) 4-chlorocatechol, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol and MA accounted for 76.9%, 3.2%, 7.1%, 12.4% and 0.4%, respectively, of the total amount excreted (Yoshida et al., 1986). 4-Chlorocatechol also appeared to be the main metabolite in the urine from two men inhalatory exposed to MCB (0.84 ppm x 415 min or 0.5 ppm x 228 min) (Ogata and Shimada, 1983).

1,4-Dichlorobenzene

Inhalatory exposure

The urinary excretion of 2,5-dichlorophenol, a metabolite of 1,4-DCB, was studied among workers exposed to 1,4-DCB in various industrial plants. A roughly linear relationship was observed between the urinary excretion of 2,5-dichlorophenol at the end of the work shift and exposure to 1,4-DCB. It was concluded that 2,5-dichlorophenol could be used as index of exposure to 1,4-DCB (Pagnotto and Walkley, 1965). In another study the relationship between occupational exposure to 1,4-DCB and urinary excretion of the unchanged compound in the urine of 4 workers was studied. A significant relationship was found between the difference of 1,4-DCB urinary concentration at the beginning and end of a working day and the 1,4-DCB environmental concentration (Ghittori et al., 1985).

Summary and conclusions "kinetics and metabolism"

MCB is absorbed after oral and inhalatory exposure. Absorption after dermal treatment of rabbits seemed minimal or negligible. Shortly after oral treatment of rats MCB was found in all tissues examined, with fat tissue containing highest amounts. A rapid tissue clearance was observed. The principal metabolites of MCB were found to be 4-chlorophenol, 4-chlorocatechol and 4-chlorophenyl-mercapturic acid. The majority of the metabolites were excreted in the urine and only small amounts were present in the faeces. After inhalatory exposure the percentage of MCB exhaled by rats increased with increasing exposure concentrations.

Experimental studies indicate that DCB's are absorbed after different routes of uptake. After oral, inhalatory or subcutaneous exposure tissue distribution appeared to be similar. Highest levels occurred in fat, liver, kidneys and lungs. After inhalatory exposure to 1,4-DCB a difference in organ distribution was observed between male and female rats; females had significant higher levels of 1,4-DCB in their livers, whereas the kidneys of the males contained significant higher levels compared to those of the female rats. 1,2-DCB and 1,4-DCB were mainly oxidized to 3,4- and 2,5-dichlorophenol, respectively, which were further conjugated. Within 24

hours after exposure 50% to 90% of the administered dose was excreted in the urine as metabolite. A small amount was excreted in the faeces. DCB's were reabsorbed via the enterohepatic circulation.

TCB's are absorbed after oral, inhalatory and dermal exposure. In rats orally treated highest amounts were found in liver, kidneys, fat tissue, bladder and gastro-intestinal tract. Increased contents were also reported for skin and muscle tissues. Tissue levels were generally highest after treatment with 1,3,5,-TCB. TCB's were metabolized to trichlorophenols, which were further conjugated with glutathion. In contrast to rats, monkeys did not use glutathion in their metabolism of TCB's. Rats excreted within 24 hours after an oral dose about 70% in the urine and about 15% in the faeces. By monkeys the excretion occurred slower; after 24 hours 40% was excreted in the urine and less than 1% in the faeces. TCB's were also reabsorbed in via the enterohepatic cyclus.

TeCB's are absorbed after oral exposure. No data are available on absorption after inhalatory or dermal exposure. In rats the tissue distribution patterns were similar after dosing the different isomers. TeCB's occurred in fat tissue, skin, kidneys, liver and guts and 1,2,4,5-TeCB resulted in the highest concentrations. From a teratogenicity study it appeared that 1,2,3,4- and 1,2,3,5-TeCB did (hardly) not accumulate in maternal rats or in fetusses. In contrast, 1,2,4,5-TeCB did accumulate in both maternal animals and fetusses. Rats and rabbits mainly metabolized TeCB's to chlorophenols, which were conjugated. In the urine of monkeys a N-acetyl-S-compound appeared to be the major metabolite. After oral treatment rats excreted within 48 hours 50% of the dose into the urine and the faeces. The excretion of 1,2,4,5-TeCB was slower; within 48 hours only 8% was excreted.

PeCB is absorbed after oral exposure. A dose-dependent accumulation in fat occurred in rats. In rabbits PeCB was metabolized to pentachlorophenol and 2,3,4,5-tetrachlorophenol. No further data were available.

HCB is absorbed after oral exposure. No data regarding absorption after inhalatory or dermal exposure were available. HCB administered in an oil solution was absorbed for about 80% and HCB in an aqueous solution for about 20%. Monkeys concentrated HCB in fat tissue, bone marrow and adrenals. Rats also had high amounts in skin- and muscle tissues. HCB has been demonstrated to transfer the placenta in various species and to

accumulate dose-related in the fetuses. The compound was metabolized slowly, the principal metabolites were pentachlorophenol, tetrachlorophenol, tetrachlorothioquinone and pentachlorothiophenol. Lower chlorinated chlorobenzenes and chlorophenols were also formed as well as different conjugates. The majority of the metabolites were excreted in the urine, whereas unchanged HCB was mainly excreted in the faeces.

The metabolic behaviour of the chlorobenzenes changes gradually with an increasing degree of chlorination. With an increasing number of chloroatoms the substances become more lipophile and accumulate to a greater extent in fat tissue and "fat-rich" organs. The biotransformation and the elimination via the urine decrease with an increasing number of chloroatoms; the difference in elimination half-life times between for example 1,4-DCB and HCB is estimated to be at least a factor of 10. Especially HCB is biotransformed very slowly and its excretion is mainly via the faeces. The tissues contain predominantly unchanged HCB.

1.2 TOXICITY

1.2.1 animal studies

Monochlorobenzene

Acute toxicity

Data on the acute toxicity of MCB are given in table 1.1. After acute exposure effects were described on the liver, kidney, lungs and central nervous system (narcotic signs and depression) (EPA, 1984).

Subacute toxicity

Oral exposure

Exposure of male and female rats to 0, 125, 250, 500, 1,000 or 2,000 mg.kg⁻¹ bw of MCB in corn oil by gavage for 14 days resulted in death at the two highest doses. In the groups receiving up to 500 mg.kg⁻¹ no adverse effects were observed (NTP, 1985a). An effect on the porphyrin metabolism was observed in rats receiving 1,140 mg.kg⁻¹ bw for 5 days. This effect was, however, much less than the effect caused by more highly chlorinated congeners (Rimington and Ziegler, 1963). Exposure of male and female mice to 0, 30, 60, 125, 250 or 500 mg.kg⁻¹ MCB in corn oil by gavage for 14 days did not result in toxicity or mortality (NTP, 1985a).

Subchronic toxicity

Oral exposure

Male and female rats and mice were exposed to 0, 60, 125, 250, 500 or 750 mg.kg⁻¹ bw of MCB (in corn oil) by gavage, 5 days a week for 13 weeks. The two highest doses decreased survival and final mean body weight of both species. An increase in urinary uroporphyrin (rats) and coproporphyrin excretion (rats, mice) was observed at these levels. At ≥ 125 mg.kg⁻¹ bw toxicity was observed in several tissues; increased liver- and kidney-weights, a dose-dependent hepatocellular necrosis, nephropathy and depletion of bone marrow, spleen and thymus in both species. Only male

animals showed an effect at $60 \text{ mg.kg}^{-1}\text{bw}$; splenic and heart weights were slightly decreased in male rats and male mice, respectively (NTP, 1985a). In a feeding study rats received 12.5, 50, 100 or $250 \text{ mg.kg}^{-1}\text{bw}$ of MCB a day for 93-99 days. The highest dose resulted in retarded growth and liver and kidney weights were increased at 100 and $250 \text{ mg.kg}^{-1}\text{bw}$. No effects were reported for the lowest doses (Monsanto, 1967, cited by Knapp et al., 1971 [abstract] and EPA, 1984). In a study conducted by Flury and Zernik rats were exposed to 14, 144 or $288 \text{ mg.kg}^{-1}\text{bw}$ for 5 days a week during 192 days. The two highest doses resulted in effects on the liver and kidneys. The no-effect-level was $14 \text{ mg.kg}^{-1}\text{bw}$ (Flury and Zernik, 1931, cited by Deichmann, 1981).

MCB was administered to dogs at doses of 27.3, 54.5 or $272.5 \text{ mg.kg}^{-1}\text{bw}$ a day on 5 days a week for 93 days by means of a capsule. Exposure to the highest dose resulted in increased mortality and effects on the liver, kidneys, gastro-intestinal mucosa and haematopoietic tissue and in changes in several blood parameters. Dogs in the mid dose group showed diarrhea, vomiting and minimal histologic changes. The no-effect-level was $27.3 \text{ mg.kg}^{-1}\text{bw}$ (Monsanto, 1967, cited by Knapp et al., 1971 [abstract] and EPA, 1984).

Inhalatory exposure

The EPA reported a number of subchronic inhalation studies (dog, rat, rabbit) with (no-) effect-levels varying widely. One east-european study (Khanin, 1977, unpublished) reported hepatic and renal effects at a dose as low as 0.1 mg.m^{-3} (rat), whereas other studies resulted in no-effect-levels of 750 mg.m^{-3} (dog) or $2,000 \text{ mg.m}^{-3}$ (rat) (Monsanto Company, 1978, unpublished). In a study reported by Dilley (1977, unpublished) a concentration of about 345 mg.m^{-3} was considered to be a marginal effect level in rats; a decreased SGOT occurred after 24 weeks of exposure, but exposure to about 100 mg.m^{-3} resulted in neurotoxic effects in other studies (EPA, 1984).

Chronic toxicity

Oral exposure

Groups of 50 male and 50 female rats and groups of 50 female mice were administered MCB in corn oil by gavage, 5 days a week for 103 weeks, at doses of 0 (vehicle control), 60 or 120 mg.kg⁻¹ bw (carcino-genicity study). A group of 50 male mice received doses of 0, 30 and 60 mg.kg⁻¹ on the same schedule. Untreated controls consisted of 50 male and 50 female rats and mice. In male rats a significant increase in liver noduli was observed only in the highest dose group. No compound-related clinical signs of toxicity were observed at any time during the studies in male or female rats or female mice. A minimal to mild hepatocellular necrosis was observed in some of the treated animals as well as in some of the controls. The evidence for mild MCB-induced hepatocellular necrosis was considered equivocal (NTP, 1985a).

Reproduction and teratogenicity

Inhalatory exposure

In a two-generation reproduction study rats were exposed to 0, 50, 150 or 450 ppm of MCB (0, 230, 690 and 2070 mg.m⁻³) for 10 weeks prior to mating, during mating, gestation and lactation. All F2 pups were observed through weaning at which time they were killed. Exposures up to 450 ppm did not have any adverse effects on reproductive performance or fertility of male or female rats. Effects on the liver and kidneys were observed in F1 and F0 male rats. In F0 and F1 males from the highest dose groups effects on the testicular germinal epithelium were seen. The relationship of these testicular changes to exposure to MCB is unclear according to the authors, because there was no increase in intensity and/or incidence among F1 males that had longer exposure (Nair et al., 1987). The embryotoxic and teratogenic potential of inhaled MCB was evaluated in rats and rabbits exposed to 0, 75, 210 or 590 ppm of MCB (0, 345, 966, 2714 mg.m⁻³) via inhalation for 6 hr/day during days 6 through 15 (rats) or days 6 through 18 (rabbits) of gestation. Inhalation of 590 ppm caused elevated liver weights in both species and decreased body weight gain and feed consumption in rats. Fetal effects were limited to a slight delay in skeletal

development which only occurred in rats exposed to 590 ppm, a maternally toxic concentration. No further embryotoxic or teratogenic effects were observed (John et al., 1984).

Dichlorobenzene (1,2-, 1,3- and 1,4-)

Acute toxicity

Data on the acute toxicity of DCB are given in table 1.1. Acute effects of 1,2-DCB and 1,4-DCB included central nervous system depression and liver and kidney damage. Acute inhalation also caused eye and nose irritation.

Subacute toxicity

Oral exposure

Groups of 5 male and female rats were given 0, 60, 125, 250, 500 or 1,000 mg.kg⁻¹ bw of 1,2-DCB in corn oil for 14 days. At the highest dose all animals died, but no increased mortality was seen up to 500 mg.kg⁻¹ bw. A dose related decrease in body weight (gain) occurred at 125 and 500 mg.kg⁻¹ bw in both sexes (depression up to 10%) (NTP, 1985b). Oral administration of 455 mg.kg⁻¹ bw 1,2-DCB for 15 days or 770 mg.kg⁻¹ bw 1,4-DCB for 5 days caused the induction of hepatic porphyria in rats (Rimington and Ziegler, 1963). Two studies were conducted with 1,2-DCB in mice. In the first study (exposure between 250 and 4,000 mg.kg⁻¹ bw) nearly all animals died, whereas in the second one (exposure between 30 and 500 mg.kg⁻¹ bw) no increased mortality occurred. The reason for the discrepancy between survival in the first versus the second study is not known. Results from a 13-week study (see subchronic exposure) were, however, more consistent with the second study than with the first one (NTP, 1985b).

Daily exposure of rats to 800 mg.kg⁻¹ bw of 1,3-DCB for 9 days caused hepatic porphyria (Poland et al., 1981, evaluated by the EPA).

Groups of 5 male and female rats were exposed to 60, 125, 250, 500 or 1,000 mg.kg⁻¹ bw of 1,4-DCB in corn oil by gavage for 14 days. No toxic effects were seen. In a 14-day study performed at higher doses increased mortality occurred at $\geq 1,000$ (in females) and a decrease in body weight (gain) was observed at 500 mg.kg⁻¹ bw (in males) (NTP, 1987). Rats administered 20-40

mg.kg⁻¹ bw 1,4-DCB for 14 days showed an increased activity of several metabolic enzymes (glucuronyltransferase and O-ethyl O-p-nitrophenylphosphonothioate). The no-effect-level was 10 mg.kg⁻¹ bw (Carlson and Tardiff, 1976). Exposure of rats (two per group) to 10, 100 or 500 mg.kg⁻¹ bw of 1,4-DCB by gavage 5 days a week for 4 weeks resulted in liver- and kidney toxicity at the highest dose. No toxic effects occurred in the lower dose groups (Hollingsworth et al., 1956).

Two 14-day studies with 1,4-DCB were conducted with mice. In the first study in which 5 male and female mice were exposed to 1,4-DCB between 250 and 4,000 mg.kg⁻¹ bw a scattered pattern of deaths in all dose groups occurred. In a second study performed at lower doses (60-1,000 mg.kg⁻¹ bw) no compound-related deaths occurred nor decreased body weight (gain) (NTP, 1987).

Subchronic toxicity

Oral exposure

Groups of 10 male and female rats and mice were administered 1,2-DCB at doses of 0, 30, 60, 125, 250 or 500 mg.kg⁻¹ bw in corn oil for 5 days a week during 13 weeks. In rats the highest dose caused increased mortality among females. Microscopically effects were found on the liver (≥ 125 mg.kg⁻¹ bw), thymus and kidney (500 mg.kg⁻¹ bw). Minimal increases in serum cholesterol levels in males (at 30 and ≥ 125 mg.kg⁻¹ bw), in serum glucose levels (at 30 and ≥ 125 mg.kg⁻¹ bw) and serum total protein levels (at all doses) in females. At the lower doses (30 and 60 mg.kg⁻¹ bw) these parameters were not consistent and/or dose-related increased. Therefore these minimal changes were considered to be not biologically significant. The no-effect-level for rats was 60 mg.kg⁻¹ bw. In mice there was also an increased mortality in the highest dose group. This dose also caused (microscopically observed) effects on the liver, thymus, muscle, spleen and heart. Only the liver showed lesions at 250 mg.kg⁻¹ bw. For mice the no-effect-level was 125 mg.kg⁻¹ bw (NTP, 1985b). Exposure of rats to 19, 188 or 376 mg.kg⁻¹ bw of 1,2-DCB (in olive oil) by gavage 5 days a week for a total of 138 doses in 192 days, resulted in slight liver- and kidney toxicity at the highest dose. The mid dose caused slight increased liver and kidney weights, whereas the lowest dose was without effects (Hollingsworth et al.,

1958). In mice, exposed to 0, 30, 60, 125, 250 or 500 mg.kg⁻¹bw in corn oil by gavage during 13 weeks, the highest dose caused increased mortality. These dose also caused toxic effects on several organs liver, kidney, thymus, spleen, heart and muscle). At 250 mg.kg⁻¹bw the only compound-related effects was liver lesions and no effect were found at 125 mg.kg⁻¹bw. The minor hematological changes occurring at the two lower doses (increase in white blood cell counts, increase in relative number of lymphocytes) and the increased relative splenic weights in females were considered to be not biologically significant. The no-effect-level appeared to be 125 mg.kg⁻¹bw (NTP, 1985b). In one study oral exposure of rats to doses varying between 0.01 and 0.1 mg.kg⁻¹bw for 5 months resulted in effects on the hematopoietic system. Because this study was not available for evaluation, it is left out of consideration (Varshavskaya, 1967, cited in EPA, 1984).

In two 13-week studies rats were exposed to 1,4-DCB at doses between 37.5 and 1,500 mg.kg⁻¹bw in corn oil by gavage. Increased mortality occurred at 900 mg.kg⁻¹bw. Male rats were more sensitive to 1,4-DCB than female rats, and the kidneys seemed to be the most sensitive organ. Renal tubular regeneration was observed in male rats receiving ≥ 300 mg.kg⁻¹bw as well as a decrease in body weight (gain) and changes in several blood parameters. The no-effect-level for rats appeared to be 150 mg.kg⁻¹bw (NTP, 1987). Exposure of female rats to 19, 188 or 376 mg.kg⁻¹bw of 1,4-DCB (in olive oil) by gavage for a total of 138 doses in 192 days, resulted in increased liver and kidney weights, focal necrosis and slight cirrhosis of the liver at the highest dose. Liver and kidney weights were increased at 188 mg.kg⁻¹bw, whereas no effects were seen at the lowest dose. Rabbits exposed to 1,000 mg.kg⁻¹bw (92 doses in 219 days) or 500 mg.kg⁻¹bw (263 doses in 367 days) of 1,4-DCB (in olive oil) by gavage showed weight loss, tremors, weakness and liver toxicity (Hollingsworth et al., 1956).

Mice were exposed to 1,4-DCB at doses between 84 and 1,800 mg.kg⁻¹bw in corn oil by gavage for 13 weeks. Increased mortality occurred at 1,500 mg.kg⁻¹bw and the liver seemed to be the most sensitive organ. A doses ≥ 600 mg.kg⁻¹bw a dose-related hepatocellular degeneration occurred in both males and females as well as a decrease in body weight (gain) and changes in blood and clinical parameters. In this study the no-effect-level for mice was 338 mg.kg⁻¹bw (NTP, 1987).

Inhalatory exposure

Exposure of 20 rats, 8 guinea pigs, 2 rabbits and 2 female monkeys to 1,2-DCB at a concentration of 93 ppm (560 mg.m^{-3} ; 7 hrs/day, 5 d/wk for up to 7 months) did not result in adverse effects. Exposure of male and female rats and guinea pigs and female mice to 49 ppm (290 mg.m^{-3} ; 7 hrs/day, 5 days a week for 6.5 months) did also not result in adverse effects (Hollingsworth et al., 1958).

In similar studies inhalation of 798 ppm of 1,4-DCB (4790 mg.m^{-3} ; 8 hrs/day 5 d/wk with a total of 20-69 exposures) caused increased mortality among rats, rabbits and guinea pigs. The symptoms included marked tremors, weakness, loss of weight, eye irritation and unconsciousness as well as histopathological changes in liver, kidney and lungs. At dose of ≥ 173 ppm (1038 mg.m^{-3} ; 7 hrs/day, 5 days a week for 16 days) several slight effects were seen in rats (on liver, kidneys and lungs), in guinea pigs (on the spleen and lungs) and in rabbits (lungs). Inhalation of 96 ppm (576 mg.m^{-3} ; 7 hrs/day, 5 d/wk during 6-7 months) was without effects (Hollingsworth et al., 1956). Subchronic exposure of male and female rats and female mice to 75 or 500 ppm of 1,4-DCB (450 or 3000 mg.m^{-3} ; 5 hrs/d on 5 d/wk) did not cause toxicity, except for male rats in the highest dose group. Male rats showed increased liver- and kidney weights and slightly elevated urinary coproporphyrin excretion (unpublished data from Riley et al., 1980, summarized by Loeser and Litchfield, 1983).

Chronic toxicity

Oral exposure

Groups of 50 male and 50 female rats and mice were administered 1,2-DCB in corn oil by gavage at doses of 0 (vehicle control), 60 or 120 mg.kg^{-1} bw, 5 days a week during 103 weeks. Untreated controls consisted of 50 male and 50 female rats and mice. An increase in tubular regeneration in the kidneys of male mice was only significant in the highest dose group (control 17%, low dose 24% and high dose 35%). No other nonneoplastic changes were observed. Data considering carcinogenicity are described in 1.4 (NTP, 1985b).

In a two year (carcinogenicity-) study groups of 50 male rats were given 1,4-DCB by gavage at doses of 0, 150 or 300 mg.kg^{-1} bw and female rats and

male and female mice were given 0, 300 or 600 mg.kg⁻¹ bw for 5 days a week during 103 weeks. In rats the incidence of nephropathy was observed in both sexes. An increased incidence of parathyroid hyperplasia occurred in males. In mice the incidences of liver lesions increased (both sexes). Effects were further seen on the kidney (nephropathy) and the haematopoietic system (lymphoid hyperplasia). Data considering the carcinogenicity are described in 1.4 (NTP, 1987).

Reproduction and teratogenicity

Oral exposure

Groups of pregnant rats were treated with 0, 250, 500, 750 or 1,000 mg.kg⁻¹ bw of 1,4-DCB a day during days 6 through 15 of gestation by gavage. The incidence of most common malformations in fetuses was not increased. A significant increase in the number of skeletal variations was observed at 750 mg.kg⁻¹ bw or more and a dose-related increase in the frequency of an extra rib was seen at 500 mg.kg⁻¹ bw or more. At doses of 500 mg.kg⁻¹ bw or more signs of maternal toxicity were seen, resulting in a reduction in food consumption and weight gain. The authors stated that the embryotoxicity could be a consequence of maternal suffering rather than of a direct effect of the chemical itself and concluded that 1,4-DCB was not teratogenic in the rat (Giavani et al., 1986).

Inhalatory exposure

Rats and rabbits were inhalatory exposed to 0, 100, 300 or 400 ppm 1,2-DCB (0, 600, 1800 or 2400 mg.m⁻³) and rabbits to 0, 100, 300 or 800 ppm 1,4-DCB (0, 600, 1800 or 4800 mg.m⁻³) for 6 hours a day on day 6 through 15 (rats) or 6 through 18 (rabbits) of gestation. Rats showed maternal toxicity was observed at all doses of 1,2-DCB and liver weight was significantly increased at 400 ppm. No teratogenic or embryotoxic effects were seen. In rabbits slight maternal toxicity (decreased body weight) occurred in the highest dose groups (400 ppm of 1,2-DCB and 800 ppm of 1,4-DCB). In rabbits there were also no teratogenic or fetotoxic effects (Hayes et al., 1985). Unpublished data indicate that 1,4-DCB did not cause embryotoxic, fetotoxic or teratogenic effects in rats at doses up to 500

ppm ($3000 \text{ mg}\cdot\text{m}^{-3}$) (Hodge et al., 1977, reported by Loeser and Litchfield, 1983).

Trichlorobenzenes (1,2,3-, 1,2,4- and 1,3,5-)

Acute toxicity

Oral LD50-values for TCB are given in table 1.1. After acute inhalatory exposure effects were seen on the liver, kidney and ganglion cells at all levels of the brain and the mucous membrane as well as irritation of the lungs and functional changes in the respiratory system (EPA, 1984).

