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The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals

122. Dichlorobenzenes

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Preface

The Nordic Council is an intergovernmental collaborative body for the five countries, Denmark, Finland, Iceland, Norway and Sweden. One of the committees, the Nordic Senior Executive Committee for Occupational Environmental Matters, initiated a project in order to produce criteria documents to be used by the regulatory authorities in the Nordic countries as a scientific basis for the setting of national occupational exposure limits.

The management of the project is given to an expert group. At present the Nordic Expert Group consists of the following member:

Vidir Kristjansson	National Board of Occupational Health, Iceland
Petter Kristensen	National Institute of Occupational Health, Norway
Per Lundberg (chairman)	National Institute for Working Life, Sweden
Vesa Riihimäki	Institute of Occupational Health, Finland
Otto Melchior Poulsen	National Institute of Occupational Health, Denmark

For each document an author is appointed by the Expert Group and the national member acts as a referent. The author searches for literature in different data bases such as Toxline, Medline, Cancerlit and Nioshtic. Information from other sources such as WHO, NIOSH and the Dutch Expert Committee is also used as are handbooks such as Patty's Industrial Hygiene and Toxicology. Evaluation is made of all relevant scientific original literature found. In exceptional cases information from documents difficult to access are used. The draft document is discussed within the Expert Group and is finally accepted as the Group's document.

Editorial work is performed by the Group's Scientific Secretary, Johan Montelius, and technical editing by Ms Karin Sundström both at the National Institute for Working Life in Sweden.

Only literature judged as reliable and relevant for the discussion is referred to in this document. Concentrations in air are given in mg/m^3 and in biological media in mol/l. In case they are otherwise given in the original papers they are if possible recalculated and the original values are given within brackets.

The documents aim at establishing a dose-response/dose-effect relationship and defining a critical effect based only on the scientific literature. The task is not to give a proposal for a numerical occupational exposure limit value.

The evaluation of the literature and the drafting of this document on Dichlorobenzenes was made by Dr Eivor Elovaara at the Finnish Institute of Occupational Health. The final version was accepted by the Nordic Expert Group May 13, 1997, as its document.

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Johan Montelius
Scientific Secretary

Per Lundberg
Chairman

Contents

1. Introduction 1
2. Substance Identification and Physical and Chemical Properties 1
3. Occurrence, Production and Use 4
    3.1. Occurrence 4
    3.2. Production 4
    3.3. Production processes 5
    3.4. Use 5
4. Occupational Exposure and Uptake 6
5. Sampling and Analysis of Work Place Exposure 7
    5.1. Established standard methods for ambient monitoring 7
6. Toxicokinetics 8
    6.1. Uptake 8
    6.2. Distribution 9
    6.3. Biotransformation and elimination 11
    6.4. Relevant kinetic interactions 17
7. Biological Monitoring 18
8. Mechanisms of Toxicity 19
    8.1. Cellular energy metabolism 20
    8.2. Tissue lesions due to metabolic activation and molecular binding 20
        8.2.1. Thyroid 20
        8.2.2. Liver 20
        8.2.3. Kidney 22
    8.3. Binding to α_{2u}-globulin 22
    8.4. DCB-induced cell proliferation 22
9. Effects In Animals and in Vitro Studies 23
    9.1. Irritation and sensitisation 23
    9.2. Single exposure toxicity 29
        9.2.1. Acute liver toxicity 29
        9.2.2. Acute kidney effects 31
        9.2.3. Acute pulmonary effects 32
    9.3. Short- and medium term exposure 32
    9.4. Long-term exposure/carcinogenicity 34
    9.5. Mutagenicity and genotoxicity 35
        9.5.1. In vitro studies 35
        9.5.2. In vivo studies 37
    9.6. Reproductive and developmental toxicity 38
    9.7. Other studies 40
10. Observations in Man 41
    10.1. Acute effects by contact and systemic distribution 42
    10.2. Effects of repeated exposure on organ systems 43
    10.3. Genotoxic effects 44
    10.4. Carcinogenic effects 45
    10.5. Reproductive and developmental effects 45
11. Dose-Effect and Dose-Response Relationships 46
12. Previous Evaluations by (Inter)National Bodies 56

12.1. Carcinogenicity classification 57
13. Evaluation of Human Health Risks 57
13.1. Groups at extra risk 57
13.2. Assessment of health risks 57
13.3. Scientific basis for an occupational exposure limit 60
14. Research Needs 60
15. Summary 62
16. Summary in Swedish 63
17. References 64
18. Data bases used in search for literature 74
Appendix 1. 75