Subacute toxicity

Oral exposure

The content of Cyt P-450 and activities of several enzymes were increased by oral administration of $250 \text{ mg}\cdot\text{kg}^{-1}$ bw 1,3,5-TCB once daily for 3 days in rats (Ariyoshi et al., 1975). Oral exposure of rats to $10\text{-}40 \text{ mg}\cdot\text{kg}^{-1}$ bw of 1,2,4-TCB for 14 days resulted in significant increased microsomal functions and enzymes (including Cyt P-450). The activity of glucuronyl-transferase decreased (Carlson and Tardiff, 1976). Induction of hepatic enzymes in rats was found for at least 16 days after administration of 1,2,4-TCB at a level of $180 \text{ mg}\cdot\text{kg}^{-1}$ bw a day during 7 days (Smith and Carlson, 1980). In rats given $0.1 \text{ mmol}\cdot\text{kg}^{-1}$ of 1,2,4-TCB by gavage daily during 14 days the serum arylesterase activity decreased and the liver arylesterase increased (Carlson, 1980). Exposure to 1,2,3-TCB ($785 \text{ mg}\cdot\text{kg}^{-1}$ bw) for 7 days and 1,2,4-TCB ($730 \text{ mg}\cdot\text{kg}^{-1}$ bw) for 15 days caused induction of hepatic porphyria in rats (Rimington and Ziegler, 1963). Rhesus monkeys were given daily oral doses of 1, 5, 25, 90, 125 or $174 \text{ mg}\cdot\text{kg}^{-1}$ bw of 1,2,4-TCB for 90 days. Effects were observed at doses of $\geq 90 \text{ mg}\cdot\text{kg}^{-1}$ bw. Additional data on this study have not been reported (Smith et al., 1978, abstract).

Inhalatory exposure

Groups of 20 rats, 4 rabbits and 2 dogs were exposed to 1,2,4-TCB (7 hr/day, 5 d/week for 6 weeks) at concentrations of 0, 30 or 100 ppm (0,

223, 742 mg.m^{-3}). In rats liver weight (both absolute and relative) and kidney weight (relative) increased at 100 ppm. In rabbits relative liver weight decreased at 30 and 100 ppm. Urinary excretion of porphyrins increased (reversible) in rats at 30 and 100 ppm (Kociba et al., 1981).

Other routes of exposure

Groups of 5 male and female rabbits were dermally exposed to undiluted TCB (about 70% 1,2,4-TCB and 30% 1,2,3-TCB) at concentrations of 0, 30, 150 or 450 mg.kg^{-1} bw for 5 days a week during 4 weeks. Topical effects were seen in all treated rabbits. A slight increase in urinary coproporphyrin levels was observed at 450 mg.kg^{-1} bw, but this was considered to be only a slight or questionable effect of treatment. Therefore it was concluded that systemic effects are unlikely to occur under conditions where there is no topical effect (Rao et al., 1982).

After immature female rats were given ip injections of 1,2,4-TCB (0, 250 or 500 mg.kg^{-1} bw) on 3 consecutive days. Liver and adrenal weights were found to be increased, whereas body weight and uterus weight decreased (Robinson et al., 1981).

Subchronic toxicity

Oral exposure

Rats were given 1,2,4-TCB at daily oral doses of 0, 50, 100 or 200 mg.kg^{-1} bw for (30, 60, 90 or) 120 days. After 120 days a significant increase in liver porphyrin was observed for all doses. A transient increase in liver weight occurred (Carlson, 1977). In an oral 90-day study rats were exposed to 1,2,4-TCB at doses of 0, 10, 20 or 40 mg.kg^{-1} bw a day. The relative liver weights increased in the highest dose group. Enzyme activities (cyt P-450, cytochrome c reductase etc.) increased at all doses. After 30-day recovery period the effects on the enzyme induction were restricted to the two highest doses (Carlson and Tardiff, 1976).

Inhalatory exposure

Groups of 20 male and female rats were exposed to vapors of 1,3,5-TCB at concentrations of 0, 10, 100 or 1,000 mg.m^{-3} for 6 hours a day, 5 days a week during 13 weeks. No changes were observed in several organs or in

blood or clinical parameters. The only effect that seemed treatment-related was found in the highest dose group; 3 males developed squamous metaplasia and hyperplasia in the respiratory epithelium of the nasal passages (due to local irritation or stress, according to the authors) (Sasmore et al., 1983). Groups of rats, rabbits and monkeys were exposed to vapors of 1,2,4-TCB at levels of about 0, 25, 50 and 100 ppm (0, 186, 370 and 742 $\text{mg}\cdot\text{m}^{-3}$). The only compound related effects observed in these studies were transient changes in liver and kidney of rats (hypertrophy of hepatocytes, granulae in the liver, biliary hyperplasia and kidney hyaline degeneration) sacrificed after 4 and 13 weeks. After 26 weeks of exposure these effects were not observed (Coate et al., 1977).

Reproduction and teratogenicity

Oral exposure

In a multi-generation study rats were exposed to 1,2,4-TCB at levels of 0, 25, 100 or 400 ppm in the drinkingwater. The study covered the period beginning with the birth of the F0 generation and continued through weaning of the F2 generation. No treatment related effects on fertility, growth, viability, locomotor activity or blood parameters were found. At the 400 ppm dose level adrenal gland enlargement was observed in both sexes of the F1 and the F2 generation at 95 days of age (Robinson et al., 1981). Female rats received 1,2,4-TCB at doses of 75, 150 or 300 $\text{mg}\cdot\text{kg}^{-1}$ bw or doses of 150, 300 or 600 $\text{mg}\cdot\text{kg}^{-1}$ bw of 1,2,3- or 1,3,5-TCB in corn oil by gavage on days 6 through day 15 of gestation. None of the TCB isomers produced any teratogenic or fetotoxic effects. With respect to maternal toxicity 1,2,4-TCB appeared to be the most toxic (effects at 150 $\text{mg}\cdot\text{kg}^{-1}$ bw) and 1,2,3-TCB the least toxic (effects at 600 $\text{mg}\cdot\text{kg}^{-1}$ bw) (Black et al., 1988). In another study female rats were given 1,2,4-TCB at doses of 0, 36, 120, 360 and 1,200 $\text{mg}\cdot\text{kg}^{-1}$ bw by gavage on days 9 through day 13 of gestation. In the two highest dose groups maternal deaths occurred (6/6 and 2/9 rats, respectively). At 120 $\text{mg}\cdot\text{kg}^{-1}$ bw and 360 $\text{mg}\cdot\text{kg}^{-1}$ bw hepatic enzyme induction in maternal rats occurred. A retarded embryonic growth was obvious at 360 $\text{mg}\cdot\text{kg}^{-1}$ bw. No teratogenic effects were observed (Kitchin and Ebron, 1983).

Tetrachlorobenzenes (1,2,3,4-, 1,2,3,5- and 1,2,4,5-)

Acute toxicity

Oral LD50-values for TeCB's are shown in table 1.1.

Subacute toxicity

Oral exposure

Rats were given daily oral doses of 660 mg.kg^{-1} bw of 1,2,3,4-TeCB (in liquid paraffin) or 905 mg.kg^{-1} bw of 1,2,4,5-TeCB (in 1% cellofas) during 5 or 10 days, respectively. The urinary excretion of porphyrines and porphyrin precursors was elevated by 1,2,3,4-TeCB, but not by 1,2,4,5-TeCB. The absence of this effect could have been due to poor absorption. Rats treated with both substances showed loss of weight and appetite. Nonnecrotic liver cell degeneration was also observed (Rimington and Ziegler, 1963).

Subchronic toxicity

Oral exposure

In a 90-day study rats were fed diets containing 1,2,3,4-, 1,2,3,5- or 1,2,4,5-TeCB at levels of 0, 0.5, 5.0, 50 or 500 mg.kg^{-1} for 90 days. Based on body weight gain and food consumption it was estimated that the amounts ingested ranged from 0.034-34 mg.kg^{-1} bw for males and from 0.042-41 mg.kg^{-1} bw for females. Morphological changes in the liver and kidneys occurred in all dose groups, including the control group. No statistical test was done, but the lesions were more frequent and more severe in the animals fed 1,2,4,5-TeCB. A significant increase in liver and kidney weights as well as haematological changes were seen at the highest dose of 1,2,4,5-TeCB. The hepatic microsomal aniline hydroxylase and aminopyrine demethylase activities were increased by 1,2,4,5-TeCB at 50 and 500 mg.kg^{-1} diet. No statistical significant effects were reported at a concentration of 5 mg.kg^{-1} diet (corresponding to 0.34 or 0.40 mg.kg^{-1} bw for males and females, respectively) (Chu et al., 1984).

Exposure of rats and rabbits to 1,2,4,5-TeCB for 8 months caused effects at doses of 0.005 and 0.05 mg.kg⁻¹bw, respectively. Because this study was not available for evaluation, it is left out of consideration (Fomenko, 1965, cited in EPA, 1984).

Chronic toxicity

Oral exposure

The 2-year study conducted by Braun et al. (1978) was considered to be inadequate and will be left out of consideration.

Reproduction and teratogenicity

Oral exposure

In a teratogenicity study all three TeCB-isomers were administered daily at doses of 50, 100 or 200 mg.kg⁻¹bw to female rats by gavage on day 6 through day 15 of gestation. Increased mortality was limited to the highest dose of 1,2,4,5-TeCB; 9 out of 10 dams died. An increase of serum cholesterol level and aniline hydroxylase activity was observed in the 50 and 100 mg.kg⁻¹bw groups of 1,2,4,5-TeCB. At the highest doses of 1,2,3,4- and 1,2,3,5-TeCB a decrease in the mean number of live fetuses per litter occurred. No teratogenic effects were seen (Kacew et al., 1984 and Ruddick et al., 1981, abstract).

Pentachlorobenzene

Acute toxicity

Oral LD50-values for PeCB are given in table 1.1. After acute oral exposure rats and mice showed tremors, weakness and labored breathing. To determine a dermal LD50 one concentration (2,500 mg.kg⁻¹bw) was tested on rats, but no toxic effect (or mortality) were seen at this dose (Linder et al., 1980).

Subacute toxicity

The content of Cyt P-450 and activities of two hepatic enzymes (aminopyrine demethylase and aniline hydroxylase) were increased by oral administration of 250 mg.kg⁻¹ bw PeCB once daily for 3 days in rats (Ariyoshi et al., 1975).

Subchronic toxicity

Oral exposure

In a study conducted by Linder et al. the effects of subchronic exposure of PeCB in rats was studied as a part of an investigation of the effects on reproduction. Weanling male rats were given dietary levels of 0, 125 or 1,000 mg.kg⁻¹ for 100 days and weanling female rats were given 0, 125, 250, 500 or 1,000 mg.kg⁻¹ for 180 days. Based on food consumption the daily dosages were estimated to be highest in the 1,000 mg.kg⁻¹ bw female group, namely 134 (in the first week) to 55 mg.kg⁻¹ bw after 6 months. After 67 days of treatment, both males and females were pair-bred with untreated partners. The pregnant rats were allowed to whelp and their litters were observed through weaning (see reproduction and teratogenicity). No mortality nor clinical signs were obvious in the adult rats. At necropsy effects were found on the liver (at 500 and 1,000 mg.kg⁻¹ bw), the kidney and adrenals (at 1,000 mg.kg⁻¹ bw. No evidence of porphyria was observed (comparison to HCB). A dietary level of 250 mg.kg⁻¹ appeared to be the no-effect-level (Linder et al., 1980).

Reproduction and teratogenicity

Groups of rats were given PeCB at dietary levels of 0, 125, 250, 500 or 1000 mg.kg⁻¹. Both males and females were treated for 67 days before mating with untreated males or females. Pregnant females continued to receive treated diets for a total of 180 days. Treatment of males had no effect on the reproduction or survival of the pups. Significant mortality occurred in the pups of females fed 1,000 mg.kg⁻¹. Tremors developed 4-14 days after birth in suckling pups of mothers fed 250 mg.kg⁻¹ or more. By the time of weaning tremors were no longer evident. An increased relative liver weight

occurred in pups of females fed 250 mg.kg^{-1} diet or more. The no-effect-level appeared to be 125 mg.kg^{-1} diet (corresponding to $6.3 \text{ mg.kg}^{-1}\text{bw}$) (Linder et al., 1980). Female rats were administered PeCB at level of 0, 50, 100 or 200 mg.kg^{-1} bw daily on days 6 through 15 of gestation. The number of live fetuses was not affected. The mean fetal weight was decreased in the highest dose group. The incidences of uni- and bilateral extra ribs were 4/127, 28/129, 21/122 and 63/100, respectively for the dose groups. The highest dose level also caused sternal effects (Khera and Villeneuve, 1975 and Villeneuve and Khera, 1975). In another study no embryotoxic (development or survival) or teratogenic effects were observed in mice treated with 50 or 100 mg.kg^{-1} bw on days 6-15 of gestation) (Courtney et al., 1977, cited in EPA, 1984).

Hexachlorobenzene

Data concerning the toxicity of HCB to livestock have been summarized in chapter 4: Agricultural crops and livestock.

Acute toxicity

LD50-values of HCB are shown in table 1.1.

Subacute toxicity

Oral exposure

HCB has been reported to induce the activity of hepatic microsomal enzymes in mice and rats. Subacute oral exposure to HCB resulted in increased cytochrome P-450 and cytochrome b5 contents and in increased activities of aminopyrine demethylase, ϕ -aminolevulinic acid (Ariyoshi et al., 1975), aryl hydrocarbon hydroxylase and aminopyrine-N-demethylase (Cantoni et al., 1987), cytochrome c reductase (Carlson, 1978), EPN (O-ethyl-o-p-nitrophenyl phenylphosphono-thioate) detoxification and azoreductase (Carlson and Tardiff, 1976), acetanilide hydroxylase and acetanilide esterase (Carlson et al., 1979), serum and liver arylesterase, procaine esterase (Carlson et al., 1980), aniline hydroxylase, 4-nitroanisole O-demethylase, biphenyl 2- and 4-hydroxylase (Turner and Green, 1974). In a

14-day study a dietary concentration of 20 mg.kg^{-1} HCB (1 mg.kg^{-1} bw) was reported to be the highest no-effect-level for enzyme induction in rats (Tonkelaar, den, and Van Esch, 1974).

The immunotoxic potential of HCB has been investigated in weaned rats and in rats with combined pre- and postnatal exposure (for these studies see reproduction and teratogenicity). In the study with weaned rats given dietary doses of 500, 1,000 or 2,000 mg.kg^{-1} ($50, 100$ or 200 mg.kg^{-1} bw) for three weeks no effects were found on the phagocytizing and killing capacity of macrophages, and on parameters of the cell-mediated immunity. With respect to humoral immunity both the primary and secondary IgM and IgG responses to tetanus toxoid were significantly increased, but no difference was found in the IgM response to *Escherichia coli* LPS. Regarding cell-mediated immunity, no significant effects were found on mortality to a *Listeria monocytogenes* infection, on rejection of skin transplants and on delayed type hypersensitivity to tuberculin. No effect was found on the response of the thymus and spleen cells to several mitogens. At the intermediate and high level rats had increased spleen weights and thymus weights were decreased at the highest level (Vos et al., 1979). In contrast, experiments conducted by Loose et al. (1978a, 1978b) showed that HCB suppresses the humoral and cell-mediated immune response in mice. In this study mice received 167 mg.kg^{-1} HCB through their diet (25 mg.kg^{-1} bw) for 3 or 6 weeks. The susceptibility to malaria (*Plasmodium berghei*) infection and to bacterial endotoxins appeared to be enhanced by treatment (Loose et al., 1978a). In addition the antibody synthesis to the antigen sheep RBC was significantly depressed. All immunoglobulin concentrations, but mainly IgA, were found to be decreased (Loose et al., 1978b).

In female beagle dogs orally treated with doses varying between 50 or 150 mg.kg^{-1} bw a day for 21 days effects included enlarged livers (with histopathological changes) and physiological changes in the central nervous system (Sundlof et al., 1981).

Increased porphyrin levels in the liver and urine have been reported for rats and rabbits. Exposure of female rats to 50 mg.kg^{-1} bw every other day during 15 weeks resulted in increased relative weights of liver, spleen, kidneys and adrenal glands. Contents of porphyrins in the liver and the urine increased. After treatment the rats were held for additional 38 weeks, in which no HCB was administered. At the end of this period organ

weights and urinary porphyrin levels returned to normal, whereas liver porphyrin levels were higher than after 15 weeks of treatment. The authors could not explain this porphyria of the liver even after treatment had stopped for longer time (Koss et al., 1978). Exposure of female rats to 100 mg.kg⁻¹ bw every other day for 6 weeks resulted in increased liver uroporphyrin and decreased coproporphyrin levels. These changes were due to a gradually decreased activity of uroporphyrinogen decarboxylase activity (UDA) during treatment until it was nearly completely inhibited. After the treatment had stopped the rats were held for another 18 months. The activity of UDA continued to be inhibited for a while before it returned to normal (Koss et al., 1983). Exposure of male mice to dietary concentrations of 2.5, 25 or 250 mg.kg⁻¹ HCB (0.125, 1.25, 12.5 mg.kg⁻¹ bw) for 21 days resulted in significant lower testosterone levels in blood at the highest dose group. Mice in this dose group also had significantly increased liver and decreased prostate and seminal vesicle weights. Increased hepatic enzyme activities occurred. In this study the no-effect-level was 25 mg.kg⁻¹ diet (1.25 mg.kg⁻¹ bw) (Elissalde and Clark, 1979).

Four animal species (rabbit, guinea pig, mice and rats) were given dietary concentrations of 0.5% HCB for 6 weeks. In rabbits the porphyrin metabolism was disturbed at this dose (150 mg.kg⁻¹ bw). In rats (250 mg.kg⁻¹ bw) HCB also affected the porphyrin metabolism but to a lesser content than in rabbits. In guinea pigs and mice (200 and 750 mg.kg⁻¹ bw, respectively) severe neurological symptoms developed (Matteis, de, et al., 1961). Five female monkeys were given daily doses varying between 8 and 128 mg.kg⁻¹ bw by gavage during 60 days. Dose-related effects were seen on the thymus, liver, kidney and ovaries (Iatropoulos et al., 1976).

Inhalatory exposure

A study with rats demonstrated that exposure to aerosols containing approximately 35 mg.m⁻³ of HCB resulted in slight changes in humoral and pulmonary cellular defenses. The slight alteration in macrophage function coupled with changes in lymphocyte responses could indicate possible immune modulation after inhalatory exposure (Sherwood et al., 1989).

Subchronic toxicity

Oral exposure

A significant increase in liver gamma-glutamyl transferase was observed in rats given daily doses of $997 \text{ mg.kg}^{-1} \text{ bw}$ for 60 or 90 days. The enzyme activity increased also, although less marked, in serum and in the small intestine (Adjarov et al., 1982).

In most subchronic studies effects on the porphyrin metabolism were reported. In female rats given orally doses of 0, 50, 100 or $200 \text{ mg.kg}^{-1} \text{ bw}$ during 30-120 days liver and urine porphyrin levels were elevated at all doses. All rats showed increased liver weights. The responses were dose- and time-dependent (Carlson, 1977). Rats given dietary concentrations of 0.2% HCB ($100 \text{ mg.kg}^{-1} \text{ bw}$) for 45-100 days showed increased liver weights and increased cytochrome P-450 and cytochrome b5 levels. After 15 days HCB feeding porphyria was observed (Stonard, 1974). Of a total of 33 rats which were fed $100 \text{ mg.kg}^{-1} \text{ bw}$ during 50 days, 13 died in the first 4 weeks. In the remaining rats a significant increase in urinary excretion of porphyrins and porphyrin precursors was noted as well as hepatomegaly and liver cell degeneration (Ockner and Schmid, 1961). Microsomal enzyme induction and an increased urinary excretion of porphyrins and porphyrinprecursors were found in rats treated with a diet containing 0.2% HCB ($100 \text{ mg.kg}^{-1} \text{ bw}$) for 100 days (Lissner et al., 1975). The livers of rats fed a diet containing 0.2% HCB ($100 \text{ mg.kg}^{-1} \text{ bw}$) for 9 weeks were studied with an light and electron microscopy. A marked enlargement of the hepatocytes was observed (Medline et al., 1973). Female rats which were fed dietary concentrations of 200 mg.kg^{-1} of HCB ($10 \text{ mg.kg}^{-1} \text{ bw}$) for 15 weeks developed a massive porphyria (due to depression of uroporphyrinogen). In males no effects were seen, which indicates that female rats are much more sensitive to porphyrinogenic effects of HCB than are males. In another study rats were given the same concentration of HCB for 90 weeks. Kidney weights were increased and a mild to severe nephrosis, especially in males, was seen. Liver UDA was significant lower than in controls and the kidney porphyrin concentration appeared to be raised (Smith et al., 1985). In a study in which male and female rats were given $14 \text{ mg.kg}^{-1} \text{ bw}$ every other day during 103 days it appeared that female rats are more susceptible to the induction

of porphyria than are males. This might be associated with a faster metabolism of HCB, perhaps under influence of oestrogen levels (Rizzardini and Smith, 1982). In the livers of female rats fed 0.01% HCB ($5 \text{ mg.kg}^{-1} \text{ bw}$) for 98 days porphyria developed (Smith et al., 1980). Rats were fed daily doses of 0, 0.5, 2, 8, 32 $\text{mg.kg}^{-1} \text{ bw}$ of HCB during 15 weeks. The two highest doses caused adverse effects; increased mortality (females), increased organ weights and an increased activity of liver enzymes. Only females developed porphyria at these doses. The results indicated that female rats are more sensitive to HCB than male rats. The no-effect-level appeared to be $2.0 \text{ mg.kg}^{-1} \text{ bw}$ (Kuiper-Goodman et al., 1977). Female rats were given 0, 0.5, 2, 8 or 32 $\text{mg.kg}^{-1} \text{ bw}$ by gavage twice a week during 29 weeks. An increased liver weight occurred in the 32 $\text{mg.kg}^{-1} \text{ bw}$ group. A dose-dependent response between the HCB concentration and the morphological alterations in the liver was observed. A marked enlargement of hepatocytes and porphyria ($\geq 8 \text{ mg.kg}^{-1} \text{ bw}$) occurred. The enlargement of hepatocytes was moderate in the 2 $\text{mg.kg}^{-1} \text{ bw}$ group and did not occur at the lowest dose. The no-effect-level was 0.5 $\text{mg.kg}^{-1} \text{ bw}$ (Böger et al., 1979). Groups of 5 male and female beagle dogs were administered 0, 1, 10, 100 or 1000 mg per dog (0.1, 1.3, 12.5 or 125 $\text{mg.kg}^{-1} \text{ bw}$) daily by means of a capsule for one year. Mortality, body weight loss and several gastrointestinal effects occurred at highest and next highest dose. Hyperplastic gastric lymphoid nodules were observed in all dose groups, including the controls, but in treated animals the lesions were more severe. The lack of evidence for porphyria suggested that dogs are insensitive to this effect of HCB (Gralla et al., 1977). Rats were exposed to diets containing 1, 5, 10 or 25 mg.kg^{-1} HCB for 3, 6 or 12 months. Gross changes were not observed, but with an electron microscope hepatic ultrastructural changes were observed at dietary doses of 5 mg.kg^{-1} diet ($0.25 \text{ mg.kg}^{-1} \text{ bw}$) and more. The no-effect-level was 1 mg.kg^{-1} diet ($0.05 \text{ mg.kg}^{-1} \text{ bw}$) (Mollenhauer et al., 1975, 1976).

In 3 male and 3 female rhesus monkeys orally exposed to 110 μg HCB (about 33 $\mu\text{g.kg}^{-1} \text{ bw}$) for 18 months (only one dose tested) no adverse effect were observed (Rozman et al., 1978).

Reproduction and teratogenicity

Oral exposure

In a two-generation study rats were exposed to 0, 0.32, 1.6, 8 or 40 mg.kg⁻¹ HCB in the diet (0, 0.016, 0.08, 0.4 or 2 mg.kg⁻¹ bw). Fertility, gestation and lactation indices were not affected by treatment. The viability index was significantly lower in the highest dose group. F₀ males from the 8- and 40 mg.kg⁻¹-groups had increased liver and heart weights. At necropsy the F₁ generation showed tumours (see carcinogenicity) (Arnold et al, 1985).

In a teratogenicity study female rats were given single oral doses of 1, 10, 20, 40, 60, 80 or 120 mg.kg⁻¹ bw during different periods (days 6-21, 6-16, 6-9 or 10-13) of gestation. Concentrations of 80 mg.kg⁻¹ bw or more caused maternal toxicity and a reduction in fetal weight. A dose-related increase in uni- and bilateral 14th rib was found in the groups treated during days 6-21, 6-16 and 10-13 of gestation. Sternal effects were observed in the experiment in which animals were treated from days 6 to 21 (Khera, 1974). Female rats were fed dietary concentrations of 0, 60, 80, 100, 120 or 140 mg.kg⁻¹ of HCB (0, 3, 4, 5, 6 or 7 mg.kg⁻¹ bw) and allowed to raise to two litters (F_{1a} and F_{1b}). No toxic signs were observed in the maternal rats. The F_{1a} and F_{1b} rats had normal birth weights, but after 4 days suckling these were significantly lower than the control rats. A dose-related increase in mortality was seen in the pups. The LD₅₀ for day 21 cumulative mortality from birth was determined to be 100 mg.kg⁻¹ (5 mg.kg⁻¹ bw) (Kitchin et al., 1982). Exposure of female rats to a diet containing 80 mg.kg⁻¹ of HCB (4 mg.kg⁻¹ bw) for 100 days (two weeks before mating to 35-36 days after weaning) did not result in effects on gestation indices or neonatal survival. About half of the dams had elevated liver porphyrin levels, suggesting a heterogeneity of response to the porphyrinogenic activity of HCB (Mendoza et al., 1979). The effects of HCB on body weight of preweanling rats after reciprocal transfer between treated and control maternal rats was examined. It was demonstrated that the transmission of HCB through milk had greater effects on body weights of rat pups than the placental transfer (Mendoza et al., 1978).

The immunotoxic potential of HCB has been investigated in rats with combined pre- and postnatal exposure to dietary levels of 50 or 150 mg.kg⁻¹. Body weights were not affected and only the highest dose group showed increased adrenal and liver weights. The cell mediated immunity (resistance to *Listeria monocytogenes* and *Trichinella spiralis*, allograft rejection and responsivity of thymus and spleen cells to T-cell mitogens) appeared to be slightly suppressed and the humoral immunity (antibody response to tetanus toxoid, to *T. spiralis*, to LPS and responsivity to B-cell mitogens) appeared to be strongly enhanced at levels of 50 mg.kg⁻¹ diet (2.5 mg.kg⁻¹ bw) (Vos et al., 1979). In a similar study with combined pre- and postnatal exposure rats were given diets containing 0, 4, 20 or 100 mg.kg⁻¹ (0.2, 1 or 5 mg.kg⁻¹ bw). From the results it was concluded that dietary levels as low as 4 mg.kg⁻¹ enhanced the humoral and cell-mediated immunity and caused intra-alveolar macrophage accumulation. The developing immune system of the rat seems to be very sensitive to HCB exposure (Vos et al., 1983). HCB was administered to hamsters and guinea pigs at doses of 0, 1.0, 10.0 or 50.0 mg.kg⁻¹ bw by gavage on days 5 through 10 of gestation for the hamster and on days 14 through 19 for the guinea pig. At the highest dose liver weights were significantly increased in hamsters. No differences in number of live fetuses or in weights of fetuses and placenta were found. In guinea pigs a loss of weight was seen at doses of 10.0 mg.kg⁻¹ bw or more. No effect on liver weight was seen nor effects on the fetus (Courtney et al., 1985).

Mice received orally doses of 100 mg.kg⁻¹ bw HCB on days 7 through 16 of gestation. A significant increased incidence of abnormal fetuses per litter occurred. Maternal liver-to-body weights were found to be increased (Courtney et al., 1976).

HCB was orally administered to lactating monkeys for 60 days at 64 mg.kg⁻¹ bw a day. Three infant-mother pairs were used. Milk concentration ranged from 7.5 to 186 ppm milk. Two of three infants died as a result of exposure, whereas the mothers remained healthy (Bailey et al., 1980).

In a four generation reproduction study rats were exposed to dietary concentrations of 0, 10, 20, 40, 80, 160, 320 or 640 mg.kg⁻¹ (0, 0.5, 1, 2, 4, 8, 16, 32 mg.kg⁻¹ bw). The two highest concentrations were toxic to the F0 generation. Decreases were seen in fertility index (≥ 320 mg.kg⁻¹) and viability and lactation indices (≥ 160 mg.kg⁻¹). The average litter size was

decreased by 160 mg.kg^{-1} . Birth weights body weights of 5-d old pups were decreased by dietary levels of 80 mg.kg^{-1} . At dietary concentrations of 40 mg.kg^{-1} liver weights were increased as well as the aniline hydroxylase activity. No effects were seen at the two lowest doses (Grant et al., 1977).

1.2.2 Human studies

Monochlorobenzene

No epidemiological studies regarding the effects of exposure to MCB are available. In a few case studies headaches, irritation of the respiratory tract and neurotoxic effects were reported (EPA, 1984).

Dichlorobenzenes

A number of case studies have been reported involving both long-term occupational exposure and (accidental) acute exposure to dichlorobezene. Of these cases 17 have involved exposure primarily through inhalation, 3 through ingestion and 3 most likely through dermal contact. Most cases involved 1,4-DCB. Although the exposures were not well defined and often involved other substances, the data suggest a common action on bone marrow and other organs of the blood-forming system (EPA, 1984).

From an industrial hygiene survey in a plant in which 1,4-DCB was handled it was reported that a faint odour was noted at doses of 15 to 30 ppm (90 to 180 mg.m^{-3}), and that the odor became strong at 30-60 ppm (180 - 360 mg.m^{-3}). Painfull irritation of the eyes and nose was reported at 80-160 ppm (480 - 960 mg.m^{-3}). At concentrations of >160 ppm ($>960 \text{ mg.m}^{-3}$) the air could not be tolerated by persons who were not acclimated. The eyes of employees, exposed to concentrations up to about 550 ppm, showed no signs of cataract. No skin irritation was observed until solid particles were held on the skin for a very long period (Hollingsworth et al., 1956).

Hexachlorobenzene

One of the most notable human exposures to HCB occurred in Southeastern Turkey from 1955-1959. More than 4,000 people were exposed through ingestion of seed grain that had been treated with HCB and was at first intended for agricultural use. The doses ingested were estimated to have been 0.5-2.0 g per day for several months to two years. It was estimated that over 3,000, mostly children in the age of 6 to 16, developed porphyria cutanea tarda (PCT). The mortality rate was 3% to 10% annually.

The PCT-patients developed bullae on sun-exposed areas, hyperpigmentation, hypertrichosis, weakness and porphyrinuria. Children under the age of 4, which were breast fed, generally did not develop PCT but a condition called 'pink-sore' (pembe yara), with a mortality rate of 95%. The children had fever, diarrhea, vomiting, anorexia, convulsions and atrophy of muscles and cutaneous lesions (Cam and Nigogosyan, 1963).

The exposed population has been the subject of several studies since. In one study the clinical features of PCT 20 years after onset were described. The clinical symptoms of PCT were still obvious, consisting of dermatological, skeletal and (in some of the people) neurological symptoms as well as hepatic cirrhosis. From urine and faeces analyses it appeared that a minor part of the group was still porphyric. Exposition to other porphyric agents might have taken place, but this was not further studied (Cripps et al., 1980). In a group of 181 patients (examined 25 years afterwards) many had an abnormal porphyrin metabolism and further similar symptoms as described in the foregoing study (Peters et al., 1982). In another group of 204 patients it was also clear that the symptoms persisted (Cripps et al., 1984).

A group of workers employed in chlorinated solvents manufacture and (among others) exposed to HCB were followed for 1-4 years. Airborn concentrations ranged between <1 and 13 ppb, as time-weighted averages. Blood levels of HCB ranged between 160 and 320 ppb. Evaluation of urinary porphyrins and several other laboratory tests did not reveal any evidence of porphyria cutanea tarda or other adverse health effects. HCB levels correlated more significantly with the years worked than with environmental levels (Currier et al., 1980).

A population exposed to HCB through transportation and disposal of "hex" wastes (a mixture of HCB, hexachlorobutadiene and other chlorinated hydrocarbons) was examined for plasma HCB levels and toxic signs. The average HCB plasma level was significantly higher in exposed persons (2.4 ppb) than in control persons (0.5 ppb). There was no evidence of (cutaneous) porphyria. The exposed group had significantly higher lactate dehydrogenase (LDH) levels than the controls (Burns and Miller, 1975).

Among a group of 20 vegetable spraymen exposed to HCB-contaminated dimethyl-1,2,3,5,6-tetrachloroterephthalate HCB residues in blood were found. The mean blood level was 40 ppb, with a range of <1 to 310 ppb. There was no relationship between HCB residues and years of employment, uroporphyrin levels, coproporphyrin levels or serum enzymes (serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase and LDH) (Burns et al., 1974).

Summary and conclusions "toxicity"

- Experimental studies

There is a clear similarity in the effects of the different chlorobenzenes. The liver and kidneys were target organs of all chlorobenzenes; exposure resulted in increased organ weights, enzyme induction and histopathological changes. In some cases necrosis occurred. All chlorobenzenes disturbed the porphyrine metabolism, but this effect was the most evident by HCB. In addition, the lower chlorinated chlorobenzenes suppressed the activities of bone marrow, spleen and thymus. The microsomal enzyme induction and the interference with the normal porphyrin metabolism were the most sensitive parameters. Microsomal enzyme induction already occurs after short exposure periods and its sensitivity dose not seem to increase after longer exposure periods. The available studies indicate that the toxicity of the chlorobenzenes increases with an increasing degree of chlorination. Most studies concerned oral exposure.

MCB - In a chronic oral (carcinogenicity-) study with mice and rats only in the highest dose group of male rats ($120 \text{ mg.kg}^{-1} \text{ bw}$) an effects was seen. In the livers of these animals a significant increase in neoplastic noduli was found. The dose-without-effct was $60 \text{ mg.kg}^{-1} \text{ bw}$. In subchronic oral

studies (dogs, rats, mice) similar doses resulted in slight effects; a dose of about 30 mg.kg⁻¹ bw was without effect. Inhalatory exposure up to 450 ppm (2070 mg.m⁻³) caused no adverse effects on reproduction or fertility of rats. Inhalation up to 590 ppm (2715 mg.m⁻³) did not result in teratogenic effects in rabbits, but this dose caused a slight delay in skeletal development in rats accompanied by maternal toxicity.

1,2-DCB - Exposure of rats to 188 mg.kg⁻¹ bw of 1,2-DCB for about half a year caused increased liver- and kidney weights; this was not reported at 19 mg.kg⁻³ bw. In an oral (carcinogenicity-) study with rats and mice an increase in tubular regeneration in the kidneys of male mice was only significant in the highest dose group (120 mg.kg⁻¹ bw); no effects were reported at 60 mg.kg⁻¹ bw. In a subchronic inhalation experiment with rats, rabbits and monkeys (small numbers) no effects were found at 93 ppm (560 mg.m⁻³). In a teratogenicity study the highest dose (400 ppm; 2400 mg.m⁻³) caused slight maternal toxicity in rats and rabbits, but no embryotoxic or teratogenic effects.

1,4-DCB - Rats exposed to 1,4-DCB (188 mg.kg⁻¹ bw) for about half a year showed increased liver- and kidney weights; no effects occurred at 19 mg.kg⁻¹ bw. In a chronic oral (carcinogenicity-) study the lowest tested doses (150 mg.kg⁻¹ bw for male mice and 300 mg.kg⁻¹ bw for female mice and male and female rats) resulted in several effects in the liver (hepatocellular degeneration) and the kidneys (nephropathy). An inhalation experiment in which rats, mice, guinea pigs, rabbits and monkeys (small numbers) were exposed for 6-7 months resulted in a dose-without-effect of 96 ppm (577 mg.m⁻³). Oral exposure of rats (250-1000 mg.kg⁻¹ bw) provided no evidence of teratogenicity; an embryotoxic effect occurred at 500 mg.kg⁻¹ bw (extra ribs). Inhalation up to 800 ppm (4800 mg.m⁻³) caused no teratogenic or embryotoxic effects in rabbits.

- TCB's - Most data concern 1,2,4-TCB, which is considered to be the most toxic isomer. In an oral 13-week study the lowest concentration (10 mg.kg⁻¹ bw) caused enzymeinduction; after a 30-day "recovery" period this effect had disappeared. A 13-week inhalation experiment with rats (1,3,5-TCB) resulted in a dose-without-effect of 100 mg.m⁻³. Inhalation of 100 ppm of 1,2,4-TCB (740 mg.m⁻³) during 26 weeks did not cause lasting effects in several species. In rabbits dermally treated with TCB's (circa 70% 1,2,4-TCB and 30% 1,2,3-TCB) topical effects occurred in all dose groups (30-450

mg.kg⁻¹ bw), only the highest dose caused slight systemic effect (increased excretion of coproporphyrins in the urine). In a more-generation reproduction study with rats, exposed through drinking water (1,2,4-TCB; 25-400 mg.l⁻¹) no effects on reproduction were found. A teratogenicity study with rats also did not result in any effects at doses up to 300 mg.kg⁻¹ bw (1,2,4-TCB) or 600 mg.kg⁻¹ bw (1,2,3- and 1,3,5-TCB). In another study with rats a dose of 360 mg.kg⁻¹ bw caused retarded embryonic growth accompanied with maternal toxicity; this was not reported at 120 mg.kg⁻¹ bw.

- TeCB's - Toxicity studies with TeCB are limited and only concern oral exposure. In a 90-day diet study with rats exposure to TeCB's caused liver- and kidney toxicity. 1,2,4,5-TeCB was the most toxic isomer; the dose-without-effect was 5 mg.kg⁻¹ diet, which corresponded to 0.34 or 0.4 mg.kg⁻¹ bw for males and females, respectively. In a teratogenicity study with rats the highest doses of 1,2,3,4- and 1,2,3,5-TeCB (200 mg.kg⁻¹ bw) caused a decrease in the number of live fetuses per litter. This dose was lethal to nearly all females in the 1,2,4,5-TeCB-group. No teratogenic effects were found.

- PeCB - Only one study (subchronic toxicity combined with reproduction toxicity) with PeCB was available. Oral exposure to 25 mg.kg⁻¹ bw caused liver- and kidney toxicity in rats; the dose-without-effect was 12.5 mg.kg⁻¹ bw. Suckling pups from mothers fed this dose developed tremors 4-14 days after birth; at a (maternal) dose of 6.3 mg.kg⁻¹ bw this effect did not occur.

- HCB - For female rats, which are more sensitive to the induction of porphyria than males, a dose-without-effect of 2 mg.kg⁻¹ bw was established. In a study with combined pre- and postnatal exposure the lowest dose tested (0.2 mg.kg⁻¹ bw) still resulted in effects on the immune system of rats. For changes in liver cells of rats a dose-without-effect of 0.05 mg.kg⁻¹ bw was established. A similar dose did also not result in effects in a small number of monkeys. No "chronic" dose-without-effect was available. In an oral two-generation study the viability index was lower at ≥ 2 mg.kg⁻¹ bw, this was not reported at 0.4 mg.kg⁻¹ bw. Effects on the reproduction were reported at doses of 8 mg.kg⁻¹ bw and higher. No teratogenic effects were found in rats (doses up to 120 mg.kg⁻¹ bw), but an embryotoxic effects occurred at 40 mg.kg⁻¹ bw.

- Human studies

Human studies were only available for HCB. One of the most notable human exposures to HCB occurred in the Southeastern of Turkey from 1955 to 1959. More than 4,000 people were exposed through the ingestion of seed grain that had been treated with HCB and was at first intended for agricultural use. The estimated ingested doses varied from 0.5 to 2.0 g a day during several months to two years. Over 3,000 people, mostly children, developed porphyria cutanea tarda (PCT), which was characterized by bullae on sun-exposed areas, hyperpigmentation, hypertrichosis, weakness and porphyrinuria. Children under the age of 4 (which were breast-fed) generally developed clinical picture named 'pink-sore' (pembe yara), with a mortality rate of 95%. The children had fever, diarrhea, vomiting, anorexia, convulsions and atrophy of muscles and cutaneous lesions.

The exposed population has been the subject of several studies since. In many patients the clinical features of PCT (consisting of dermatological, skeletal and sometimes also neurological symptoms as well as liver cirrhosis and porphyria) were still obvious, 20 years after onset.

In workers among others exposed to HCB, no evidence of porphyria (or other HCB-induced effects) were found, although HCB levels in blood were in one study higher than those of controls.

Table 1.1 Acute toxicity of chlorobenzenes (mg/kg bw)

Chlorobenzene	route	species	parameter		reference
MCB	oral	rat	LD50	400-2,144	EPA '84, Allen et al.'79
	oral	rabbit	LD50	2,830	EPA '84
	i.p.	rat	LD50	7,400	EPA '84
	i.p.	guinea pig	LD50	4,100	EPA '84
	i.p.	mouse	LD50	1,355	Mohtashamipur et al. '87
	inh.	rat	LC50	2,965	EPA '84
	inh.	mouse	LC50	1,886	EPA '84
1,2-DCB	dermal	rabbit	LD50	>10,000	EPA '84
	oral	rat	LD50	2,138	EPA '84
	oral	rabbit	LD50	1,875	EPA '84
	oral	mouse	LD50	2,000	EPA '84
	oral	guinea pig	LD50	3,375	EPA '84
	oral	rat	LD50	500	Allen et al.'79
1,3-DCB	i.p.	mouse	LD50	1,228	Mohtashamipur et al. '87
	i.p.	mouse	LD50	1,062	Mohtashamipur et al. '87
1,4-DCB	oral	rat	LD50	500	EPA '84
	oral	rabbit	LD50	2,812	EPA '84
	s.c.	mouse	LD50	5,145	EPA '84
	i.p.	mouse	LD50	2,000	Mohtashamipur et al. '87
1,2,3-TCB	i.p.	mouse	LD50	1,390	Mohtashamipur et al. '87
1,2,4-TCB	oral	rat	LD50	756	EPA '84
	oral	mouse	LD50	766	EPA '84
	i.p.	mouse	LD50	1,223	Mohtashamipur et al. '87
1,3,5-TCB	i.p.	mouse	LD50	2,260	Mohtashamipur et al. '87
1,2,4,5-TeCB	oral	rat, rabbit	LD50	1,500	EPA '84
	oral	mouse	LD50	1,035	EPA '84
TeCB-isomers	oral	rat	LD50	1,200-3,000	EPA '84
PeCB	oral	rat	LD50	250	Allen et al. '79
	oral	rat	LD50	940-1,125	EPA '84
	oral	mouse	LD50	1,175-1,370	EPA '84
HCB	oral	rat	LD50	32,100	Allen et al. '79
	oral	rat	LD50	3,500-10,000	EPA '84
	oral	rabbit	LD50	2,600	EPA '84
	oral	cat	LD50	1,700	EPA '84
	oral	mouse	LD50	4,000	EPA '84

i.p. = intraperitoneal; inh. = inhalatory; s.c. = subcutaneous; LD50 = lethal dose for 50% of the exposed animals

1.3 GENOTOXICITY

- Experimental systems

The results from *in vitro* and *in vivo* genotoxicity tests with chlorobenzenes are summarized in table 1.2 and 1.3, respectively.

- *in vitro*

MCB was negative in the the *Salmonella typhimurium* gene mutation test (=Ames test), either with or without metabolic activation. The results of a mouse lymphoma (L5178Y) assay was equivocal when tested without activation, whereas with activation it was positive. In this positive test no dose-response relationship existed. MCB did not increase the frequency of chromosome aberrations in chinese hamster ovary cells. 1,2-DCB and 1,3-DCB were only tested in the Ames test; negative results were obtained. 1,4-DCB was negative in the Ames test. The results of a mouse lymphoma test were considered to be equivocal, both with and without activation (according to the NTP the test with activation was positive). In chinese hamster ovary cells 1,4-DCB did not increase the frequency of SCE's or chromosomal aberrations (NTP, 1987). 1,2,3-TCB, 1,2,4-TCB and 1,3,5-TCB were not mutagenic in the Ames test and did not increase the frequency of chromosome aberrations in chinese hamster ovary cells. 1,2,3,4-TeCB, 1,2,3,5-TeCB, 1,2,4,5-TeCB, PeCB and HCB were only tested in the Ames test; the results were all negative.

Prasad (1970) conducted fungal mutation tests with MCB, 1,2-, 1,3- and 1,4-DCB. Besides the fact that this test is not a current test system, the "positive" results were considered equivocal. Therefore these results will not be taken into account.

- *in vivo*

In one mouse micronucleus test in bone marrow clear positive results were obtained for all chlorobenzenes that were included in the test: MCB, 1,2-DCB, 1,3-DCB, 1,4-DCB, 1,2,3-TCB, 1,2,4-TCB and 1,3,5-TCB. The test was conducted according to a commonly accepted procedure (two intraperitoneal injections). The purity of the test substances was at least 98%. Beside that, for all chlorobenzenes positive dose-response relationships were

found (Mohtashamipur et al., 1987). 1,4-DCB did not show an increased number of micronucleated cells among erythrocytes from peripheral blood (NTP, 1987). In a dominant lethal test 1,4-DCB was found to be negative at any maturation stage of the 8 week spermatogenic cycle in mice exposed up to 450 ppm (4610 mg.m^{-3}) (unpublished results from Anderson and Hodge (1976), evaluated by Loeser and Litchfield, 1983). In bone-marrow cells of rats exposed to levels up to 680 ppm (6965 mg.m^{-3}) no increase in the number of observable chromosomal aberrations could be detected (unpublished results from Anderson and Richardson (1976), evaluated by Loeser and Litchfield, 1983). 1,2,4,5-TeCB did not cause an increased frequency of sex-linked mutations in *Drosophila melanogaster*. Two dominant lethal tests with HCB in rats gave negative results.

- Human studies

In peripheral lymphocytes of a group of 8 males and 18 females accidentally exposed to vapors of 1,2-DCB (no quantitative data) during 4 working days a significant increase in chromosomal aberrations (9%) was found compared with a control group (2%). The chromosomal aberrations were divided into two groups: single or double breaks. No further specification of type of chromosomal aberration was given. A decrease (4%) in number of chromosomal aberrations in the exposed groups was found 6 months after exposure, which indicated an reversible effect. Although the concentration of the vapor was not determined, the symptoms of most exposed persons were consistent with those usually observed at concentrations above 100 ppm. Because of the fact that no types of chromosome aberrations were specified and the large variation in percentage of cells with chromosome aberrations (0-22%) the results are difficult to interpret and therefore the study will be left out of consideration (Zapata-Gayon et al., 1982).

In peripheral lymphocytes of workers exposed to 1,2,4,5-TeCB in producing organophosphate insecticides a significant increase in the frequency of chromosome aberrations was found compared to a control group (consisting of "normal" healthy persons). The concentration to which these workers were exposed was not determined. Although the authors considered chromosomal mutagenicity of 1,2,4,5-TeCB proven, this study is left out of

consideration in the present evaluation because the workers had been simultaneously exposed to other substances (Kiraly et al., 1979).

- Additional data

Various types of mitotic and chromosomal anomalies were observed in root tips of several plant species treated with 1,4-DCB (Carey and McDonough, 1943, Sharma and Bhattacharyya, 1956, Srivastava, 1966, Sarbhoy, 1980).

In several *in vivo* and *in vitro* tests it was demonstrated that MCB binds covalently to DNA and other macromolecules. About one day after an intraperitoneal injection into rats and mice, MCB was found to be covalently bound to DNA of the liver, kidneys and lung. The binding of MCB with DNA was found *in vitro* to be mediated by liver microsomes. The involvement of Cyt P-450 was indicated (Prodi et al., 1986, Grilli et al., 1985).

24 Hours after *i.p.* injections into mice and rats 1,2-DCB was covalently bound to DNA, RNA and proteins of the liver, kidney, lung and stomach (Colacci et al., 1990).

Summary and conclusions "genotoxicity"

Data on genotoxicity are rather limited. With regard to *in vitro* tests it appeared that all chlorobenzenes were negative in the *Salmonella typhimurium* gene mutation test (=Ames test). A number of chlorobenzenes (MCB, 1,4-DCB and TCB's) were tested in mammalian cells for one or more of the following end points: gene mutations, chromosome aberrations and "sister chromatid exchanges". From these tests only one was equivocal (1,4-DCB) or equivocal/positive (MCB), while all other tests were negative. Therefore, it is concluded that the *in vitro* studies show no clear indications for genotoxic activities of chlorobenzenes.

With regard to *in vivo* genotoxicity a few studies with negative results were available; this concerned 1,4-DCB (mous micronucleus test in peripheral blood, mous dominant lethal test, chromosome aberrations in rat bone marrow cells) and HCB (test with *Drosophila melanogaster*, and two dominant lethal tests with rats). However, in one mous micronucleus test in bone marrow, clear positive results were obtained for all chlorobenzenes that were included in the test: MCB, 1,2-DCB, 1,3-DCB, 1,2,3-TCB, 1,2,4-TCB

and 1,3,5-TCB). The test was conducted according to a commonly accepted procedure (two intraperitoneal injections) with substances that a purity of at least 98%. Beside that for all chlorobenzenes a positive dose-response relationship was found. As a result of this test and the chemical affinity of the substances it can not be excluded that the other chlorobenzenes would also have been positive in this test. It must be noted that the results of this test need to be verified. As yet, it is concluded that the data are too limited to consider the chlorobenzenes as genotoxic. Two studies reported an increased frequency of chromosomal aberrations in peripheral lymphocytes of humans exposed to 1,2-DCB or 1,2,4,5-TeCB. For several reasons (type of chromosomal aberrations not specified, no individual data, mixed exposure) these studies will be left out of consideration.