1. Introduction

Dichlorobenzene isomers *ortho*-dichlorobenzene (*o*-DCB) and *para*-dichloro benzene (*p*-DCB) are produced in high amounts, whereas the production of *meta*-dichlorobenzene (*m*-DCB) is low and poorly documented. Commercial products may contain some amounts of the other DCB isomers as well as other (poly)-chlorobenzenes. *o*-DCB is used mainly as a solvent, chemical intermediate, and deodoriser, and *p*-DCB as a deodoriser and insecticide. The use of the *meta* isomer is very limited; it is used as an intermediate compound in the synthesis of other chemicals. Only *o*-DCB and *p*-DCB isomers are commercially important, but *m*-DCB is not. This may explain why the toxicological data on the long-term effects of *m*-DCB are very scarce. The two other isomers, on the other hand, have been studied extensively, including rodent cancer bioassays. In spite of the fact that there are no natural sources for the dichlorobenzenes, they are all found as common environmental pollutants. Hence, all three have been studied for a variety of environmental effects of concern to public health (including non-occupational exposure due to the common use of products containing *p*-DCB, e.g. in homes), and regulations/guidelines have been established for acceptable concentrations in ambient air and standards for the control of drinking water. Dichlorobenzenes have been held to be compounds with a low intrinsic toxicity, requiring activation to induce toxic effects in target organs like the liver, kidneys, thyroid, or spleen. In the literature, most of the toxicological data that are based on industrial experience of dichlorobenzenes (*ortho* and *para*) come from surveys conducted long ago; few reports describe the exposure levels and health effects in work places today.

2. Substance Identification and Physical and Chemical Properties

The substance identification data (Table 1) and the physical and chemical property data (Table 2) of the three dichlorobenzenes are presented in tabular form. Dichlorobenzenes are produced as high purity liquid grade (98-99%), as well as technical grades. Commercial products may contain various amounts of related isomers; e.g., technical *o*-DCB may contain up to 19% of the other two isomers. *p*-DCB is also available as crystals in several particle sizes containing no detectable impurities (88, 89, 153).

Table 1. Substance identification of dichlorobenzene isomers

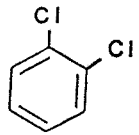
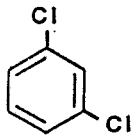
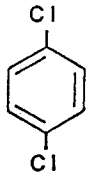
	o-Dichlorobenzene	m-Dichlorobenzene	p-Dichlorobenzene
Common name	o-DCB, ortho-DCB or 1,2-DCB	m-DCB, meta-DCB or 1,3-DCB	p-DCB, para-DCB or 1,4-DCB
CAS name	1,2-dichlorobenzene	1,3-dichlorobenzene	1,4-dichlorobenzene
CAS No	95-50-1	541-73-1	106-46-7
EEC No	602-034-00-7	602-067-00-7	602-035-00-2
EINECS No	202-425-9	208-792-1	203-400-5
IUPAC name	o-dichlorobenzene	m-dichlorobenzene	p-dichlorobenzene
Synonyms	Orthosol, ODB; ODCB; o–dichlorobenzol; o-chlorophenyl chloride; o-phenylene dichloride	m–Dichlorobenzol; MDB; MDCB; m–chlorophenyl chloride	p–Dichlorobenzol; PDB; PDCB; p–chlorophenyl chloride
Trade names	Chloroben; chloroden; dilatin; dizene; Dowtherm E; Termitkil		Di-chloricide; Evola; Paracide; Paradi; Paradow; Paramoth; Parazene; Persia-Perazol; Santochlor
Molecular formula	$C_6H_4Cl_2$	$C_6H_4Cl_2$	$C_6H_4Cl_2$
Molecular weight	147.01	147.01	147.01
Structural formula			
	1,2-Dichlorobenzene (o-DCB)	1,3-Dichlorobenzene (m-DCB)	1,4-Dichlorobenzene (p-DCB)