Table 1.2 Genotoxicity of chlorobenzenes in vitro

Species or test system	Endpoint	Dose	Purity test-subst.	Test results		Reference
				without act.	with act.	
MCB						
S. typh. TA98, 100, 1537, 1538	gene mut.	0-333 µg/plate _t	"pure"	-	-	NTP, 1985a
S. typh. TA98, 1537, 1538	gene mut.	-	-	-	-	Lawlor et al. '79 abstract
S. typh. TA98, 100, 1535, 1537	gene mut.	0-333 µg/plate	-	-	-	[1] Haworth et al. '83
S. typh. TA98, 100, 1535, TA1537, 1538.	gene mut.	0-1.28 µl/plate	98	-	-	[1] Shimizu et al. '83
Mouse lymphoma cells (L5178Y)	gene mut.	0-200 µg/µl	-	±	+	[5] McGregor et al. '88
CHO cells	chrom. ab.	0-400 µg/ml	-	-	-	[1] Sofuni et al. '85
1,2-DCB						
S. typh. TA98, 1537, 1538	gene mut.	-	-	-	-	Lawlor et al. '79 abstract
S. typh. TA98, 100, 1535, 1537	gene mut.	0-100 µg/plate _t	-	-	-	[1] Haworth et al. '83
S. typh. TA98, 100, 1535, 1537	gene mut.	0-333 µg/plate _t	-	-	-	NTP '85b
S. typh. 8 strains	gene mut.	1-5 µl/plate	≥90	-	-	[3] Andersen et al. '72
S. typh. TA98, 100, 1535, TA1537, 1538.	gene mut.	0-1.28 µl/plate	98	-	-	[1] Shimizu et al. '83
1,3-DCB						
S. typh. TA98, 100, 1535, 1537	gene mut.	0-100 µg/plate	-	-	-	[1] Haworth et al. '83
S. typh. TA98, 100, 1535, TA1537, 1538.	gene mut.	0-1.28 µl/plate	98	-	-	[1] Shimizu et al. '83
1,4-DCB						
S. typh. TA98, 1537, 1538	gene mut.	-	-	-	-	Lawlor et al. '79 abstract
S. typh. TA98, 100, 1535, 1537	gene mut.	0-100 µg/plate	-	-	-	[1] Haworth et al. '83
S. typh. TA98, 100, 1535, TA1537, 1538.	gene mut.	0-13105 µl/plate	99	-	-	[1] Shimizu et al. '83
S. typh. TA98, 100, 1535, 1537	gene mut.	0-100 µg/plate	-	-	-	NTP '87
mouse lymphoma cells (L5178Y)	gene mut.	0-100 µg/µl	-	±	±	[6] McGregor et al. '88
mouse lymphoma cells (L5178Y)	gene mut.	0-100 µg/µl	-	-	-	NTP '87
CHO cells	SCE's	0-150 µg/µl	-	-	-	NTP '87
CHO cells	chrom. ab.	0-150 µg/µl	-	-	-	NTP '87
CHO cells	chrom. ab.	0-200 µg/ml	-	-	-	[1] Sofuni et al. '85
1,2,3-TCB						
S. typh. TA98, 100, 1535, 1537	gene mut.	0-100 µg/plate _t	-	-	-	[1] Haworth et al. '83
CHO cells	chrom. ab.	0-63 µg/ml	-	-	-	[1] Sofuni et al. '85
1,2,4-TCB						
S. typh. TA98, 1537, 1538	gene mut.	-	-	-	-	Lawlor et al. '79 abstract
S. typh. TA98, 100, 1535, 1537	gene mut.	0-100 µg/plate _t	-	-	-	[1] Haworth et al. '83
S. typh. TA98, 100, 1535, 1537	gene mut.	0-1599 µg/plate _t	-	-	-	[7] Schoeny et al. '79
CHO cells	chrom. ab.	0-125 µg/ml _t	-	-	-	[1] Sofuni et al. '85

Table 1.2 continued

Species or test system	Endpoint	Dose	Purity test-subst.	Test results		Reference
				without act.	with act.	
1,3,5-TCB						
S. typh. TA98, 100, 1535, 1537	gene mut.	0-100 µg/plate _t	-	-	-	[1] Haworth et al. '83
CHO cells	chrom. ab.	0-125 µg/ml _t	-	-	-	[1] Sofuni et al. '85
TCB (isomer not given)						
S. typh. 8 strains	gene mut.	1-5 µl/plate	-	-	-	[3] Andersen et al. '72
1,2,3,4-TeCB						
S. typh. TA98, 100, 1535, 1537	gene mut.	0-33 µg/plate _t	-	-	-	[1] Haworth et al. '83
1,2,3,5-TeCB						
S. typh. TA98, 1537, 1538	gene mut.	-	-	-	-	Lawlor et al. '79 abstract
S. typh. TA98, 100, 1535, 1537	gene mut.	0-20 µg/plate _t	-	-	-	[1] Haworth et al. '83
1,2,4,5-TeCB						
S. typh. TA98, 1537, 1538	gene mut.	-	-	-	-	Lawlor et al. '79 abstract
S. typh. TA98, 100, 1535, 1537	gene mut.	0-1000 µg/plate _t	-	-	-	[2] Haworth et al. '83
PeCB						
S. typh. TA98, 1537, 1538	gene mut.	-	-	-	-	Lawlor et al. '79 abstract
S. typh. TA98, 100, 1535, 1537	gene mut.	0-1000 µg/plate _s	-	-	-	[1] Haworth et al. '83
NCB						
S. typh. TA98, 100, 1535, 1537	gene mut.	0-1000 µg/plate _s	-	-	-	[1] Haworth et al. '83

abn. mit. = abnormalities in behaviour of chromosomes during mitosis
 act. = metabolic activation
 chrom. ab. = chromosome aberrations
 gen. mut. = gene mutation
 S. typh. = Salmonella typhimurium
 t/s = highest concentration is limited by toxicity to the bacteria or to solubility.

[1] Test compound solved in dimethyl sulfoxide

[2] Test compound solved in acetone

[3] Presence of metabolic activation not stated

[5] The overall (NTP-) conclusions of the tests conducted with or without activation ("positive" and "inconclusive", respectively) were confirmed by RIVM-experts (from the Laboratory of mutagenesis and carcinogenesis).

[6] The overall (NTP-) conclusion of the tests conducted without activation ("inconclusive") was confirmed by RIVM-experts. The overall (NTP-) conclusion of the tests with activation ("positive") has not been confirmed; the results were considered to be "inconclusive" by RIVM-experts.

[7] Concentrations ranging from 102-1.4 x 10⁵ µg/plate were tested, but the toxic dose was determined as 1599 µg/plate. 1,2,4-TCB was negative for mutagenicity in the presence of S9-mix prepared from uninduced rats or from rats induced by Arcolor 1254 or 1,2,4-TCB. The mutagenicity of 2-aminoanthracene appeared to be affected by 1,2,4-TCB-induced S9-mix.

Table 1.3 Genotoxicity of chlorobenzenes in vivo

Species or test system	Exposure	Result	Purity test-substance	Reference
MCB				
mouse micronucleus	2 intraperitoneal injections of 113-450 mg/kg bw	positive	≥98%	Mohtashamipur et al. '87
1,2-DCB				
mouse micronucleus	2 intraperitoneal injections of 94-375 mg/kg bw	positive	≥98%	Mohtashamipur et al. '87
1,3-DCB				
mouse micronucleus	2 intraperitoneal injections of 88-350 mg/kg bw	positive	≥98%	Mohtashamipur et al. '87
1,4-DCB				
mouse micronucleus	0-1,800 mg/kg bw by gavage 5 days a week for 13 weeks	negative	>99%	[2] NTP '87
mouse micronucleus	2 intraperitoneal injections of 178-710 mg/kg bw	positive	≥98%	Mohtashamipur et al. '87
mouse dominant lethal	inhalatory exposure to 75, 225 or 450 ppm, 6 hrs/day for 5 days	negative	--	[3] unpublished data from Anderson and Hodge '76
chromosome aberrations in rat bone marrow cells	single or multiple exposure to 75-680 ppm	negative	--	[3] unpublished data from Anderson and Richardson '76
1,2,3-TCB				
mouse micronucleus	2 intraperitoneal injections of 125-500 mg/kg bw	positive	≥98%	Mohtashamipur et al. '87
1,2,4-TCB				
mouse micronucleus	2 intraperitoneal injections of 105-420 mg/kg bw	positive	≥98%	Mohtashamipur et al. '87
1,3,5-TCB				
mouse micronucleus	2 intraperitoneal injections of 213-850 mg/kg bw	positive	≥98%	Mohtashamipur et al. '87
1,2,4,5-TeCB				
<i>Drosophila melanogaster</i>	3,5 mM in medium	no increased frequency of sex-linked recessive lethal mutations	--	[1] Parádi and Lovenyák '81
MCB rat dominant lethal	0-221 mg/kg bw by gavage for 5 consecutive days	negative	--	Simon et al. '79
rat dominant lethal	0-60 mg/kg bw by gavage for 10 consecutive days	negative	--	Khera '74

[1] The BASC technique was used for measuring X-linked recessive lethals.

[2] Peripheral erythrocytes were used.

[3] Evaluated by Loeser and Litchfield (1983).

1.4 CARCINOGENICITY

Monochlorobenzene

Groups of 50 male and 50 female F344/N rats and groups of 50 female B6C3F1 mice were administered chlorobenzene in corn oil by gavage, 5 days a week for 103 weeks, at doses of 0 (vehicle control), 60 or 120 mg.kg⁻¹ bw. A group of 50 male mice received doses of 0, 30 and 60 mg.kg⁻¹ on the same schedule. Untreated controls consisted of 50 male and 50 female rats and mice. A significant increase of the incidence of neoplastic noduli of the liver at the high dose was observed in male rats. The incidences were 4/50 in untreated controls, 4/50 in vehicle controls, 4/49 in the low dose group and 8/49 in the high dose group. Hepatocellular carcinomas occurred in two male rats in the vehicle control group. No increased incidence in liver adenomas or carcinomas was observed in female rats or in male or female mice. A significant decrease of pituitary adenomas or carcinomas was observed in male and female rats in the higher dose group compared to the vehicle controls. A significant decrease in endometrial stromal polyps in the low dose female rat group. The reasons for a decrease in these tumours are unknown, as stated by the NTP. On the basis of these studies it is concluded that there is no evidence for carcinogenicity of MCB in experimental animals (NTP, 1985a, Kluwe et al., 1985).

1,2-Dichlorobenzene

Groups of 50 male and 50 female F344/N rats and B6C3F1 mice were administered 1,2-DCB in corn oil by gavage at doses of 0 (vehicle control), 60 or 120 mg.kg⁻¹, 5 days a week during 103 weeks. Untreated controls consisted of 50 male and 50 female rats and mice. A dose-related increase in the incidence of malignant histiocytic lymphomas was observed in male and female mice. The incidences of all types of malignant lymphoma, which is considered to be a more appropriate comparison, were not significantly increased. Therefore, the NTP discounted the increase in malignant histiocytes, and concluded that, under the conditions of the present study, there was no evidence of carcinogenicity of 1,2-DCB in experimental animals (NTP, 1985b).

1,4-Dichlorobenzene

Groups of 50 male F344/N rats were given 1,4-DCB by gavage at doses of 0, 150 or 300 mg.kg⁻¹ bw and female rats and male and female B6C3F1 mice were given 0, 300 or 600 mg.kg⁻¹ bw for 5 days a week during 103 weeks. In male rats the incidences increased of renal tubular cell adenocarcinoma (1/50, 3/50 and 7/50), tubular cell adenoma and adenocarcinoma (combined; 1/50, 3/50 and 8/50) (both only significant in the high dose group) and pelvic epithelial cell hyperplasia (significant in low and high dose groups). In males the incidence of pelvis epithelial cell hyperplasia was significantly increased. No renal tumours were found in female rats. In mice the incidences increased of hepatocellular adenomas (5/50, 13/49 and 16/60 in males and 10/50, 6/48 and 21/50 in females) and hepatocellular carcinomas (14/50, 11/49, 32/50 in males and 5/50, 5/48, 19/50 in females), both significant in the higher dose groups. In the higher dose groups the incidence of hepatoblastoma and several types of pheochromocytoma were increased in male mice. On the basis of these data the NTP concluded that there is clear evidence of carcinogenicity of 1,4-DCB in male rats and male and female mice, but not in female rats (NTP, 1987).

However, some critical observations must be added to this conclusion. Recent studies indicate that the development of renal tumours in male F344 rats is based on a very specific mechanism. 1,4-DCB seems to bind reversible to the male rat-specific protein alpha(2u)-globulin. This complex is resistant to proteolytic hydrolysis, leading to accumulation in renal lysosomes and subsequent cytotoxicity and cell death. This results in increased cell proliferation that persists (providing exposure continues) which is thought to promote initiated cells to form preneoplastic and renal neoplasia in male rats. This syndrome is highly species and sex specific; humans do not synthesize alpha(2u)-globulin (Swenberg et al., 1989, Charbonneau et al., 1989). In mice hepatocellular adenomas and carcinomas were found. Since these tumours are common neoplasm in mice of this strain (spontaneous incidence from 16% up to 60% in males and from 2% to 20% in females) (Seiler et al., 1991), the relevance of these tumours is considered equivocal. On the basis of these data it is concluded that there is limited evidence of carcinogenicity of 1,4-DCB in experimental animals.

Hexachlorobenzene

In a group of 7 female Agus rats fed dietary concentrations of 100 mg.kg^{-1} HCB ($6-8 \text{ mg.kg}^{-1} \text{ bw}$) for 90 weeks all animals developed liver-cell tumours. The livers of the treated rats was twice the size of that of the controls. The onset of porphyria (in the urine) was seen but no other effects (Smith and Cabral, 1980). In another 90-week study in which F344/N rats were given dietary concentrations of 200 mg.kg^{-1} HCB ($10 \text{ mg.kg}^{-1} \text{ bw}$) all surviving females (10) had either neoplastic noduli or hepatocellular carcinoma. Only 2 out of 12 surviving males seemed to have "liver tumours", but histological examination of portions of the liver showed no noduli or carcinomas. Male livers showed hypertrophy, fatty degeneration and bile duct hyperplasia (Smith et al., 1985). Exposure of Sprague-Dawley rats to 0, 75 or 150 mg.kg^{-1} HCB in the diet (0, 4 or $8 \text{ mg.kg}^{-1} \text{ bw}$) for 2 years resulted in increased incidences of renal cell adenoma (7/54, 7/56 and 15/54 in females and 7/54, 41/52 and 42/56 in males), renal cell carcinoma in females (1/52, 2/56 and 2/54), hepatic lesions, hepatocarcinoma (0/54, 3/52 and 4/56 in males and 0/52, 36/56 and 48/55 in females) and bile duct adenoma or carcinoma (0/54, 2/52 and 2/56 in males and 1/52, 19/56 and 29/55 in females) (Lambrecht et al., 1983a, 1983b, abstracts).

Syrian golden hamsters and Sprague-Dawley rats of both sexes were given 0, 200 or 400 mg.kg^{-1} HCB in the diet for 90 days. In the hamster-experiment the liver was the most severely involved organ; (pre-) cirrhotic lesions, bile-duct hyperplasia and hepatomas (2/28 and 2/22 in the 200 and $400 \text{ mg.kg}^{-1} \text{ bw}$ groups, respectively) were found (Lambrecht et al., 1982a, abstract). Rats showed besides liver neoplasms and lymphatic leukemias a variety of renal lesions as well as renal adenomas (males and females) and carcinomas (only females) (Lambrecht et al., 1982b, abstract). Exposure of Syrian golden hamsters to dietary concentrations of 0, 50, 100 or 200 mg.kg^{-1} HCB (0, 4, 8 or $16 \text{ mg.kg}^{-1} \text{ bw}$) for lifespan resulted in increased mortality and a decline in body weight at the highest dose. A dose-related increase in hepatomas (0/39, 14/30, 17/30 and 51/60 for females and 0/40, 14/30, 26/30 and 49/57 for males) and liver haemangio-endotheliomas (0/39, 0/30, 2/30 and 7/60 for females and 0/40, 1/30, 6/30 and 20/57 for males) was observed. In the mid and highest dose group some hamsters also developed thyroid adenomas or spleen haemangioendotheliomas (Cabral et al.,

1977). Exposure of male and female Swiss mice to dietary concentrations of 0, 50, 100 or 200 mg.kg⁻¹ for 101-120 weeks resulted in increased mortality at the highest dose. The incidence of liver cell tumours was increased in the 100 and 200 mg.kg⁻¹ dose group but not in the 50 mg.kg⁻¹ dose groups. The incidences were 3/30 females and 3/29 males in the 100 mg.kg⁻¹ group and 14/41 females and 7/44 males in the 200 mg.kg⁻¹ group. With regard to lymphomas and lung adenomas the incidences in the control group were found to be higher (!) than in the treated groups. In a separate experiment groups of 30 male and female mice were fed dietary doses of 300 mg.kg⁻¹ HCB (36 mg.kg⁻¹ bw) for 15 weeks. After 120 weeks, when the experiment was terminated, an increase in mortality was seen in males. Two treated mice developed liver cell tumours (1 male and 1 female) compared to none of the controls (Cabral et al., 1979). On the basis of these studies it is concluded that there is sufficient evidence of carcinogenicity of HCB in experimental animals.

Additional data

In a two generation feeding study rats were given 0, 0.32, 1.6, 8.0 or 32 mg.kg⁻¹ HCB in the diet. At necropsy it appeared that the F1 generation had pituitary and subcutaneous tumours (Arnold et al., 1985).

In a feeding study male ICR mice were exposed to HCB and polychlorinated terphenyl (PCT) singly and in combination for 24 weeks. Administration of HCB in concentrations of 10 or 50 mg.kg⁻¹ HCB did not increase tumor incidences. However, when administered in combination with PCT, it seemed to enhance the induction of liver tumours by PCT (Shirai et al., 1978).

Summary and conclusions "carcinogenicity"

Data on carcinogenicity are limited; only MCB, 1,2-DCB, 1,4-DCB and HCB were tested in experimental animals. There are no epidemiological studies. After MCB was orally administered to rats and mice (60 and 120 mg.kg⁻¹ bw) for 2 years in male rats a significant increase in the incidence of neoplastic noduli of the liver was found. No increased incidences of tumours were observed in female rats or male and female mice. On the basis

of these studies it is concluded that there is no evidence of carcinogenicity of MCB in experimental animals.

Similar studies were carried out with 1,2-DCB (60 or 120 mg.kg⁻¹ bw) and 1,4-DCB (150, 300 or 600 mg.kg⁻¹ bw) in mice and rats. In the study with 1,2-DCB a dose-related increase in malignant histiocytic lymphoma was observed in mice. The incidence of all types of malignant lymphomas, which was considered to be more relevant, was not increased. Therefore it was concluded that there was no evidence for carcinogenicity of 1,2-DCB in experimental animals. The "International Agency for Research on Cancer" (IARC) concluded that there was inadequate evidence of carcinogenicity of 1,2-DCB to animals (IARC, 1987b).

The carcinogenicity of 1,4-DCB was tested in mice and rats. Male rats were exposed to 0, 150 or 300 mg.kg⁻¹ bw of 1,4-DCB by gavage for 2 years, and female rats and male and female mice received doses of 0, 300 or 600 mg.kg⁻¹ bw. Male rats from the highest dose group had significantly increased incidences of renal carcinoma and adenoma. In male and female mice increased incidences were found of liver adenomas and carcinomas as well as of some types of pheochromocytoma. However, recent studies indicate that renal tumours in male rats develop as a result of alpha (2u)-globulin nephropathy. This protein is male rat specific and therefore the whole development of renal tumours is highly species and sex specific. Humans do not synthesize this protein. With respect to the hepatocellular tumours in mice it is emphasized that these types of neoplasm are common in this strain of mice (spontaneous incidence from 16% to 60% in males and from 2% to 20% in females). On the basis of these data it is concluded that there is limited evidence of carcinogenicity of 1,4-DCB in experimental animals.

The carcinogenicity of HCB was tested in (sub-)chronic oral studies with rats, mice and hamsters. Exposure of rats to HCB (≥ 4 mg.kg⁻¹ bw) resulted in increased incidences of hepatocellular carcinoma, renal cell adenoma and carcinoma and bile duct adenoma and carcinoma. The development of these tumours was clearly accompanied by the occurrence of toxicity in the target organs (increased organ weights, noduli and hyperplasia). In hamsters (exposed to 4-16 mg.kg⁻¹ bw), HCB mainly affected the liver; liver cirrhosis, hyperplasia of the bile duct and hepatomas. Mice exposed to 6-32 mg.kg⁻¹ bw during 2 years, showed an increased incidence of liver tumours. These HCB-induced tumours could not be explained by a specific mechanism, as

was the case with renal tumours in male rats exposed to 1,4-DCB, because HCB induced tumours in several organs of both males and females of several species. The development of tumours was clearly accompanied by toxicity in the target organs. On the basis of these studies it is concluded that there is sufficient evidence of carcinogenicity of HCB in experimental animals.

2 ECOTOXICITY I - AQUATIC ORGANISMS

2.1 ACCUMULATION

Bioaccumulation of the chlorobenzenes is determined by aqueous solubility (S), lipid solubility as indicated by the n-octanol/water partition coefficient (Kow) and by the number of chlorine atoms. Most experimental data on bioaccumulation concerned freshwater fish; table 2.1 gives an overview of reported "whole-body" or "lipid" bioconcentration factors (BCF's) for these organisms [*The BCF is the concentration in organisms divided by the concentration in water*]. From the data it can be concluded that all chlorobenzenes bioconcentrate in freshwater fish, with the tendency to increase with degree of chlorination (Neely et al., 1974, Könemann and Van Leeuwen, 1980, Kenaga, 1980, Mackay, 1982, Oliver and Niimi, 1983, Hoornstra, 1988, Van der Naald and Bruggeman, 1988). From a study conducted by Giam et al. (1980) it appeared that HCB also accumulates in marine fish (*Fundulus similis*), but to a lesser extent than freshwater fish; a BCF of 375 was determined. In a bioconcentration study the marine bivalve *Mytilus edulis* was exposed to PeCB for 21 days. Steady state was not completely reached, but at the end of this period the BCF was about 3,900 (Renberg et al., 1985).

The bioconcentration of a mixture of several chlorobenzenes (1,2-DCB, 1,3-DCB, 1,4-DCB, 1,3,5-TCB, 1,2,4-DCB, 1,2,3-TCB, 1,2,4,5-TeCB, 1,2,3,4-TeCB, PeCB and HCB and the chlorinated hydrocarbons hexachlorobutadiene and hexachloroethane) in rainbow trout were determined in a laboratory study. Exposure levels were either 'low' (ranging from 47 ng.l⁻¹ for 1,2-DCB to 0.32 ng.l⁻¹ for hexachlorobutadiene) or 'high' (ranging from 930 to 3.4 ng.l⁻¹ for the same substances). There was a high correlation between BCF's and Kow, except for HCB. Whole-body BCF's (high exposure group) were 560 for 1,2-DCB, 740 for 1,3-DCB, 720 for 1,4-DCB, 4,100 for 1,3,5-TCB, 3,200 for 1,2,4-TCB, 2,600 for 1,2,3-TCB, 13,000 for 1,2,4,5-TeCB, 12,000 for 1,2,3,4-TeCB and 20,000 for PeCB and HCB. BCF's in the 'low' exposure group were about a factor two lower. "Lipid"-BCF's were found by multiplying whole-body BCF's by 12 (Oliver and Niimi, 1983).

Larval stages of the midge *Chironomus decorus* were exposed to sediment-bound chlorobenzenes (MCB, 1,2-DCB, 1,2,4-TCB or HCB) in a flowthrough exposure system. Larvae were exposed to high- and low-organic content sediments. Experiments were carried out with water that contained no test chemical (nonequilibrium flow) or a concentration that was in equilibrium with that in the sediment. It appeared that the accumulation was mediated by the uptake of compounds from the interstitial water. BCF's calculated on the basis of interstitial water were comparable in all experiments (about 5 for MCB, 30 for 1,2-DCB, 200 for 1,2,4-TCB and 800 for HCB). Interstitial water BCF's highly correlated with K_{ow} -values. BCF's calculated on the basis of sediment were <1 in all experiments. BCF's based on the concentration in overlying water under equilibrium exposure conditions correlated also strongly with K_{ow} -values (Knezovich and Harrison, 1988).

The uptake of sediment-bound HCB by the deposit feeding clam *Macoma nasuta* was determined using a clam ventilation chamber (mass balance study). 10 possible uptake routes were studied. It appeared that uptake of HCB by the gut from ingested solids was the single most important route (accounting for 63% to 84% of HCB residues) (Boese et al., 1990). In an experiment with the deposit-feeding marine bivalve *Abra nitida* it was found that the bioaccumulation of HCB in a water-sediment system increased in the presence of suspended solids (Ekelund et al., 1987). The fish *Pimephales promelas*, the worm *Lumbriculus variegatus* and the amphidods *Hyaella azteca* and *Gammarus lacustris* were exposed to HCB in water with or without a bed of HCB-spiked sediment for about 28 days. The waterborn HCB concentration was similar in both tests. BCF's were significantly higher in aquaria without sediment in the *L. variegatus* test (25,000 versus 6,700) and in one of the two tests with *P. promelas* (94,000 versus 50,000). The BCF's for *H. azteca* and *G. lacustris* were similar in both test systems; about 23,000 and 42,000, respectively. In the other test with *P. promelas* the BCF was about 90,000. The sediment appeared to be a more efficient sink for HCB than the organisms (Schuytema et al., 1990).

The uptake and bioconcentration of 37 chemicals (including 1,2-, 1,3- and 1,4-DCB, 1,2,3-, 1,2,4- and 1,3,5-TCB, 1,2,3,4- and 1,2,4,5-TeCB, PeCB and HCB) from Lake Ontario sediments by worms has been studied in laboratory aquaria. A sediment sample (OM=4.6%) was collected from the lake and prepared for the experiment by adding the chemicals slowly over a period of

several days. After a few days the "contaminated" sediment was placed in aquaria and allowed to settle. The tanks were filled with filtered water from Lake Ontario. Then worms, mainly *Tubifex tubifex* and *Limnodrilus hoffmeisteri*, were added to the tanks and exposed for 79 days. BCF's (concentration in worm dry weight/concentration in sediment) were <1 for all chlorobenzenes except for PeCB and HCB, which were 1.9 and 3.1, respectively. BCF's expressed as the concentration in worms dry weight/ the concentration in interstitial water for PeCB and HCB were 19,000 and 24,000, respectively, very similar to those obtained with fish (Oliver, 1987).

In a model ecosystem five aquatic species were exposed to HCB to study bioaccumulation rates. HCB-treated soils (0.1, 1 or 10 mg.kg⁻¹) were placed in tanks which were then filled with water. After one day daphnids (*Daphnia magna*), snails (*Helisoma* sp.), a few strains of alga (*Oedogonium cardiacum*) and water containing diatoms, rotifers etc. were added. At day 30 some daphnids were taken out and two mosquito fish (*Gambusia affinis*) were added. Three days later all organisms were harvested and two catfish (*Ictalurus punctatus*) were added and exposed for 8 days. Mean whole-body BCF's were 740, 1500, 910, 1600 and 10,610 for algae, snails, daphnids, mosquito fish and catfish, respectively. In a similar experiment soil was used that had been treated with HCB a year before. The water concentration was higher and tissue concentrations lower, resulting in much lower BCF's; 570, 75, 120 and 400 for algae, snails, daphnids and fish, respectively (Isensee et al., 1976).

Quantitative structure activity relationships (QSAR's)

Many QSAR's have been established to relate the BCF's of (groups of chemicals including) chlorobenzenes to either the aqueous solubility (S) or the n-octanol/water partition coefficients (Kow). Könemann and Van Leeuwen (1980), for example, studied the accumulation and elimination of 6 chlorobenzenes in the fish *Poecilia reticulata*. BCF's on the basis of fat weight were calculated and related to Kow. Linear regression resulted in a good correlation when HCB was excluded from the calculations: $\log \text{BCF} = 0.980 \log \text{Kow} - 0.063$ ($r=0.991$). For compounds with Kow-values of about 6 (like HCB) a decrease in bioaccumulation is expected, caused by a sharp decrease

in magnitude of uptake rate constant beyond the optimum value (Könemann and Van Leeuwen, 1980). Other QSAR's based on fish data were, among others, calculated by Veith et al. (1979), Van der Naald and Bruggeman (1988) and Neely et al. (1974).

2.2 TOXICITY

INTRODUCTION

All tests were evaluated on the basis of the primary literature source and were conducted according to current guidelines for aquatic toxicity testing. MCB, DCB's and TCB's are highly volatile compounds. Therefore, tests with these compounds were only taken into account when a) a closed or continuous flow system was used, or b) toxicant concentrations were analyzed during the test or when c) both criteria were fulfilled. The following studies were thus left out of consideration: Abernethy et al. (1986), Bringmann and Kühn (1980), Dawson et al. (1977), Huthchinson et al. (1980), LeBlanc (1980), Millington et al. (1988) and Pickering and Henderson (1966) for freshwater organisms and Abernethy et al. (1986), Curtis et al. (1979), Dawson et al. (1977) and Heitmuller et al. (1981) for marine organisms.

Freshwater organisms short-term

Short-term toxicity tests with freshwater organisms resulting in reliable L(E)C50-values are summarized in table 2.3.

Additional information

After an acute intraperitoneal injection of $1,103 \text{ mg.kg}^{-1} \text{ bw}$ of MCB to *Salmo gairdneri* behavioural changes were observed as well as hepatotoxicity (Dalich et al., 1982). HCB was not acutely toxic to freshwater organisms at concentrations up to or exceeding the water solubility of the compound, appearing from tests with *Procambarus clarki* (Laska et al., 1978), the alga *Selenastrum capricornutum*, the water flea *Daphnia magna* and the fish *Salmo gairdneri* and *Brachydanio rerio* (Calamari et al., 1982). In

addition, exposure of the fish *Micropterus salmoides* up to 10 mg.l^{-1} of HCB for 15 days caused no toxic effects neither did intraperitoneal injections of HCB (125 mg.kg^{-1}) dissolved in peanut oil given to the fish *Fundulus grandis* or *P. clarki* (Laska et al., 1978).

Freshwater organisms long-term

Data on long-term toxicity tests with freshwater organisms resulting in NOL(E)C- and L(E)C50-values are summarized in table 2.4 and 2.5, respectively.

Additional information

The survival of two gram negative bacteria *Serratia liquefaciens* and *Pseudomonas aeruginosa* was affected at 300 and $800 \mu\text{g.ml}^{-1}$ of HCB (in a benzene-ethanol solution), respectively (Hamdy, 1988). Incubation of the alga *Chlorella pyrenoidosa* over a period of 46 h with HCB (0,001, 0,01, 0,1, 1 and 10 mg.l^{-1} in nutrient solution which contained acetone) led to a decrease of all growth parameters studied (chlorophyll content, total nitrogen etc.) in a dose-dependent way. After an incubation period of three months only the highest concentration (10 mg.l^{-1}) had a slight negative effect on algal growth measured as chlorophyll content, while 0.1 and 1 mg.l^{-1} had a significant stimulating effect (Geike and Parasher, 1976a). Incubation of the alga *Tetrahymena pyriformis* with HCB ($0.001\text{-}0.5 \text{ mg.l}^{-1}$) for 10 days decreased growth measured as dry weight, carbohydrates and total nitrogen (Geike and Parasher, 1976b). Exposure to a concentration of $5 \mu\text{g.l}^{-1}$ for 2 to 68 days under continuous flow conditions did not cause toxic effects in the cladoceran *Daphnia magna*, the amphipods *Hyaella azteca* and *Gammarus lacustris*, the worm *Lumbricus variegatus* and the fish *Pimephales promelas* (Nebeker et al., 1989). Long-term exposure (period not exactly given) to HCB at concentrations up to and exceeding its water solubility did also not cause toxicity in the crustacean *Procambarus clarki*, or in the fish *Poecilia latipinna*, *Fundulus grandis* and *Micropterus salmoides* (Laska et al., 1978).

The effect of 1,2,4-TCB on freshwater plankton was studied in an outdoor-model-ecosystem. A natural pond, rich in *Daphnia* and phytoplankton species, was divided into compartments. The investigation period covered two weeks and three weeks post-application phases. The mean initial (measured) concentration of 1,2,4-TCB was $215 \mu\text{g.l}^{-1}$ decreasing to less than $40\text{-}80 \mu\text{g.l}^{-1}$ within 20 days. With regard to phytoplankton no effects on diversity or abundance were observed. In contrast, 1,2,4-TCB was toxic to the daphnid-population. The mean number of daphnids from the treated compartments was less than 10% of the controls during post-application period. A regeneration phase seemed to begin at day 21, when the concentration was $50\text{-}100 \mu\text{g.l}^{-1}$ (Lay et al., 1985).

Marine organisms short- and longterm

Short- and long-term toxicity tests with marine organisms resulting in L(E)C50- or NOL(E)C-values are summarized in table 2.6.

Additional information - short-term toxicity

The effects of exposure to 1,4-DCB and TCB (isomer not given) on the embryonic development and survival/development of larvae of the clam *Mercenaria mercenaria* and the oyster *Crassostrea virginica* were studied. The test compounds (in acetone solution) were renewed every second day. The tests with TCB resulted in 48-h EC50-values (based on development of eggs) of 3.1 mg.l^{-1} and $>10 \text{ mg.l}^{-1}$ for *C. virginica* and *M. mercenaria*. The 12-d LC50-value (larvae survival) for TCB was reported to be $>10 \text{ mg.l}^{-1}$ for *M. mercenaria*. The tests with 1,2-DCB resulted in an 48-h LC50-value of $>100 \text{ mg.l}^{-1}$ and a 12-d LC50-value of $>100 \text{ mg.l}^{-1}$ for *M. mercenaria* (Davis and Hidu, 1969). The toxicity of HCB in seawater and sandy sediment (97% sand, 0.28% organic carbon) to the shrimp *Crangon septemspinosa* was determined in 96-h static tests. In both tests no mortality was found at the highest concentrations tested: $7.2 \mu\text{g.l}^{-1}$ in the water-test and 300 mg.kg^{-1} in the sediment-water test (McLeese and Metcalf, 1980).

Additional information - long-term toxicity

Exposure of marine phytoplankton (mixed laboratory cultures of a diatom *Thalassiosira pseudonana* and a green alga *Dunaliella tertiolecta*) to 50 or

100 $\mu\text{g.l}^{-1}$ of HCB (dissolved in acetone) for 3 days did not result in effects on algal growth or size of progeny (Biggs et al., 1979). Exposure of the alga *Skeletonema costatum* to MCB in a closed static system for 5-days resulted in a NOEC-value of 100 mg.l^{-1} (nominal), based on total cell count or total cell volume (Cowgill et al. 1989).

The toxicity of sediment-bound 1,2,4-TCB to the shrimp *Palaemonetes pugio* and the amphioxus *Branchiostoma caribaeum* was investigated. Sediments contaminated with 10,000 $\mu\text{g.kg}^{-1}$ were not lethal to *P. pugio* in both a static and a flow-through test. Exposure to 10,000 or 240,000 $\mu\text{g.kg}^{-1}$ of 1,2,4-TCB in sediment caused 0% and 100% mortality, respectively, to *B. caribaeum*. The 10-d LC50 was determined at 200,000 $\mu\text{g.kg}^{-1}$. It must be noted that 240,000 $\mu\text{g.kg}^{-1}$ TCB in sediment would yield a concentration of 13,000 $\mu\text{g.l}^{-1}$ in the overlying water, exceeding the lethal concentration for this organism (10,000 $\mu\text{g.l}^{-1}$) (Clark et al., 1987).

Quantitative structure activity relationships (QSAR's)

The toxicity of chlorobenzenes, especially the acute toxicity, correlates strongly with both the K_{ow} and the aqueous solubility (S) of the substances. QSAR's have been developed using toxicity data derived from experiments with algae (Wong et al., 1984), daphnids (Bobra et al., 1985) and fish (Könemann, 1980, 1981, Veith et al., 1983, Neely, 1984) (see table 2.2). A high quality QSAR might indicate that the working mechanism of the group of chemicals is similar. The lethal effect of the chlorobenzenes is probably caused by membrane perturbation and this seems to be a minimum effect: a hydrophobic substance is (within error) at least as toxic as is calculated from its QSAR, unless it is strongly metabolized (Könemann, 1981).

A QSAR-analysis was performed on substituted benzenes for which toxicity values have been obtained over 96-h in the fathead minnow *Pimephales promelas*. The additive toxicity of several substituent groups was determined. A decreasing contribution to toxicity was found to be $\text{Cl} > \text{Br} > \text{NO}_2 > \text{CH}_3 > \text{OCH}_3 > \text{NH}_2 > \text{OH}$. With regard to the chlorobenzenes as a separate group it was found that the toxicity increases with the number of chlorine atoms and that the position of the chlorine atom on the benzene ring does not have a significant influence on the toxicity (Hall et al., 1984).

Summary and conclusions "Aquatic organisms"

Accumulation

Most experimental data of bioaccumulation concern freshwater organisms (mainly fish); "whole body" BCF's [the concentration in organisms divided by the concentration in water] ranged from 12-450, 66-740, 700-4100, 2400-13000, 3400-20000 and 7880-75000 for MCB DCB's, TCB's, TeCB's, PeCB and HCB, respectively. It appeared that all chlorobenzenes bioaccumulate, with the tendency increasing with the number of chlorine atoms. Bioaccumulation is mainly determined by lipid solubility as indicated by the n-octanol/water partition coefficient (K_{ow}), which increases with the degree of chlorination. Many quantitative structure activity relationships (QSAR's) have been established to relate the BCF's of chlorobenzenes to either the water solubility or the lipid solubility.

Chlorobenzenes also accumulate in marine organisms (limited data), but to a lesser extent than freshwater organisms. A number of studies (worms and larvae of midges) indicate that the uptake of sediment-bound chlorobenzenes was mediated by uptake from interstitial water.

Toxicity to freshwater organisms

For most chlorobenzenes acute toxicity data (48 h L(E)C50-values) were available for algae, crustaceans and fish. The lowest L(E)50-values from "single species" tests were $660 \mu\text{g.l}^{-1}$ for MCB (a short-term "early life stage" test with fish), $700 \mu\text{g.l}^{-1}$ for DCB's, $350 \mu\text{g.l}^{-1}$ for TCB's, $860 \mu\text{g.l}^{-1}$ for TeCB's, $250 \mu\text{g.l}^{-1}$ for PeCB and $<30 \mu\text{g.l}^{-1}$ for HCB. In some studies with higher chlorinated chlorobenzenes (1,2,3,5- and 1,2,4,5-TeCB, PeCB and HCB) L(E)C50-values were reported at concentrations up to or exceeding the water solubility. The relevance of these values, which were obtained using solvents, is debatable.

With respect to chronic toxicity data are rather limited. In most cases NOE(L)C-values were only available for crustaceans (mainly *Daphnia magna*) and fish (various species). Data on the toxicity of chlorobenzenes to developmental stages (embryo, larval, juvenile) of fish were reported for all (groups of) isomers. The lowest NOE(L)C-values were $320 \mu\text{g.l}^{-1}$ for MCB, (\geq) $122 \mu\text{g.l}^{-1}$ for DCB's, $40 \mu\text{g.l}^{-1}$ for TCB's, $10 \mu\text{g.l}^{-1}$ for TeCB's, $10 \mu\text{g.l}^{-1}$ for PeCB and $1.8 \mu\text{g.l}^{-1}$ for HCB. These NOEC-values show an

increasing toxicity with an increase in degree of chlorination. Toxicity is also (similar to bioconcentration) mainly determined by lipid solubility (K_{ow}). QSAR's have been established to relate the, especially acute, toxicity of chlorobenzenes to either the water solubility or the lipid solubility (see table 2.2).

Toxicity to marine organisms

Data on the toxicity of chlorobenzenes to marine organisms are very limited, especially with regard to chronic toxicity. The lowest 48/96 h L(E)C50-values were $540 \mu\text{g.l}^{-1}$ for 1,2,4-TCB, $3700 \mu\text{g.l}^{-1}$ for 1,2,3,5-TeCB, $330 \mu\text{g.l}^{-1}$ for 1,2,4,5-TeCB and $800 \mu\text{g.l}^{-1}$ for PeCB. Only one long-term study (fish) was available, which resulted in a NOLC of $90 \mu\text{g.l}^{-1}$.

Table 2.1 Overview of "whole-body" or "lipid" BCF's for freshwater organisms

Chlorobenzene	Species	BCF (2)	Reference(s)
MCB	fish	12-450	Kenaga '80; EPA, '84
1,2-DCB	fish	89-560	Barrows et al. '80; Oliver & Niimi '83
1,3-DCB	fish	66-740	Barrows et al. '80; Carlson & Kosian '87; Oliver & Niimi '83
1,4-DCB	fish	15-720	Barrows et al. '80; Neely et al. '74 Calamari et al. '82; Kenaga '80; Carlson & Kosian '87; EPA '84; Oliver & Niimi '83
1,2,3-TCB	fish	1,800 *	Könemann '80
	fish	700-2,600	Könemann & van Leeuwen '80
1,2,4-TCB	fish	13,000 *	Könemann '80
	fish	182-3,200	Barrows et al. '80; Veith et al. '79; EPA '84; Oliver & Niimi '83
1,3,5-TCB	fish	760-4,100	Könemann & van Leeuwen '80; Oliver & Niimi '83
1,2,3,4-TeCB	fish	14,000 *	Könemann '80
	fish	2,400-12,000	Carlson & Kosian '87; EPA '84; Oliver & Niimi '83
1,2,3,5-TeCB	fish	1,800-3,900	Barrows et al. '80; Konemann & van Leeuwen '80
1,2,4,5-TeCB	fish	72,000 *	Könemann '80
	fish	4,000-13,000	Kenaga '80; EPA '84; Oliver & Niimi '83
PeCB	fish	3,400-20,000	Barrows et al. '80; Kenaga '80; Renberg et al. '85; Carlson & Kosian '87 Könemann & van Leeuwen '80; Oliver & Niimi '83
HCB	fish	260,000 *	Könemann '80
	fish	7,880-22,000	Neely et al. '74; Kenaga '80; Veith et al. '79; Carlson & Kosian '87; Nebeker et al. '89; Oliver & Niimi '83
	crus.	13,200-75,000	Nebeker et al. '89
	fish	354,000-6000,000 *	
	fish	177,000-306,000 *	Nebeker et al. '89; Könemann '80

* = "lipid"-BCF

(2) Whole body BCF, under flow-through conditions.

Table 2.2 Overview of a selected number of quantitative structure activity relationships
Toxicity - physical/chemical factors

Species or test-system	(group) of chemicals	QSAR	Reference
Acute toxicity			
Ankistrodesmus falcatus	benzene and 12 chlorobenzenes	$\log 1/EC50 = -0.587 \log S + 2.419$ $\log 1/EC50 = 0.985 \log K_{ow} - 2.626$ ($r=0.985$)	* Wong et al. '84
Daphnia magna	6 chlorobenzenes	$\log EC50 = -0.301 - 0.548 \log S$ ($r=0.989$) $\log EC50 = 3.55 - 0.659 \log K_{ow}$ ($r=0.981$)	Bobra et al. '85
Poecilia reticulata 2-3 months old	benzene and 12 chlorobenzenes	$\log 1/LC50 = 0.845 \log K_{ow} - 4.63$ ($r=0.980$)	** Könemann '80
idem	benzene and 12 chlorobenzenes	$\log LC50 = 0.85 \log K_{ow} - 1.37$ ($r=0.980$)	Hoornstra '88
Daphnia magna	chlorobenzenes	$\log 1/LC50 = 0.90 \log K_{ow} - 2.65$ ($r=0.924$)	Richter et al. '83
Chronic toxicity			
Daphnia magna	4 chlorobenzenes and 4-chlorotoluene	$\log 1/NOEC_r = 0.67 \log K_{ow} - 2.82$ ($r=0.995$)	De Wolf et al. '88
Branchydanio rerio Pimephales promelas	4 chlorobenzenes and 4-chlorotoluene	$\log 1/NOEC = 0.67 \log K_{ow} - 2.82$ ($r=0.995$)	Van Leeuwen et al. '90

r = regression coefficient

NOEC_r = no-effect-concentration based on reproduction (in $\mu\text{mol/l}$)

NOEC_g = no-effect-concentration based on growth (in $\mu\text{mol/l}$)

* HCB was left out of these equations because of its low toxicity.

** The validity of this QSAR ends at $\log K_{ow} = \pm 6$. This can be caused by the very slight solubility of very hydrophobic substances and by possible theoretical deviation from linearity of the QSAR at $\log K_{ow} > 6$ (Könemann, 1980).

Table 2.3 Freshwater organisms - short-term toxicity tests with chlorobenzenes: L(E)C50-values

Organism	A	Test- type	Test sub. purity	Test- water.	pH	Hardness mg CaCO ₃ /l	Exp.- time	Crite- rion	Result µg/l	Reference
Monochlorobenzene										
Algae										
Selenastrum capricornutum	+	c-S	ag	s.w.	-	--	3-h	EC50	33,000 [1]	Calamari et al. '83
Ankistrodesmus falcatus	-	c-S	--	n.m.	8	--	4-h	EC50 _g	49,980	Wong et al. '84
Crustaceans										
Daphnia magna	+	c-S	ag	s.w.	-	--	24-h	EC50 _i	4,300 [1]	Calamari et al. '83
Daphnia magna	-	c-S	--	lake	8.0	160	48-h	LC50	13,000	Cowgill et al. '85
Daphnia magna	-	c-S	≥97%	s.w.	-	--	48-h	EC50 _i	5,810	Abernethy et al. '86
Ceriodaphnia dubia/affinis	-	c-S	--	lake	8.0	90	48-h	LC50	7,900	Cowgill et al. '85
Fish										
Branchydanio rerio	+	c-S	ag	s.w.	7.4	320	48-h	LC50	10,500 [1]	Calamari et al. '83
Salmo gairdneri	+	c-S	ag	s.w.	7.4	320	48-h	LC50	4,100 [1]	Calamari et al. '83
Lepomis macrochirus	-	c-S	≥80%	r.w.	6.7-7.8	32-48	96-h	LC50	16,000 [4]	Buccafusco et al. '81
Pimephales promelas (fry)	-	c-S	rg	s.w.	7.6-8.3	96-125	96-h	LC50	22,300	Mayes et al. '83
Carassius auratus egg <1-d old --> hatching	+	c-CF	--	r.w.	7.3-8.1	200	3.5-d	LC50	4,080 α	Birge et al. '79
Micropterus salmoides egg <1-d old --> hatching	+	c-CF	--	r.w.	7.3-8.1	50	3.5-d	LC50	660 α	Birge et al. '79
1,2 - dichlorobenzene										
Algae										
Selenastrum capricornutum	+	c-S	ag	s.w.	-	--	3-h	EC50	2,200 [1]	Calamari et al. '83
Scenedesmus pannonicus	+	c-S	99.9	s.w.	-	--	24-h	EC50	17,000 α	Canton et al. '85
Ankistrodesmus falcatus	-	c-S	--	n.m.	8	--	4-h	EC50 _g	20,000	Wong et al. '84
Crustaceans										
Daphnia magna	+	c-S	ag	s.w.	-	--	24-h	EC50 _i	780 [1]	Calamari et al. '83
Daphnia magna	-	c-S	≥97%	s.w.	-	--	48-h	EC50 _i	2,350	Abernethy et al. '86
Daphnia magna	+	c-S	99.9	s.w.	-	--	48-h	EC50 _i	740 α	Canton et al. '85
								LC50 _i	2,200 α	Canton et al. '85
Daphnia magna	-	c-S	--	tap	7.6-7.7	16	24-h	EC50	1,700	Kühn et al. '89
Fish										
Salmo gairdneri	+	c-S	ag	s.w.	7.4	320	48-h	LC50	10,000 [1]	Calamari et al. '83
Branchydanio rerio	+	c-S	ag	s.w.	7.4	320	48-h	LC50	6,800 [1]	Calamari et al. '83
Lepomis macrochirus	-	c-S	≥80%	r.w.	6.7-7.8	32-48	96-h	LC50	5,600 [4]	Buccafusco et al. '81
1,3 - dichlorobenzene										
Algae										
Scenedesmus pannonicus	+	c-S	99.4	s.w.	-	--	24-h	EC50	31,000 α	Canton et al. '85
Ankistrodesmus falcatus	-	c-S	--	n.m.	8	--	4-h	EC50 _g	22,930	Wong et al. '84

Table 2.3 Freshwater organisms - short-term toxicity tests with chlorobenzenes: L(E)C50-values (continued)

Organism	A	Test-type	Test sub. purity	Test water.	pH	Hardness mg CaCO ₃ /l	Exp.-time	Crite- rion	Result µg/l	Reference
Crustaceans										
Daphnia magna	+	S	98%	lake	7.1-7.7	45	48-h	LC50	7,400	α Richter et al. '83
								EC50 _i	4,200	α Richter et al. '83
Daphnia magna	+	c-S	99.4%	s.w.	-	--	48-h	EC50 _i	1,200	α Canton et al. '85
							48-h	LC50	6,800	α Canton et al. '85
Daphnia magna	-	c-S	--	tap	7.6-7.7	16	24-h	EC50	7,000	Kühn et al. '89
Fish										
Pimephales promelas (30-d old)	+	CF	98%	lake	7.3-7.6	45	96-h	LC50	7,800	α Carlson & Kosian '87
Pimephales promelas	+	CF	--	lake	7.5	42-46	96-h	LC50	7,800	Veith et al. '83
Lepomis macrochirus	-	c-S	≥80%	r.w.	6.7-7.8	32-48	96-h	LC50	5,000 [4]	Buccafusco et al. '81
1,4 - dichlorobenzene										
Algae										
Selenastrum capricornutum	+	c-S	ag	s.w.	-	--	3-h	EC50 _g	5,200 [1]	Calamari et al. '82 Calamari et al. '83
Scenedesmus pannonicus	+	c-S	99.7	s.w.	-	--	24-h	EC50	31,000	α Canton et al. '85
Ankistrodesmus falcatus	-	c-S	--	n.m.	8	--	4-h	EC50 _g	20,000	Wong et al. '84
Crustaceans										
Daphnia magna	+	c-S	ag	s.w.	-	--	24-h	EC50 _i	1,600 [1]	Calamari et al. '82 Calamari et al. '83
Daphnia magna	+	c-S	99.4%	s.w.	-	--	48-h	EC50 _i	700	α Canton et al. '85
							48-h	LC50	2,200	α Canton et al. '85
Daphnia magna	-	c-S	--	tap	7.6-7.7	16	24-h	EC50	3,200	Kühn et al. '89
Fish										
Salmo gairdneri	+	c-S	ag	s.w.	7.4	320	48-h	LC50	1,180 [1]	Calamari et al. '83
Branchydanio rerio	+	c-S	ag	s.w.	7.4	320	48-h	LC50	4,250 [1]	Calamari et al. '83
Pimephales promelas (30-d old)	+	CF	97%	lake	7.3-7.6	45	96-h	LC50	4,200	α Carlson & Kosian '87
Pimephales promelas	+	S	--	r.s.w.	7.2-7.9	40-48	96-h	LC50	2,400	α Curtis et al. '79 [5] Curtis & Ward '81
Pimephales promelas	+	CF	--	lake	7.5	42-46	96-h	LC50	4,000	Veith et al. '83
Pimephales promelas (fry)	-	c-S	rg	s.w.	7.6-8.3	96-125	96-h	LC50	3,600	Mayes et al. '83
Lepomis macrochirus	-	c-S	≥80%	r.w.	6.7-7.8	32-48	96-h	LC50	4,300 [4]	Buccafusco et al. '81
1,2,3 - trichlorobenzene										
Algae										
Selenastrum capricornutum	+	c-S	ag	s.w.	-	--	3-h	EC50	2,200 [1]	Calamari et al. '83
Ankistrodesmus falcatus	-	c-S	--	n.m.	8	--	4-h	EC50 _g	5,990	Wong et al. '84

Table 2.3 Freshwater organisms - short-term toxicity tests with chlorobenzenes: L(E)C50-values (continued)

Organism	A	Test- type	Test sub. purity	Test- water.	pH	Hardness mg CaCO ₃ /l	Exp.- time	Crite- rion	Result µg/l	Reference
Crustaceans										
Daphnia magna	+	c-S	ag	s.w.	-	--	24-h	EC50 _i	350 [1]	Calamari et al. '83
Daphnia magna	-	c-S	>97%	s.w.	6-7	--	48-h	EC50 _i	2,720	Bobra et al. '83
Daphnia magna	-	c-S	≥97%	s.w.	-	--	48-h	EC50 _i	1,450	Abernethy et al. '86
Fish										
Salmo gairdneri	+	c-S	ag	s.w.	7.4	320	48-h	LC50	710 [1]	Calamari et al. '83
Branchydanio rerio	+	c-S	ag	s.w.	7.4	320	48-h	LC50	3,100 [1]	Calamari et al. '83
<u>1,2,4 - trichlorobenzene</u>										
Algae										
Selenastrum capricornutum	+	c-S	ag	s.w.	-	--	3-h	EC50	3,900 [1]	Calamari et al. '83
Ankistrodesmus falcatus	-	c-S	--	n.m.	8	--	4-h	EC50 _g	5,990	Wong et al. '84
Crustaceans										
Daphnia magna	+	c-S	ag	s.w.	-	--	24-h	EC50 _i	1,200 [1]	Calamari et al. '83
Daphnia magna	+	S	--	lake	7.1-7.7	45	48-h	LC50	2,100 α	Richter et al. '83
Fish										
Pimephales promelas (30-d old)	+	CF	--	lake	7.3-7.6	45	96-h	LC50	2,800 α	Carlson & Kosian '87
Pimephales promelas	+	CF	--	lake	7.5	42-46	96-h	LC50	2,900	Veith et al. '83
Salmo gairdneri	+	c-S	ag	s.w.	7.4	320	48-h	LC50	1,950 [1]	Calamari et al. '83
Branchydanio rerio	+	c-S	ag	s.w.	7.4	320	48-h	LC50	6,300 [1]	Calamari et al. '83
Lepomis macrochirus	-	c-S	≥80%	r.w.	6.7-7.8	32-48	96-h	LC50	3,400 [4]	Buccafusco et al. '81
<u>1,3,5-trichlorobenzene</u>										
Algae										
Ankistrodesmus falcatus	-	c-S	--	n.m.	8	--	4-h	EC50 _g	9,070	Wong et al. '84
<u>1,2,3,4 - tetrachlorobenzene</u>										
Algae										
Ankistrodesmus falcatus	-	c-S	--	n.m.	8	--	4-h	EC50 _g	4,100	Wong et al. '84
Fish										
Pimephales promelas (30-d old)	+	CF	99%	lake	7.3-7.6	45	96-h	LC50	1,100 α	Carlson & Kosian '87
Pimephales promelas	+	CF	--	lake	7.5	42-46	96-h	LC50	1,100	Veith et al. '83
<u>1,2,3,5 - tetrachlorobenzene</u>										
Algae										
Chlamydomonas angulosa	-	S	--	n.m.	6.5	--	3-h	EC50	1,580	Hutchinson et al. '80

Table 2.3 Freshwater organisms - short-term toxicity tests with chlorobenzenes: L(E)C50-values (continued)

Organism	A	Test-type	Test sub. purity	Test water.	pH	Hardness mg CaCO ₃ /l	Exp.-time	Crite- rion	Result µg/l	Reference
Chlorella vulgaris	-	S	--	n.m.	6.5	--	3-h	EC50	2,500	Hutchinson et al. '80
Ankistrodesmus falcatus	-	c-S	--	n.m.	8	--	4-h	EC50 _g	3,022	Wong et al. '84
Crustaceans										
Daphnia magna < 1-d old	-	S	≥80%	r.w.w.	7.4-9.4	173	48-h	LC50	9,700 [3]	LeBlanc '80
Daphnia magna	-	c-S	>97%	s.w.	6-7	--	48-h	EC50 _i	1,730	Bobra et al. '83
Daphnia magna	-	c-S	>97%	s.w.	-	--	48-h	EC50 _i	860	Abernethy et al. '86
Fish										
Lepomis macrochirus	-	c-S	≥80%	r.w.	6.7-7.8	32-48	96-h	LC50	6,400 [4]	Buccafusco et al. '81
<u>1,2,4,5 - tetrachlorobenzene</u>										
Algae										
Ankistrodesmus falcatus	-	c-S	--	n.m.	8	--	4-h	EC50 _g	4,965	Wong et al. '84
Crustaceans										
Daphnia magna < 1-d old	-	S	≥80%	r.w.w.	7.4-9.4	173	48-h	LC50	>530,000 [3]	LeBlanc '80
Fish										
Salmo gairdneri early fry	-	R	97%	s.w.	7.2	50	96-h	LC50	1,200 [2]	Van Leeuwen et al. '85
Lepomis macrochirus	-	c-S	≥80%	r.w.	6.7-7.8	32-48	96-h	LC50	1,600 [4]	Buccafusco et al. '81
<u>pentachlorobenzene</u>										
Algae										
Ankistrodesmus falcatus	-	c-S	--	n.m.	8	--	4-h	EC50 _g	1,250	Wong et al. '84
Crustaceans										
Daphnia magna <24-h old	-	S	≥80%	r.w.w.	7.4-9.4	173	48-h	LC50	5,300 [3]	LeBlanc '80
Daphnia magna	-	c-S	>97%	s.w.	6-7	--	48-h	EC50 _i	1,250	Bobra et al. '83
Daphnia magna	-	S	≥97%	s.w.	-	--	48-h	EC50 _i	300	Abernethy et al. '86
Fish										
Lepomis macrochirus	-	c-S	≥80%	r.w.	6.7-7.8	32-48	96-h	LC50	250 [4]	Buccafusco et al. '81
<u>hexachlorobenzene</u>										
Algae										
Selenastrum capricornutum	+	c-S	ag	s.w.	-	--	3-h 96-h	EC50 EC50 _g	30 [1] <30 [1]	Calamari et al. '83 Calamari et al. '83

Table 2.3 Freshwater organisms - short-term toxicity tests with chlorobenzenes: L(E)C50-values (continued)

Organism	A	Test- type	Test sub. purity	Test- water.	pH	Hardness mg CaCO ₃ /l	Exp.- time	Crite- rion	Result µg/l	Reference
Crustaceans										
Daphnia magna	+	c-S	ag	s.w.	-	--	24-h	EC50 _i	<30 [1]	Calamari et al. '83
Fish										
Salmo gairdneri	+	c-S	ag	s.w.	7.4	320	48-h	LC50	<30 [1]	Calamari et al. '83
Branchydanio rerio	+	c-S	ag	s.w.	7.4	320	48-h	LC50	<30 [1]	Calamari et al. '83

rg = reagent-grade quality; ag = analytical grade quality; n.m. = nutrient medium; s.w. = standard water; r.w. = reconstituted water; w.w. = well water; r.w.w. = reconstituted well water; r.s.w. = reconstituted soft water; i = immobility; g = growth.

For explanation, see "list of abbreviations tables 2.3 to 2.6".

- [1] For all chlorobenzenes tested the loss of compound did not exceed 10% of the initial value, except for the Daphnia test in which it did not exceed 15%.
- [2] Tests were performed with life stages from 0-77 days; egg 0-h, egg 24-h, early eyed egg 14-d, late eyed egg 28-d, sac fry 42-d and early fry 77-d. The early fry stage appeared to be the most critical stage, resulting in a 96-h LC50-value of 1,200 µg/l. For all other egg stages the 96-h LC50-values were 10,000 µg/l.
- [3] The range of pH-values is based on all tests that were conducted (including those on other compounds). The vessels with test solutions were covered with a plastic wrap secured with an elastic band.
- [4] The range of pH-values is based on all tests that were conducted (including those on other compounds). The range of dissolved oxygen concentration was 9.7 (at start) to 0.3 (after 96-h) mg/l. The acute toxicity of some chemicals was tested above their water solubility. Therefore in the tests with MCB, 1,3-DCB and 1,2,4,5-TeCB undissolved chemical was present in the test solution, whereas the test solution of 1,4-DCB, 1,2,3,5-TeCB, 1,2,4,5-TeCB and PeCB contained precipitates. The vessels were "capped" in an effort to control volatilization.
- [5] On the basis of the same test Curtis and Ward (1981) reported 96-h LC50-values based on nominal concentrations of 57 and 30 mg/l for 1,2-DCB and 1,4-DCB, respectively. The concentrations of 1,2-DCB were not measured, therefore no reliable LC50-value can be given.

Table 2.4 Freshwater organisms - long-term toxicity tests with chlorobenzenes: NOE(L)C-values

Organism	A	Test- type	Test- subst. purity	Test- water.	pH	Hardness mg CaCO ₃ /l	Exp.- time	Crite- rion	Result µg/l	Reference
monochlorobenzene										
Crustaceans										
Daphnia magna	+	R	--	s.w.	-	--	16-d	NOEC _r NOLC _r	320 1,000	[4] Hermens et al. '84 Hermens et al. '84
Daphnia magna	+	R	--	s.w.	-	--	16-d	NOEC NOEC _g	320 1,000	[6] De Wolf et al. '88 De Wolf et al. '88
Daphnia magna	+	c-R	ag	s.w.	-	--	2-w	NOEC _r	1,050	[8] Calamari et al. '83
Fish										
Salmo gairdneri	-	CF	--	w.w.	-	--	30-d	NOLC	2,900	[9] Dalich et al. '82
Branchydanio rerio egg <1-d old --> fry	+	R	>99	r.w.	7.4-8.4	210	28-d	NOEC NOLC _g	4,800 α 8,500 α	Van Leeuwen et al. '90 Van Leeuwen et al. '90
1,2-dichlorobenzene										
Crustaceans										
Daphnia magna	+	c-R	--	tap	6.6-7.6	16	21-d	NOEC _r NOLC _r	505 α 505 α	[9] Kühn et al. '89 Kühn et al. '89
Daphnia magna	+	c-R _d	ag	s.w.	-	--	2-w	NOEC _r	185	[8] Calamari et al. '83
1,3-dichlorobenzene										
Crustaceans										
Daphnia magna	+	R	95-99	lake	6.6-7.9	45	28-d	NOEC _r NOEC _g	690 α 690 α	Richter et al. '83 Richter et al. '83
Daphnia magna	+	c-R	--	tap	6.6-7.6	16	21-d	NOEC _g	650 α	[9] Kühn et al. '89
Daphnia magna	+	CF	98%	lake	7.4-6.9	42	28-d	NOEC _r	690	Call et al. '83
Fish										
Pimephales promelas embryo->early juvenile	+	CF	98%	lake	7.3-7.6	44-46	32-d	NOEC NOLC _g	1,000 α 2,400 α	Carlson & Kosian '87 Carlson & Kosian '87
1,4-dichlorobenzene										
Algae										
Selenastrum capricornutum	+	c-S	ag	s.w.	-	--	96-h	NOEC _g	570	Calamari et al. '83
Crustaceans										
Daphnia magna	+	c-R	--	tap	6.6-7.6	16	21-d	NOEC	400 α	[9] Kühn et al. '89
Daphnia magna	+	c-R	ag	s.w.	-	--	8-d	NOEC _r	220	Calamari et al. '82
Daphnia magna	+	c-R	ag	s.w.	-	--	2-w	NOEC _r	320	[8] Calamari et al. '83
Fish										
Pimephales promelas embryo --> early juvenile	+	CF	97%	lake	7.3-7.6	44-4	32-d	NOEC NOLC _g	570 α 570 α	Carlson & Kosian '87 Carlson & Kosian '87

Table 2.4 Freshwater organisms - long-term toxicity tests with chlorobenzenes: NOE(L)C-values (continued)

Organism	A	Test-type	Test-subst. purity	Test-water.	pH	Hardness mg CaCO ₃ /l	Exp.-time	Crite- rion	Result µg/l	Reference
Salmo gardneri eggs --> alevin	+	c-CF	ag	s.w.	-	--	60-d	NOEC _{m,h}	≥122	[3] Calamari et al. '82
Branchydanio rerio eggs <1-d old --> fry	+	R	99%	r.w.	7.4-8.4	210	28-d	NOEC _g NOLC _r	650 α 2,100 α	Van Leeuwen et al.'90 Van Leeuwen et al.'90
<u>1,2,3-trichlorobenzene</u>										
Algae										
Selenastrum capricornutum	+	c-S	ag	s.w.	-	--	96-h	NOEC _g	110	Calamari et al. '83
Crustaceans										
Daphnia magna	+	c-R	ag	s.w.	-	--	2-w	NOEC _r	40	[8] Calamari et al. '83
Fish										
Branchydanio rerio eggs <1-d old --> fry	+	R	99%	r.w.	7.4-8.4	210	28-d	NOEC _g NOLC _r	250 α 450 α	Van Leeuwen et al.'90 Van Leeuwen et al.'90
<u>1,2,4-trichlorobenzene</u>										
Algae										
Selenastrum capricornutum	+	c-S	--	s.w.	-	--	96-h	NOEC _g	190	Calamari et al. '83
Crustaceans										
Daphnia magna	+	R	95-99%	lake	6.6-7.9	45	28-d	NOEC _r NOEC _r	360 α 360 α	Richter et al.'83 Richter et al.'83
Daphnia magna	+	c-R	ag	s.w.	-	--	28-d	NOEC _g	190	Calamari et al.'83
Daphnia magna	+	R	--	s.w.	-	--	16-d	NOEC _r NOLC _r	100 320	[4] Hermens et al. '84 Hermens et al. '84
Daphnia magna	+	R	--	s.w.	-	--	16-d	NOEC _g NOEC _g	100 181	[6] De Wolf et al. '88 De Wolf et al. '88
Daphnia magna	+	CF	99%	lake	7.3-6.6	45	28-d	NOEC _r	360	Call et al. '83
Daphnia magna	+	c-R	ag	s.w.	-	--	2-w	NOEC _r	160	[8] Calamari et al. '83
Fish										
Pimephales promelas embryo --> early juvenile	+	CF	--	lake	7.3-7.6	44-46	32-d	NOEC _g	500 α	Carlson & Kosian '87
<u>1,2,3,4-tetrachlorobenzene</u>										
Crustaceans										
Daphnia magna	+	R	98	s.w.	-	--	16-d	NOEC _r NOLC _r	10 100	[4] Hermens et al. '84 Hermens et al. '84
Daphnia magna	+	R	--	s.w.	-	--	16-d	NOEC _g NOEC _r	55 55	[6] De Wolf et al. '88 De Wolf et al. '88

Table 2.4 Freshwater organisms - long-term toxicity tests with chlorobenzenes: NOE(L)C-values (continued)

Organism	A	Test- type	Test- subst. purity	Test- water.	pH	Hardness mg CaCO ₃ /l	Exp.- time	Crite- rion	Result µg/l	Reference
Fish										
Pimephales promelas embryo --> early juvenile	+	CF	99%	lake	7.3-7.6	44-46	33-d	NOEC NOLC ^g	250 α 250 α	Carlson & Kosian '87 Carlson & Kosian '87
Branchydanio rerio eggs <1-d old --> fry	+	R	--	r.w.	7.4-8.4	210	28-d	NOEC NOLC ^g	100 α 310 α	Van Leeuwen et al.'90 Van Leeuwen et al.'90
<u>pentachlorobenzene</u>										
Crustaceans										
Daphnia magna P < 1-d old	-	R	≥98%	lake	8.1	225	3-w	NOEC _g NOLC NOEC	56 100 100	Van Leeuwen et al.'87 Van Leeuwen et al.'87 Van Leeuwen et al.'87
Daphnia magna	+	R	--	s.w.	-	--	16-d	NOEC ^r NOEC ^g	31 31	[6] De Wolf et al. '88 De Wolf et al. '88
Daphnia magna	+	R	--	s.w.	-	--	16-d	NOEC ^r NOLC ^r	10 100	[4] Hermens et al. '84 Hermens et al. '84
Daphnia magna exponentially growing populations	+	CF	≥98%	lake	8.1	225	2-w	NOEC _y	50 α	[2] Van Leeuwen et al. '87
Fish										
Pimephales promelas embryo-early juvenile	+	CF	98%	lake	7.3-7.6	44-46	31-d	NOEC NOLC ^g	≥55 α ≥55 α	[1] Carlson & Kosian '87 Carlson & Kosian '87
Branchydanio rerio eggs < 1-d old --> fry	+	R	98	r.w.	7.4-8.4	210	28-d	NOEC NOLC ^g	34 α 110 α	Van Leeuwen et al.'90 Van Leeuwen et al.'90
<u>hexachlorobenzene</u>										
Algae										
Selenastrum capricornutum	+	c-S	ag	s.w.	-	--	96-h	NOEC _g	14	[7] Calamari et al. '83
Worms										
Lumbriculus variegatus	+	CF	--	w.w.	7.0	25-35	49-d	NOEC NOEC ^g NOLC ^r	≥4.7 ≥4.7 ≥4.7	Nebeker et al. '89 Nebeker et al. '89 Nebeker et al. '89
Crustaceans										
Daphnia magna	+	CF	--	w.w.	7.0	25-25	7-d	NOLC	≥5	Nebeker et al. '89
Gammarus lacustris	+	CF	--	w.w.	7.0	25-35	28-d	NOLC	1.8	Nebeker et al. '89
Hyaella azteca	+	CF	--	w.w.	7.0	25-35	30-d	NOEC ^r NOEC ^g NOLC ^g	≥4.7 ≥4.7 ≥4.7	Nebeker et al. '89 Nebeker et al. '89 Nebeker et al. '89
Fish										
Pimephales promelas embryo --> early juvenile	+	CF	97%	lake	7.3-7.6	44-46	32-d	NOEC NOLC ^g	≥4.8 α ≥4.8 α	[1] Carlson & Kosian '87 Carlson & Kosian '87
Pimephales promelas	+	CF	--	w.w.	7.0	25-35	28-d	NOEC NOLC ^g	≥3.8 ≥3.8	Nebeker et al. '89 Nebeker et al. '89

n.m. = nutrient medium; s.w. = standard water; w.w. = well water. g = growth; r = reproduction; m = mortality; h = histopathological changes; d = development; y = yield.

For explanation, see "list of abbreviations tables 2.3 to 2.6".

- [1] Non-toxic at highest mean concentration that could be maintained in the test chambers.
- [2] At 50 $\mu\text{g/l}$ the yield (mean number of daphnids) was reduced 10%; the calculated EC50 was 120 $\mu\text{g/l}$.
- [3] Concentrations ranging from 1.8 to 122 $\mu\text{g/l}$ were tested. Neither macroscopic malformations nor histological changes were found at the time of hatching. The cumulative mortality of in treated groups was not increased as compared to the control groups.
- [4] The decrease in concentrations during the tests, till renewing the solutions, were reported to be maximal 20%.
- [5] Fish exposed to 2.1 and 2.9 mg/l did not show mortality, but changes in behaviour and liver toxicity (elevated GTP activity).
- [6] The average decrease in concentration during the tests was 9%, with a maximum of 26%.
- [7] A slight inhibition (12% in respect to control cultures) was seen at the maximum tested concentration; about 27 $\mu\text{g/l}$ (90% of the solubility). The NOEC has been estimated using a factor 2.
- [8] A 16% reproductive impairment was reported at 2,100 $\mu\text{g/l}$ for MCB, 370 $\mu\text{g/l}$ for 1,2-DCB, 640 $\mu\text{g/l}$ for 1,4-DCB, 80 $\mu\text{g/l}$ for 1,2,3-TCB and 320 $\mu\text{g/l}$ for 1,2,4-TCB. The NOEC-values have been calculated from these EC16-values using a factor 2.
- [9] The given NOEC-value is an average between the nominal NOEC-value and the "minimal" NOEC-value, representing the lowest analysed concentration obtained during the test.

Table 2.5 Freshwater organisms - long-term toxicity tests with chlorobenzenes: L(E)C50-values

Organism	A	Test- type	Test- subst. purity	Test- water.	pH	Hardness mg CaCO ₃ /l	Exp.- time	Crite- rion	Result µg/l	Reference
Monochlorobenzene										
Algae										
Selenastrum capricornutum	+	c-S	ag	s.w.	-	--	96-h	EC50 _g	12,500	[1] Calamari et al. '83 Galassi & Vighi '81
Crustaceans										
Daphnia magna	+	c-R	--	s.w.	-	--	14-d	EC50 _r	2,500	Calamari et al. '83
Daphnia magna	+	R	--	s.w.	-	--	16-d	LC50 _r	4,000	[3] Hermens et al. '84
							16-d	EC50 _r	1,100	Hermens et al. '84
Fish										
Poecilia reticulata	-	R	--	s.w.	-	--	14-d	LC50	19,120	Könemann '79
Micropterus salmoides eggs <1-d old --> 4-d post-hatching	+	CF	--	r.w. 7.3-8.1	50	7.5-d	LC50	50	50	Birge et al. '79
Carassius auratus eggs <1-d old --> 4-d post-hatching	+	CF	--	r.w. 7.3-8.1	50	7.5-d	LC50	880	880	Birge et al. '79
1,2 - dichlorobenzene										
Algae										
Selenastrum capricornutum	+	c-S	ag	s.w.	-	--	96-h	EC50 _g	2,200	[1] Calamari et al. '83 Galassi & Vighi '81
Crustaceans										
Daphnia magna	+	c-R	--	s.w.	-	--	14-d	EC50 _r	550	Calamari et al. '83
Fish										
Poecilia reticulata	-	R	--	s.w.	-	25	14-d	LC50	5,850	Könemann '79
1,3 - dichlorobenzene										
Fish										
Poecilia reticulata	-	R	--	s.w.	-	25	14-d	LC50	7,370	Könemann '79
1,4 - dichlorobenzene										
Algae										
Selenastrum capricornutum	+	c-S	ag	s.w.	-	--	96-h	EC50 _g	1,600	[1] Calamari et al. '83 Galassi & Vighi '81
Crustaceans										
Daphnia magna	+	c-R	--	s.w.	-	--	14-d	EC50 _r	930	Calamari et al. '83

Table 2.5 Freshwater organisms - long-term toxicity tests with chlorobenzenes: L(E)C50-values (continued)

Organism	A	Test- type	Test- subst. purity	Test- water.	pH	Hardness mg CaCO ₃ /l	Exp.- time	Crite- rion	Result µg/l	Reference
Fish										
Pimephales promelas	+	CF	--	lake	-	43-49	8-d	LC50	3,530	Hall et al. '84
Poecilia reticulata	-	R	--	s.w.	-	25	14-d	LC50	3,960	Könemann '79
Salmo gairdneri alevins	+	c-CF	--	s.w.	-	--	14-d	LC50	800	Calamari et al. '82
<u>1,2,3 - trichlorobenzene</u>										
Algae										
Selenastrum capricornutum	+	c-S	ag	s.w.	-	--	96-h	EC50 _g	900	[1] Calamari et al. '83 Galassi & Vighi '81
Crustaceans										
Daphnia magna	+	c-R	--	s.w.	-	--	14-d	EC50 _r	200	Calamari et al. '83
Fish										
Poecilia reticulata	-	R	--	s.w.	-	25	14-d	LC50	2,340	Könemann '79
<u>1,2,4 - trichlorobenzene</u>										
Algae										
Selenastrum capricornutum	+	c-S	≥98%	s.w.	-	--	96-h	EC50 _g	1,400	Calamari et al. '83 Galassi & Vighi '79
Crustaceans										
Daphnia magna	+	R	--	s.w.	-	--	16-d 16-d	LC50 EC50 _r	560 270	[3] Hermens et al. '84 Hermens et al. '84
Fish										
Pimephales promelas	+	CF	--	lake	-	43-49	8-d	LC50	1,815	Hall et al. '84
Poecilia reticulata	-	R	--	s.w.	-	25	14-d	LC50	2,390	Könemann '79
<u>1,3,5 - trichlorobenzene</u>										
Fish										
Poecilia reticulata	-	R	--	s.w.	-	25	14-d	LC50	3,300	Könemann '79
<u>1,2,3,4 - tetrachlorobenzene</u>										
Crustaceans										
Daphnia magna	+	R	--	s.w.	-	--	16-d 16-d	LC50 EC50 _r	320 43	[3] Hermens et al. '84 Hermens et al. '84
Fish										
Poecilia reticulata	-	R	--	s.w.	-	25	14-d	LC50	800	Könemann '79

Table 2.5 Freshwater organisms - long-term toxicity tests with chlorobenzenes: L(E)C50-values (continued)

Organism	A	Test- type	Test- subst. purity	Test- water.	pH	Hardness mg CaCO ₃ /l	Exp.- time	Crite- rion	Result µg/l	Reference
<u>1,2,3,5 - tetrachlorobenzene</u>										
Fish										
Poecilia reticulata	-	R	--	s.w.	-	25	14-d	LC50	800	Könemann '79
Pimephales promelas	+	CF	--	lake	-	43-49	8-d	LC50	800	Hall et al. '84
<u>1,2,4,5 - tetrachlorobenzene</u>										
Fish										
Poecilia reticulata	-	R	--	s.w.	-	25	14-d	LC50	300	Könemann '79
Pimephales promelas	+	CF	--	lake	-	43-49	8-d	LC50	300	Hall et al. '84
<u>pentachlorobenzene</u>										
Crustaceans										
Daphnia magna P < 1-d old	-	R	≥98%	lake	8.1	225	3-w	LC50	240	Van Leeuwen et al. '87
Daphnia magna P < 1-d old	-	R	≥98%	lake	8.1	225	3-w	EC50 _y	120	Van Leeuwen et al. '87
Daphnia magna	+	R	--	s.w.	-	--	16-d 16-d	LC50 EC50 _r	110 25	[3] Hermens et al. '84 Hermens et al. '84
Fish										
Poecilia reticulata	-	R	--	s.w.	-	25	14-d	LC50	180	Könemann '79
<u>hexachlorobenzene</u>										
Crustaceans										
Daphnia magna	+	c-R	--	s.w.	-	-	14-d	EC50 _r	16	Calamari et al. '83
Fish										
Poecilia reticulata	-	R	--	s.w.	-	25	14-d	LC50	>300	[2] Könemann '79

n.m. = nutrient medium; w.w. = well water; g = growth; r = reproduction; y = yield.
For explanation, see "list of abbreviations tables 2.3 to 2.6".

[1] For all chlorobenzenes tested the loss of compound did not exceed 10% of the initial value, except for the Daphnia test in which it did not exceed 15%.

[2] No mortality was observed at concentrations far more than its solubility in water.

[3] The decrease in concentrations during the tests, till renewing the solutions, were reported to be maximal 20%.

Table 2.6 Marine organisms - short- and longterm toxicity tests chlorobenzenes: L(E)C50- and NO(L)EC-values

Organism	A	Test- type	Test- subs. purity	Test- medium	Salinity o/oo	Exp.- time	Crite- rion	Result µg/l	Reference
<u>1,2,4 - trichlorobenzene</u>									
Amphibiens									
Branchiostoma caribaeum	+	CF	--	n.s.	-	96-h	NOLC	1,500	[1] Clark et al. '87
	+	CF	--	n.s.	-	96-h	LC50	>1,500	Clark et al. '87
Crustaceans									
Palaemonetes pugio	+	CF	--	n.s.	-	96-h	LC50	540	[1] Clark et al. '87
	+	CF	--	n.s.	-	96-h	NOLC	240	Clark et al. '87
Fish									
Cyprinodon variegatus (14-28 days old)	-	S	≥80%	n.s.	10-31	96-h	LC50	21,000	Heitmuller et al. '81
<u>1,2,3,5 - tetrachlorobenzene</u>									
Fish									
Cyprinodon variegatus (14-28 days old)	-	S	≥80%	n.s.	10-31	96-h	LC50	3,700	Heitmuller et al. '81
<u>1,2,4,5 - tetrachlorobenzene</u>									
Fish									
Cyprinodon variegatus (14-28 days old)	-	S	≥80%	n.s.	10-31	96-h	LC50	800	Heitmuller et al. '81
Cyprinodon variegatus	+	CF	--	n.s.	-	96-h	LC50	330 α	Ward et al. '81
Cyprinodon variegatus	+	CF	--	n.s.	12-28	>28-d	NOEC	300 α	Ward et al. '81
eggs <1-d old --> 28 days							NOLC ^g	90 α	Ward et al. '81
post-hatching							NOEC _h	≥520 α	Ward et al. '81
<u>pentachlorobenzene</u>									
Fish									
Cyprinodon variegatus (14-28 days old)	-	S	≥80%	n.s.	10-31	96-h	LC50	800	Heitmuller et al. '81

n.s. = natural seawater; g = growth; h = hatchability
 For explanation, see "list of abbreviations tables 2.3 to 2.6".

[1] Measured concentrations were between 75% and 95% of nominal concentrations.

List of abbreviations tables 2.3 to 2.6

A	+ Test substance analysed in test solution - Test substance not analysed in solution or : no data
α	Value based on actual (measured) concentrations in test solutions, as mentioned explicitly in the literature source. Values not indicated by " α " are considered to be nominal concentrations.
> and \geq	Value indicated is highest concentration used in the test.
< and \leq	Value indicated is lowest concentration used in the test.
Test type	S: static; R: renewal; CF: continuous flow; c- : closed system
Test time	hr: hour(s); w: week(s); m: month(s)
Criterion	LC50: Lethal concentration for 50% of the organisms exposed EC50: Effect concentration for 50% of the organisms exposed NOLC: No-observed-lethal-concentration NOEC: No-observed-effect-concentration

CHAPTER 3 ECOTOXICITY - TERRESTRIAL ORGANISMS

3.1 ACCUMULATION

In a long-term experiment (11-w) using a sandy soil (pH=6.1, OM=1%) the BCF based on fresh weight of HCB in the earthworm *Lumbricus terrestris* was found to be about 2. In a soil with a higher OM (exact percentage not given) the BCF (based on fresh weight) was determined to be 0,5. After exposure of the snail *Deroceras reticulatum* to 1.0 mg.kg⁻¹ of HCB in soil, the snails contained 1.4 µg.kg⁻¹ bw (Ebing et al., 1984). BCF's in the earthworms *L. terrestris* and *Allolobophora longa*, about 55 days exposed to 1,2,3,5-TeCB and HCB in soil (2.6% OM, pH=5.1), were about 4 (Lord et al., 1980). In three wild mammals the HCB content in adipose tissues were determined. In animals known to feed on small animals (mice and invertebrates) higher HCB residues were found than in animals feeding exclusively on plant material. According to the authors this could be an indication of accumulation of HCB via the food chain (Koss and Manz, 1976). The disposition of four ¹⁴C-labeled pesticides, including HCB, was examined as a seed-protectant coating in a terrestrial microcosm chamber, containing a synthetic soil medium, agricultural crops, numerous invertebrates and two gravid gray-tailed vole (*Microtus canicaudus*). After 45 days the percentage total recovery was 61% for HCB. Some accumulation of HCB in plants was found, HCB was present mainly as extractable parent compound. Due to lack of parent material in the invertebrates, as well as poor recovery of the organisms, no index for "ecological magnification" (= whole body concentration/concentration in soil; EM) could be given. For the vole an EM of 118 was determined (Gille and Gillet, 1979).

3.2 TOXICITY

- Soil processes

The effects of HCB on soil processes were studied using a pine forest microcosm during 21 days. HCB-contaminated litter (0, 0.085, 0.730, 7.42 mg.cm⁻³) was applied to replicate soils in filtered, flow-through microcosm

systems. The two studied parameters, CO₂ efflux and Ca loss, were affected by HCB: CO₂ efflux decreased and Ca loss increased (Ausmus et al., 1979).

- Invertebrates

The results of toxicity tests with earthworms resulting in L(E)C50-values are summarized in table 3.1.

Additional data

The toxicity of 1,4-DCB to the earthworms *Dendroboena rubida* and *Lumbricus terrestris* was determined using a soil containing 5% peat and 5% cow-dung (pH=7) which was repeatedly treated. The LC50-values for both earthworms were found to be 390 mg.kg⁻¹ dry weight. The oral LD50-value for the insect *Apis mellifera* has been reported to be >5 µg 1,4-DCB per insect (Caprioli et al., 1984). The effect of HCB on postembryonic stages of the free-living nematode *Panagrellus redivivus* was studied in a 96-h assay. Test animals were L2 juveniles (second-stage juvenile), which develop subsequently into the third stage (L3), the fourth stage (L4) and the adult stage. The testconditions were established such that 50% of the L2 animals will reach the adult stage in a 96-h growth period. HCB had no effect on "overall" (L2 →adult) survival over the range tested (10⁻⁸ - 10⁻³ mol.l⁻¹). It was also reported that the lower but not the higher concentrations (≤10⁻⁵) had effects on intermediate stages (L2-L3, L3-L4 and L4 to adult) (Samoiloff et al., 1980).

- Vertebrates

The quail *Coturnix coturnix* was given a single oral dose of 1,4-DCB, followed by an observation period of 14 days. The LD50-value was 2400 mg.kg⁻¹ bw (Caprioli et al., 1984).

After a single oral dose of 1,2,4-TCB (200 mg.kg⁻¹ bw or more) a significant increase in liver porphyrin levels and a liver enzyme was observed in adult Japanese quails. Repeated administration of 50 or 200 mg.kg⁻¹ bw significantly increased several liver enzymes, including cyt P-450 (Miranda et al., 1983).

Quails *Coturnix coturnix japonica* were given oral doses of 500 mg.kg⁻¹ bw HCB for 1, 2, 5, or 10 days. After one dose the birds already developed porphyria. Porphyrin levels were increased as well as the activity of some liver enzymes (including Cyt P450 enzymes). A decrease in body weight was observed (Carpenter et al., 1985a, Carpenter et al., 1985b). In another experiment an oral dose of 100 mg.kg⁻¹ bw HCB for 10 days caused an increase in liver enzymes and a slight increase in liver porphyrin concentrations in *C. coturnix japonica*. A consecutive treatment (same dose for 1, 5, 10, or 15 days) caused a further increase in porphyrin levels (Carpenter et al., 1985c). In a 90-day study quails were exposed to dietary concentrations of 0, 1, 5, 20 or 80 mg.kg⁻¹ (0, 0.1, 0.5, 2.2 or 8.8 mg.kg⁻¹ bw). At the highest dose an increased mortality and several toxic effects were seen. At a concentrations of 5 mg.kg⁻¹ diet and more an increased liver weight and slight liver damage was observed. The no-effect level was established at 1 mg.kg⁻¹ diet (which equals 0.1 mg.kg⁻¹ bw) (Vos et al., 1971). In a reproduction study minks and ferrets were fed HCB at dietary concentrations of 0, 1, 5, 25, 125 or 625 mg.kg⁻¹ (0, 0.04, 0.2, 1, 5, 25 mg.kg⁻¹ bw) for nearly one year. Diets containing 125 or 625 were lethal to the adults. Diets containing 1 mg.kg⁻¹ or more resulted in reduced reproductive performance, as indicated by decreased litter size, increased percentage of still births, increased kit mortality and decreased growth. Minks were more sensitive than ferrets. From cross-fostering experiments it appears that exposure via milk also increases kit mortality, although mortality was lower than among kits exposed in utero (Bleavins et al., 1984). Female mink were exposed to HCB at dietary levels of 0, 1 or 5 mg.kg⁻¹ and bred with males on the same treatments. Female offspring were allowed to mature to 16-17 weeks and killed. A profound effect on the survival of kits was found: 44% in the lowest dose group and 77% in the high dose group, versus 8% in the control group. At 17 weeks of age the only effect was enzym induction in the highest dose group (Rush et al., 1983).

Summary and conclusions "Terrestrial organisms"

- Accumulation

Data on the accumulation of terrestrial organisms from soil are very limited. Studies with earthworm species (2) exposed to HCB and 1,2,4,5-TeCB, resulted in BCF's of 2 and ± 4 . In a terrestrial laboratory microcosm, containing agricultural crops, numerous invertebrates and two voles, no indication of bioconcentration was found.

- Toxicity

The long-term toxicity of 1,2,3-TCB to the earthworms *Eisenia andrei* and *Lumbricus rubellus* was determined in four different soils; humic sand, a very humic sand, an artificial (OECD) soil and a peaty soil. The 2-w LC50-values for *E. andrei* ranged from 134 to 547 mg.kg⁻¹ dry weight and those for *L. rubellus* from 115 to 563 mg.kg⁻¹ dry weight. The effects of 1,2,4-TCB were studied in 4 earthworm species using an artificial soil. LC50-values ranged from 127 to 251 mg.kg⁻¹ dry weight. The LC50-values of 1,4-DCB for the earthworms *Dendroboena rubida* and *L. terrestris* were found to be 390 mg.kg⁻¹ dry weight.

The effects of short- and longterm exposure of the quail *Coturnix coturnix* to chlorobenzenes, mostly HCB, was studied several times. An LD50-value of 2400 mg.kg⁻¹ bw was reported for 1,2-DCB, whereas subacute exposure to 50 mg.kg⁻¹ bw of 1,2,4-TCB induced liver enzyme activity. Long-term oral exposure of quails to HCB caused an increased mortality, toxic effects on the liver (increased liver weights and liver enzyme induction) and porphyria, resulting in increased liver porphyrin concentrations. The dose without effect was 1 mg.kg⁻¹ diet, corresponding to 0.1 mg.kg⁻¹ bw.

In reproduction studies with ferrets and minks it appeared that the lowest tested dietary concentration (1 mg.kg⁻¹, corresponding to 0.04 mg.kg⁻¹ bw) caused adverse effects on the reproductive performance (decreased litter size, increased percentage of still births, etc.). Minks were more sensitive than ferrets. The survival of kits from minks fed 1 mg.kg⁻¹ diet, also was significantly lower than those in the control group.

Table 3.1 Earthworms - toxicity tests with chlorobenzenes: LC50-values (laboratory studies)

Organism	Soil	pH	%OM	%Clay	Temp. °C	Exp.- time	Criterion	Result in test soil (mg/kg dry weight)	Calculated [*] value in 10% OM soil
<u>1,2,3,-trichlorobenzene</u>									
Eisenia andrei	mod. humic sand	4.8	3.7	1.4	19	2-w	LC50	134	362
	very humic sand	5.3	6.1	2.4	19	2-w	LC50	240	393
	art.(OECD) soil	3.8	16	9	19	2-w	LC50	133	83
	peaty soil	6.5	8.1	8.1	19	2-w	LC50	547	675
Lumbricus rubellus	mod. humic sand	4.8	3.7	1.4	19	2-w	LC50	115	310
	very humic sand	5.3	6.1	2.4	19	2-w	LC50	207	340
	art.(OECD) soil	3.8	16	9	19	2-w	LC50	195	122
	peaty soil	6.5	8.1	8.1	19	2-w	LC50	563	695
Van Gestel & Ma '90									
<u>1,2,4-trichlorobenzene</u>									
Eisenia fetida	art. soil	6.0	10	20	20	2-w	LC50	197	197
Allolobophora tuberculata						2-w	LC50	251	251
Eudrilus eugeniae						2-w	LC50	127	127
Perionyx excavatus						2-w	LC50	180	180
Neuhauser et al. '86									

For explanation, see "list of abbreviations tables 3.1 and 4.1".

CHAPTER 4 AGRICULTURAL CROPS AND LIVESTOCK

4.1 AGRICULTURAL CROPS

- Accumulation

In experiments under outdoor conditions the toxicity of ^{14}C -labelled 1,2,4-TCB, PeCB and HCB to barley and cress plants was determined. The plants were grown for one vegetation period on a soil containing 2 mg.kg^{-1} dry weight of the substances and analyzed after varying time intervals. The soil used contained 34% clay, 27% silt and 32% sand (pH= 6.4, OM=2.1%). After 11 days the BCF's (concentration in dry plant matter/ concentration in dry soil) varied between 2.1 (HCB in cress plants) and 36 (1,2,4-TCB in barley plants). In course of time (125 and 80 days for barley and cress plants, respectively) the BCF's were about a factor 10 lower. This effect is due to growth dilution, since the absolute amounts of radioactive substances in plants increased with time (Topp et al., 1989). In another study with the same plants the uptake of ^{14}C -labelled substances (by roots and by leaves via the air) was described. The same soil as described in the preceding study was used. After about 60 days the BCF's of PeCB and HCB were about 1. For the total group of substances a positive relationship was found between barley root BCF's and n-octanol/water partition coefficient. The uptake of chemicals via leaves correlated strongly to volatilization from the soil. For the cress plant these correlations were poor (Topp et al., 1986). In a laboratory soil-plant system the uptake of ^{14}C -labelled HCB by barley plants from a humus sand (pH=6.9, OM=2.3%) containing 2 mg.kg^{-1} HCB was examined. The plants contained 3.6 mg.kg^{-1} , which resulted in a BCF of 1.9 (Kloskowski et al., 1981).

HCB residues may be present in lettuce and witloof- chicory as a result of earlier application of quintozone (a fungicide) to lettuce foliage or soil and to witloof-chicory soil. HCB is normally present as an impurity in technical grade quintozone in amounts varying between 1% and 6.2% (Dejonckheere et al., 1975, 1976).

- Toxicity

Toxicity studies with lettuce resulting in 2-w EC50- and NOEC-values are summarized in table 4.1.

Additional data

The toxicity of 1,2,4,5-TeCB on the germination and seedling vigor of barley, oats and wheat was studied using a sandy soil, a sandy loam, a clay loam and a clay soil (no % organic matter given). The crops were planted 1 to 125 days after treating the soil 6 times, resulting in a concentration corresponding to 12-980 mg.kg⁻¹. The lowest concentration was already detrimental after one day, except for the clay soil. In this soil effects were seen at concentrations ≥ 320 mg.kg⁻¹. The adverse effects decreased when the time between treatments of the soil and planting increased (Ameen et al., 1960)

Summary and conclusions "Agricultural crops"

- Accumulation

Data on accumulation of chlorobenzenes by agricultural crops are limited. In an outdoor experiment it was found that the BCF's of 1,2,4-TCB, TCB (isomer not given), PeCB and HCB in barley and cress varied between 2 (HCB in cress plants) and 36 (1,2,4-TCB in barley plants). In course of time (about 100 days) the BCF's were a factor of 10 lower. In another study with the same plants the uptake of PeCB and HCB by roots and by leaves via the air was studied. A positive correlation was found between barleys root BCF's and Kow-values. The uptake of chemicals via leaves correlated strongly to the volatilization from the soil. For cress these correlations were poor.

-Toxicity

Data on the toxicity of chlorobenzenes to agricultural crops are limited to one study with lettuce and one with cereals. In the first study the long-term toxicity of chlorobenzenes to *Lactuca sativa* was determined, resulting in 2-w EC50-values (based on growth) of 248 mg.kg⁻¹ for MCB, 1-4

mg.kg⁻¹ for 1,2,3-TCB, 48 mg.kg⁻¹ for 1,2,4-TCB, 123 mg.kg⁻¹ for 1,3,5-TCB, 32 mg.kg⁻¹ for 1,2,3,4-TeCB, 1.3 mg.kg⁻¹ for 1,2,3,5-TeCB, 2 mg.kg⁻¹ for 1,2,4,5-TeCB and 56 mg.kg⁻¹ for PeCB. For a number of chlorobenzenes NOEC-values were reported as well: 10 mg.kg⁻¹ for 1,4-DCB, 1 mg.kg⁻¹ for 1,2,3-TCB, 10 mg.kg⁻¹ for 1,2,4-TCB, 1,3,5-TCB, 1,2,3,4-TeCB and PeCB and 100 mg.kg⁻¹ for HCB. Concentrations of ≥ 12 mg.kg⁻¹ of 1,2,4,5-TeCB were toxic to cereals in several soils.

4.2 LIVESTOCK

- Accumulation

Growing chickens were exposed to HCB for 6 months at dietary levels of 0, 0.1, 1, 10 or 100 mg.kg⁻¹ (0, 0.0125, 0.125, 1.25 or 12.5 mg.kg⁻¹ bw). The amounts found in the tissues increased with the dietary level and were roughly proportional to the fat content of the tissues. After 6 months the concentrations in body fat, egg yolk, liver and muscle were 2,000, 450, 57 and 8.6 mg.kg⁻¹, respectively, at the highest dose (Avrahami and Steele, 1972c). Laying pullets were given dietary concentrations up to 100 mg.kg⁻¹ HCB (12.5 mg.kg⁻¹ bw) for 6 months. The concentrations in the fat were 20-30 times the dietary concentrations (up to 2,900 mg.kg⁻¹ at the highest dose). Relative HCB concentrations in fat, yolk, liver and muscle were 75, 40, 4 and 1, respectively (Avrahami and Steele, 1972b).

HCB was fed to pigs at concentrations between 0.05 and 50 mg.kg⁻¹ bw a day for 90 days. HCB accumulated in fat and blood at all doses; the concentrations in fat (mg.kg⁻¹) being about 300 times those administered (mg.kg⁻¹ bw). Tissues of control animals also contained significant HCB concentrations, which was, according to the authors, due to cross contamination. The (lowest) dose groups could have been contaminated as well. Therefore, the concentration factor of 300, even for the lowest dose, is considered to be an overestimation (Tonkelaar, den et al., 1978). In growing swines, orally exposed to HCB for 13 weeks, HCB also accumulated in fat to concentrations 5-7 times the dietary concentration (0, 1, 10 or 100 mg.kg⁻¹) (Hansen et al., 1977). In lambs the HCB levels in fat reached a level approximately 10 times those in the diet (0, 0.01, 0.1 or 1 mg.kg⁻¹)

at the end of the 90-day exposure period. The highest levels in other tissues were in the brain and in the liver (Mull et al., 1978). Sheep were dosed orally with HCB at 0.1, 1, 10 or 100 mg.kg⁻¹ (0.004, 0.04, 0.4 or 4 mg.kg⁻¹ bw HCB) a day during 18 weeks. The sheep stored HCB in their body fat to the extent of about 8 times the daily intake. In the blood the concentrations were about 1,000 times lower than in fat. The undosed controls were also found to accumulate HCB in their fat. The primary source of HCB in these controls could have been the faeces of the dosed sheep that grazed in the same paddocks (Avrahami and Steele, 1972a). HCB was fed to pregnant sows at concentrations of 0, 1 or 20 mg.kg⁻¹ diet (0, 0.025 or 0.5 mg.kg⁻¹ bw) a day throughout gestation and nursing (over 200 days). In the sows highest residue concentrations were found in fat and bone marrow, with levels of up to 7 and 90 for the lower and higher dietary levels, respectively. The pigs accumulated fat residues that were higher than those of the sows (Hansen et al., 1979). Two groups of 3 cows were fed either 5 or 25 mg of HCB per day for 60 days. Residues were determined in milk at 5-day intervals during the exposure period and for 60 days after dosing was stopped. The average concentrations in milk fat for 40th to 60th days of dosing were about 2 and 9 mg.kg⁻¹ for the low and high dose groups, respectively. The concentrations in subcutaneous body fat were about 2 and 35 for the low and high dose groups, respectively. After exposure had stopped the concentrations in declined rapidly (Fries and Marrow, 1975).

- Toxicity

Single oral doses of 800 mg.kg⁻¹ bw of MCB, 1,4-DCB or 1,2,4-TCB produced an increase in total porphyrin content of liver of one day-old chicks. The administration of similar concentrations to chick embryos failed to produce an induction of liver porphyrins (Miranda et al., 1984). Feeding of HCB at dietary levels up to 100 mg.kg⁻¹ (12.5 mg.kg⁻¹ bw) to growing chickens for 6 months did not cause any effects (Avrahami and Steele, 1972c). In another study, in which HCB was fed to laying pullets at concentrations up to 100 mg.kg⁻¹ (12.5 mg.kg⁻¹), no adverse effects were seen on the pullets nor on the egg fertility or hatchability (Avrahami and Steele, 1972b). Groups of laying hens were fed HCB at levels of 0, 1, 5, 125 or 625 mg.kg⁻¹ diet (0, 0.2, 0.9, 0,21.9 or 110 mg.kg⁻¹ bw) for 12 weeks. The highest dose caused

decreased body weight, increased relative liver weight and induced drug enzyme activity. No histopathological abnormalities were found (Kan et al., 1979). Laying pullets were given oral doses of HCB ranging from 1 to 100 mg.kg⁻¹ for 7 days. At the higher doses HCB (≥ 10 mg.kg⁻¹) delayed the onset of full egg production but also appeared to offer protection against development of hemorrhagic fatty liver (Hansen et al., 1978).

Groups of growing swines were exposed to dietary concentrations of 0, 1, 10 or 100 mg.kg⁻¹ HCB (0, 0.04, 0.4 or 4 mg.kg⁻¹ bw) for 13 weeks. No toxic effects were observed. In the highest dose group larger livers with hypertrophy were observed (Hansen et al., 1977). In an 90-day test pigs were exposed orally to 0.05, 0.5, 5 or 50 mg.kg⁻¹ bw HCB a day. In the highest dose group clear signs of porphyria were seen. At 0.5 and 5.0 mg.kg⁻¹ bw an increased excretion of coproporphyrin and an induction of liver enzymes was found. An increased liver weight occurred at 5.0 mg.kg⁻¹ bw. The no-effect-level was judged to be 0.05 mg.kg⁻¹ bw a day for HCB (Tonkelaar, den et al., 1978).

Pregnant sows were exposed to dietary concentrations of 0, 1 or 20 mg.kg⁻¹ HCB (0, 0.025, 0.5 mg.kg⁻¹ bw) throughout gestation and nursing (over 200 days). No adverse effects were seen at the lower dose. The higher dose caused slight toxic effects (Hansen et al., 1979).

Three studies (6, 16 and 68 weeks) were conducted to study the effects of the addition of a mixture of organochlorine pesticides to the feed of broilers or laying hens. The total concentration of the pesticides was maximal 3.1 mg.kg⁻¹ diet, including 0.10 mg.kg⁻¹ HCB. No adverse effects were seen (Kan and Tuinstra, 1976, Kan and Jonker-den Rooyen, 1978, Kan et al., 1978).

Summary and conclusions "Livestock"

With regard to the accumulation of chlorobenzenes in livestock the only data that were available concern HCB. The accumulation of HCB after subchronic oral administration was examined in growing chickens, laying pullets, pigs, swines, lambs, sows and sheep. In general, the amounts of HCB found in the tissues increased with the dietary level and with the fat

content of the tissues. In growing chickens and laying pullets the fat contained between 20 and 30 times the dietary level. The concentrations in egg yolk was about 4 times the dietary level. In pigs fed HCB up to levels of 50 mg.kg^{-1} bw a day the concentrations in the fat were about 500 times higher than those in blood ($15,500 \text{ mg.kg}^{-1}$ in fat). Growing swines, lambs and sheep concentrated HCB in their fat to the extent of about 10 times the dietary levels. Other organs that contained higher levels were bone marrow, liver and brain.

Most data on the toxicity of chlorobenzenes to livestock concern HCB. The only data that were available on the toxicity of other chlorobenzenes indicate that single doses of 800 mg.kg^{-1} bw of MCB, 1,4-DCB and 1,2,4-TCB produce an increase in prophyrin contents of the liver of one-day old chickens. HCB was tested in several domestic animals; chickens, laying hens, swines, lambs and sows. Feeding of HCB to chickens at levels up to 12.5 mg.kg^{-1} bw for 6 months did not produce adverse effects. In cattle HCB caused effects on the liver, resulting in liver enzyminduction. Th dose-without effect was 0.025 mg.kg^{-1} bw.

Table 4.1 Toxicity tests with chlorobenzenes: EC50- and NOEC-values (laboratory studies)

species	Soil	pH	%OM	%Clay	Temperature. °C	Exp.- time	Criterion	Result in test soil (mg/kg dry weight)	Calculated value in 10% OM soil (mg/kg dry weight)	*
<u>1,4-dichlorobenzene</u>										
Lactuca sativa	brook bed	7.8	1.4	12	20	2-w	EC50	248	1250	[1]
						7-d	NOEC ^g	10	50	[1]
<u>1,2,3-trichlorobenzene</u>										
Lactuca sativa	brook bed	7.8	1.4	12	18-26	2-w	EC50	1	5	[2]
Lactuca sativa	brook bed	7.8	1.4	12	20	2-w	EC50 ^g	4	20	[1]
						2-w	NOEC ^g	1	5	[1]
<u>1,2,4-trichlorobenzene</u>										
Lactuca sativa	brook bed	7.8	1.4	12	20	2-w	EC50	48	240	[1]
						2-w	NOEC ^g	10	50	[1]
<u>1,3,5-trichlorobenzene</u>										
Lactuca sativa	brook bed	7.8	1.4	12	20	2-w	EC50	123	615	[1]
						7-d	NOEC ^g	10	50	[1]
<u>1,2,3,4-tetrachlorobenzene</u>										
Lactuca sativa	brook bed	7.8	1.4	12	20	2-w	EC50	32	160	[1]
						2-w	NOEC ^g	10	50	[1]
<u>1,2,3,5-tetrachlorobenzene</u>										
Lactuca sativa	brook bed	7.8	1.4	12	20	2-w	EC50 ^g	1.3	6.5	[1]
<u>1,2,4,5-tetrachlorobenzene</u>										
Lactuca sativa	brook bed	7.8	1.4	12	18-26	2-w	EC50 ^g	2	10	[2]
<u>pentachlorobenzene</u>										
Lactuca sativa	brook bed	7.8	1.4	12	20	2-w	EC50	56	280	[1]
						2-w	NOEC ^g	10	50	[1]
<u>hexachlorobenzene</u>										
Lactuca sativa	brook bed	7.8	1.4	12	20	2-w	NOEC ^g	100	500	[1]

g = growth

For explanation, see "list of abbreviations tables 3.1 and 4.1".

[1] Data evaluated by Denneman and Van Gestel (1990), RIVM

[2] Hulzebos et al. (1989)

List of abbreviations tables 3.1 and 4.1

Test time hr: hour(s); w: week(s); m: month(s)
Criterion LC50: Lethal concentration for 50% of the organisms
 exposed
 EC50: Effect concentration for 50% of the organisms
 exposed
 NOLC: No-observed-lethal-concentration
 NOEC: No-observed-effect-concentration

Soil characteristics:

OM: organic matter
dw: dry weight

OECD artificial soil:

10% sphagnum peat, 20% kaolin clay, 69% fine sand,
1% calcium carbonate to adjust pH.

The indicated soil characteristics are based on measurements in several tests.

* Calculated value in 10% OM soil = Experimental value x $10/\%OM-t$
 $\%OM-t$ = % organic matter in test soil.

5 RISK ASSESSMENT

5.1 RISK ASSESSMENT FOR MAN

Generally, a toxicological limit value is only established when a certain "minimum toxicological dataset" concerning genotoxicity, carcinogenicity, reproductive and/or teratogenicity and (sub)chronic toxicity is available. Consequently, a toxicological limit value for oral exposure could only be established for MCB, 1,2-DCB, 1,4-DCB and HCB. No limit values could be given for the remaining chlorobenzenes. However, because of the fact that a group of related substances is involved which behave rather similar or at least predictable (with respect to metabolism, toxicology and genotoxicity), it was decided to give indicative toxicological limit values for oral exposure for the other chlorobenzenes (1,3-DCB, 1,2,3-TCB, 1,2,4-TCB, 1,3,5-TCB, 1,2,3,4-TeCB, 1,2,3,5-TeCB, 1,2,4,5-TeCB and PeCB). With respect to inhalatory exposure the data were for all chlorobenzenes insufficient to establish a (indicative) toxicological limit value.

The metabolic behaviour of the different chlorobenzenes changes gradually with the degree of chlorination. With an increase in the number of chloro-atoms the substances become more lipophile and accumulate to a greater extent in fat and fatty tissues. The biotransformation and elimination via the urine decrease with the number of chloro-atoms; the difference in halflife times between for example 1,4-DCB and HCB was estimated to be at least a factor of 10. Especially HCB is metabolized very slowly and the excretion is mainly via the faeces. The tissues contain predominantly unchanged HCB.

The toxicity of the chlorobenzenes, when administered repeatedly, also increases with the degree of chlorination. This may be partly explained by the decrease in elimination and the increase in accumulation.

Microsomal enzyme induction and the interference with the normal synthesis of porphyrines are considered to be sensitive toxicological parameters for all chlorobenzenes. Microsomal enzyme induction is already obvious after a relative short exposure period, and the sensitivity does not seem to increase after longer exposure periods. With respect to chlorobenzenes, enzyme induction was one of the studied parameters in nearly all subchronic

experiments. Instead, this parameter was generally not studied in the chronic experiments, which mostly concerned carcinogenicity. If possible, this evaluation was based on data on enzyme induction.

As was already stated, the chlorobenzenes might have genotoxic activities. This suspicion was, however, based on only one study, in which MCB, DCB's and TCB's were tested for their potential to induce micronuclei in the bone marrow of mice. In all other test systems, both *in vitro* as *in vivo*, the chlorobenzenes showed no or (hardly no) activity. The evidence for genotoxicity is therefore considered to be very weak.

From carcinogenicity experiments with a number of chlorobenzenes, it appeared that the development of tumours is mostly accompanied by toxicity in the target organ. In addition, it is striking that for the most toxic chlorobenzene, HCB, the indications of carcinogenicity are most distinct. In three species exposed to HCB, toxicity (in the liver) was accompanied by the development of tumours. On the basis of the limited indications of genotoxicity and the correlation between toxicity and carcinogenicity it seems as yet justified to use a threshold extrapolation method for risk assessment.

- Oral exposure

Monochlorobenzene

On the basis of experimental studies there is no evidence of carcinogenicity of MCB in animals. There is limited evidence of genotoxicity. Oral reproductive or teratogenicity studies were not found.

A two-year (carcinogenicity-) study resulted in a dose without effect of 60 mg.kg⁻¹bw. From subchronic experiments it appeared that at similar doses slight effects were occurred in rats, mice and dogs (for example slight increases of heart- and splenic weights); the dose without effect was (±) 30 mg.kg⁻¹ bw. Taking into account a safety factor of 100 (for extrapolation from animal data to human beings) a maximal acceptable daily intake of 0.3 mg.kg⁻¹bw for was determined for lifetime exposure.

1,2-Dichlorobenzene

On the basis of experimental studies there is no evidence of carcinogenicity of 1,2-DCB in animals. There is limited evidence of genotoxicity. No oral reproductive or teratogenicity studies were found.

From a chronic (carcinogenicity-) study it appeared that only in the highest dose group ($120 \text{ mg.kg}^{-1} \text{ bw}$) a significant effect (tubular regeneration of the kidneys) occurred. The lower dose (60 mg/kg bw) was without effect. Taking into account a safety factor of 100, a maximal acceptable daily intake of 0.6 mg/kg bw was calculated for lifetime exposure.

1,4-Dichlorobenzene

On the basis of experimental studies there is limited evidence of carcinogenicity of 1,4-DCB in animals. Based on the limited evidence of genotoxicity and the correlation between toxicity and carcinogenicity it seems justified to use a threshold extrapolation method as yet. There is no evidence of teratogenicity and an embryotoxic effect was reported at doses of 500 mg/kg bw or more.

In a chronic (carcinogenicity-) study the lowest tested dose resulted in effects on the liver and kidneys of rats. In another experiment with rats (exposed for circa 6 months) the lowest dose ($19 \text{ mg.kg}^{-1} \text{ bw}$) did not result in any effects. Because of the fact that in this experiment only increased liver- and kidneyweights were found at the next lowest dose, which was a factor of 10 higher than the dose-without effect, it was concluded that a safety factor of 100 was sufficient. Based on a dose of $19 \text{ mg.kg}^{-1} \text{ bw}$ a maximal acceptable daily intake of $0,2 \text{ mg.kg}^{-1} \text{ bw}$ was calculated.

Hexachlorobenzene

On the basis of experimental studies there is sufficient evidence of carcinogenicity in animals. There is limited evidence of genotoxicity, but as yet a threshold extrapolation method is used. There is no evidence of a teratogenic effect or effects on the reproduction. Embryotoxicity occurred at doses of $\geq 40 \text{ mg.kg}^{-1} \text{ bw}$.

An oral subchronic experiment with female rats resulted in a dose without effect of $2 \text{ mg.kg}^{-1} \text{ bw}$ for the induction of porphyria and liver enzymes. In a study with combined pre- and postnatal exposure the lowest dose (0.2

mg.kg⁻¹bw) resulted in effects on the immune system of rats. The dose without effect for changes in liver cells of rats was 0.05 mg.kg⁻¹bw. A similar dose did also not cause effects in a small group of monkeys. It was not possible to derive a dose without effect from the chronic studies. In spite of the fact that the dose without effect was derived from a subchronic experiment, a safety factor of 100 is considered sufficient because the parameters are considered to be toxicologically sensitive. The maximal acceptable daily intake thus becomes 0.5 μg.kg⁻¹bw for lifetime exposure.

Other chlorobenzenes

For the other chlorobenzenes the data were limited; therefore only an indicative toxicological limit value can be derived. In the introduction of this section it was already stated that a threshold extrapolation method can be used (as yet) and that as much as possible data concerning enzyme induction were used.

Trichlorobenzenes

At the end of a 13-week study with rats the lowest dose (10 mg.kg⁻¹ bw) resulted in enzyminduction. After a 30-day "recovery" period this effect had disappeared. A study with monkeys (13 weeks exposed) resulted in a dose without effect of 25 mg.kg⁻¹bw. There was no evidence of teratogenic effects or effects on the reproduction. Embryotoxicity, which was accompanied by maternal toxicity, occurred at doses ≥ 360 mg.kg⁻¹bw.

In establishing an indicative limit value, a safety factor of 500 is used because of the fact that at a dose of 10 mg.kg⁻¹bw still effect were found. This results in a indicative value of 0.02 mg.kg⁻¹bw.

Tetrachlorobenzenes

With respect to toxicity (enzyme induction) one subchronic study with rats was available; 1,2,4,5-TeCB appeared to be the most toxic isomer. The dose without effect was 5 mg.kg⁻¹ in the diets (corresponding to 0.34 and 0.4 mg.kg⁻¹bw for males and females, respectively). There was no evidence of teratogenicity. An embryotoxic effect occurred at 200 mg.kg⁻¹bw of

1,2,3,4- and 1,2,3,5-TeCB. A similar dose of 1,2,4,5-TeCB caused mortality in nearly all mother-animals.

Using the dose without effect of $0.4 \text{ mg.kg}^{-1}\text{bw}$ and a safety factor of 100 an indicative limit value of $4 \mu\text{g.kg}^{-1}\text{bw}$ for TeCB's was established.

Pentachlorobenzene

One subchronic and reproduction toxicity study with rats was available, which resulted in a dose without effect of $12.5 \text{ mg.kg}^{-1}\text{bw}$. However, suckling pups of mothers from this dose group developed tremors. The dose without effect was $6.3 \text{ mg.kg}^{-1}\text{bw}$.

Because of the fact that no data are available on enzyminduction of PeCB no indicative toxicological limit value is given.

5.2 RISK ASSESSMENT FOR THE ENVIRONMENT

5.2.1 Aquatic organisms

The extrapolation methods, which can be used in establishing toxicological limit values, are being discussed at the moment. At present, an "preliminary hazard assessment" method (modification of the EPA-method) will be applied when the available number of NOEC-values resulting from long-term studies is less than 4. When there are at least 4 NOEC-values (from at least three different taxonomic groups) an "refined hazard assessment" method will be applied.

For all chlorobenzenes the first extrapolation method had to be applied. The results of this method are summarized in table 5.1. In principle, lowest NOE(L)C- or L(E)C50-values were used. However, if more values based on the same parameter were available for the same test species, then the geometric mean of these values was used. No data from short-term toxicity tests were used, when three NOEC-values (for algae, crustaceans and fish) were available (see 1,4-DCB, 1,2,3-TCB, 1,2,4-TCB and HCB). Because of the limited number of data the calculated values have to be considered as indicative of maximally acceptable risk-levels (MTR's).

The data show an increasing toxicity with an increase in degree of chlorination. This has been confirmed by values estimated on the basis of

QSAR's. For example, on the basis of the chronic QSAR for crustaceans (De Wolf et al., 1988) the following NOEC-values were estimated: $940 \mu\text{g.l}^{-1}$ for MCB, $540 \mu\text{g.l}^{-1}$ for DCB's, $300 \mu\text{g.l}^{-1}$ for TCB's, $170 \mu\text{g.l}^{-1}$ for TeCB's, $90 \mu\text{g.l}^{-1}$ for PeCB and $45 \mu\text{g.l}^{-1}$ for HCB. The experimental data do not show a difference in toxicity between isomers with a similar number of chloro-atoms. Therefore, only one indicative MTR has been derived for isomers with a similar number of chloro-atoms. The indicative MTR's were in the first place based on that compound from which most data were available. In addition, the MTR's were influenced by the theoretical difference in toxicity, appearing from QSAR-data. This resulted in the following indicative MTR's: $30 \mu\text{g.l}^{-1}$ for MCB, $20 \mu\text{g.l}^{-1}$ for DCB's, $10 \mu\text{g.l}^{-1}$ for TCB's, $5 \mu\text{g.l}^{-1}$, $2.5 \mu\text{g.l}^{-1}$ for PeCB and $0.2 \mu\text{g.l}^{-1}$ for HCB.

The limited number of toxicity data on marine organisms are comparable to those of freshwater organisms. Therefore, the indicative MTR's for seawater are similar to those for fresh water.

5.2.2 Terrestrial organisms

For terrestrial organisms, the numbers of toxicity values are (very) limited. Therefore, the "preliminary hazard assessment" method (modification of the EPA-method) has been used. The results of this extrapolation method are summarized in table 5.2. The NOEC- and EC50-values from this table have been converted to a "standard soil" containing 10% organic matter, to correct for differences in toxicity caused by the use of different soils. Accordingly, the values calculated with the extrapolation method refer to a 10% OM standard soil. This results in indicative MTR's varying between 0.005 mg.kg^{-1} to 0.4 mg.kg^{-1} for 1,4-DCB, TCB's, TeCB's and PeCB and 50 mg.kg^{-1} for HCB. The toxicity values for terrestrial organisms do not show a clear trend of increasing toxicity with increasing degree of chlorination, not even in identical tests. This may be caused by different ways of applications (water soluble compounds in aqueous solution, not water soluble compounds as solids) and therefore variable bioavailability. For this reason a range of 0.01 to 1 mg.kg^{-1} dry weight is given as indicative for MTR's, for individual compounds, in a 10% standard soil. It must be noted that plants seemed to be relative sensitive to some chlorobenzenes, such as 1,2,3-TCB and 1,2,3,5-TeCB.

Tabel 5.1. Calculated "acceptable" concentrations ($\mu\text{g/l}$) of chlorobenzenes in fresh water, according to the "preliminary hazard assessment" (PHA) method ("modification" of EPA-method; OECD Workshop, 1990).

Compound	Input (a)		result ($\mu\text{g/l}$)		indicative "MTR"
	lowest NOEC	lowest L(E)C50	EPA-modification		
	I (n)	II (n)	I (b)	II (c)	
MCB	320 (2)	660 (3)	32	6,6	30
1,2-DCB	340 (1*)	1230 (3)	34	12	20
1,3-DCB	680 (2)	3270 (3)	68	33	20
1,4-DCB	304 (3)		30		20
1,2,3-TCB	40 (3)		4		10
1,2,4-TCB	190 (3)		19		10
1,3,5-TCB	260 (**)	490 (1**)	26	4,9	10
1,2,3,4-TeCB	25 (2)	340 (2*)	2,5	3,4	5
1,2,3,5-TeCB	180 (**)	1580 (3)	18	15,8	5
1,2,4,5-TeCB	150 (**)	(d)	15		5
PeCB	35 (2)	250 (3)	3,5	2,5	2,5
HCB	1,8 (3)		0,18		0,2

"MTR": maximally acceptable risk-levels

(n) The number of taxonomic groups for which NOE(L)C- or L(E)C50-values were available. Numbers indicate experimental values, whereas (*) indicate the number of values estimated on the basis of QSAR's. The following QSAR's were used: $\log 1/\text{EC50} = 0,69 \log \text{Kow} - 3,18$ and $\log 1/\text{NOEC} = 0,67 \log \text{Kow} - 2,82$ for crustaceans (De Wolf et al., 1988) and $\log 1/\text{LC50} = 0,94 \log \text{Kow} - 4,62$ and $\log 1/\text{NOEC} = 1,06 \log \text{Kow} - 4,57$ for fishes (Van Leeuwen et al., 1990).

(a) In principle, lowest NOE(L)C- or L(E)C50-values were used. If more values based on the same parameter were available for the same test species, then the geometric mean was used.

(b) An extrapolationfactor of 10.

(c) An extrapolationfactor of 100 is applied in case there is at least 1 "reliable" L(E)C50-values for each of the following taxonomic groups: algae, crustaceans and fish; in all other cases an extrapolationfactor of 1000 is applied.

(d) Toxicity values for 1,2,4,5-TeCB were equal to exceeded the water solubility threshold ($560 \mu\text{g/l}$).

Table 5.2. Calculated "acceptable" concentrations of chlorobenzenes according to the "preliminary hazard assessment" (PHA) method (modification of the EPA-method, OECD Workshop, 1990) (in mg/kg dry weight)

Compound	input (a)		results (mg/kg)		
	lowest NOE(L)C I (n)	lowest L(E)C50 II (n)	EPA-modification I (b) II (c)		indicative "MTR"
	MCB				
1,2-DCB					
1,3-DCB					
1,4-DCB	50 (1)	390 (1)	5	0.4	0.4
1,2,3-TCB	5 (1)	5 (2)	0.5	0.005	0.005
1,2,4-TCB	50 (1)	127 (2)	5	0.1	0.1
1,3,5-TCB	50 (1)	615 (1)	5	0.6	0.6
1,2,3,4-TeCB	50 (1)	160 (1)	5	0.2	0.2
1,2,3,5-TeCB		7 (1)		0.007	0.007
1,2,4,5-TeCB		10 (1)		0.01	0.01
PeCB	50 (1)	280 (1)	5	0.3	0.3
HCB	500 (1)		(50)		

(n) The number of taxonomic groups of which NOEC- or EC50-values were available.

(a) The experimental values (V_e) have been converted into estimated values (V_s) into a "standard soil" containing 10% organic matter (% OM-s = 10%), on the basis of the percentage of organic matter in the test soil (% OM-t), using the following equation:

$$V_s = V_e \times 10 / \% \text{ OM-t.}$$

In the tests with plants, the % OM in the test soil was 1.4%, in these cases a percentage of 2% has been used in the equation.

(b) Extrapolationfactor of 10.

(c) Extrapolationfactor of 1000 is applied in all cases.

(An extrapolationfactor of 100 is only applied in case at least one "reliable" L(E)C50-value for at least 3 of the following taxonomic groups: microbial processes, earthworms, plants and arthropods is available).)

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