Table 2. Physical and chemical properties of dichlorobenzene isomers

	o-Dichlorobenzene	m-Dichlorobenzene	p-Dichlorobenzene
Description	Colourless liquid	Colourless liquid	Colourless or white volatile crystals with a penetrating odour. Sublimes at ordinary temperatures.
Melting point	-17 °C	-25 °C	53 °C
Boiling point	180 °C	173 °C	174 °C
Vapour pressure (volatility)	0.20 kPa (25 °C)	0.31 kPa (25 °C)	1.33 kPa (54.8 °C)
Vapour density (air = 1)	5.07	5.07	5.07
Flash point	65 °C	63 °C	65 °C
Autoignition temperature	648 °C	648 °C	648°C
Explosive limits	2.2 - 9.2 %	2.2 - 9.2%	2.2 - 9.2 %
Density	1.306 (20 °C)	1.288 (20 °C)	1.2417 (60 °C)
Refractive index	1.5510 (20 °C)	1.5460 (20 °C)	1.5285 (60 °C)
Solubility in water	140 mg/l (25 °C)	123 mg/l (25 °C)	79 mg/l (25 °C)
Solubility in organic solvents	Miscible with alcohol, ether, benzene	Soluble in alcohol, ether, acetone, benzene	Soluble in alcohol, ether, acetone, benzene, chloroform, carbon disulfide
Partition coefficient (octanol/water)	log P_{OW}: 3.38	log P_{OW}: 3.60	log P_{OW}: 3.37
Other partition coefficients	Water/air: 9.0 Olive oil/air: 39900 Blood/air: 423	Water/air: 5.5 Olive oil/air: 27100 Blood/air: 201	Water/air: 10
Odour threshold	0.3 ± 4.2 ppm (v/v) in air (mean ± S.E.)		0.18 ± 4.1 ppm (v/v) in air (mean ± S.E.)
Conversion factors in air (25 °C, 101.3 kPa)	1 mg/m^3 = 0.1663 ppm 1 ppm = 6.01 mg/m^3	1 mg/m^3 = 0.1663 ppm 1 ppm = 6.01 mg/m^3	1 mg/m^3 = 0.1663 ppm 1 ppm = 6.01 mg/m^3

References for data in Tables 1 and 2: (1, 2, 5, 36, 45, 50, 85-87, 111, 149, 178)

3. Occurrence, Production and Use

3.1. Occurrence

Dichlorobenzenes (*ortho*-, *meta*-, and *para*-) are not known to occur in nature (88). They all are commercially available at high purity levels, usually as a volatile liquid. *p*-DCB is found as liquid mixtures of differential grades of purity, and in pure crystalline forms (153).

Dichlorobenzenes are environmental pollutants with widespread occurrence at varying levels in ambient air, in water, and sediments, in soil, plants, and animal feeds; and in food (drinking water, milk, eggs, pork, chicken, fish, and muscles) (89, 169, 182). For example, a mean atmospheric pollution concentration of 30-60 ppt of *o*-DCB, *m*-DCB, and *p*-DCB was used for describing the average exposure of the population in Netherlands in year 1980 and for calculation of an average daily intake of DCBs to be 7 μg/day (for 20 m^3 of inhaled air and assuming 50% lung retention) (70). Results from Total Exposure Assessment Methodology studies carried out in six cities in US showed that *p*-DCB was an indoor air pollutant (6-71 $\mu g/m^3$), outweighing its presence in outdoor air (0.3-2 $\mu g/m^3$) by more than 20:1 (179). Notably, chlorobenzene congeners (mono- to pentachloroisoforms) are common environmental pollutants due to losses during manufacture and sources relating to their use, and due to environmental fate processes influenced by rates of bioaccumulation and biodegradation (182).

3.2. Production

The most important manufacturing regions for dichlorobenzenes are Western Europe, USA, and Japan. The annual production levels estimated in the USA (182) were in 1980 for *o*-DCB 22 000 tonnes and for *p*-DCB 24 000 tonnes. The production amounts of the *m*-DCB are not available. The annual production volumes of *p*-DCB are high in USA; in 1990 it was 59 000 tonnes (85, 87, 156) . The United States export about 25% of its *p*-DCB production volume (85, 87). The main producer/importer countries of *p*-DCB are in Europe Germany, France, and Italy, and the estimated annual production of *p*-DCB in Europe is 50 000-100 000 tonnes (91). Dichlorobenzenes are not produced in Nordic countries but are imported (Table 3).

Table 3. Annual uses (import) of dichlorobenzenes in the Nordic countries

	Denmark[a] 1994	Finland[b] 1993	Norway[c] 1994	Sweden[d] 1994	Island
o-Dichlorobenzene	5 t		80 t	< 1 t	no data
p-Dichlorobenzene	< 500 kg	}15 t	7 t	no data	no data
m-Dichlorobenzene	< 5 kg	no data	no data	no data	no data

a) Personal communication/Dr. A. Schaich Fries (National Inst. of Occupational Health, Copenhagen, Denmark)

b) National Board of Customs, Finland: Report on Foreign trade, vol. 1 (1993)

c) Personal communication/Dr. P. Kristensen (National Inst. of Occupational Health, Oslo, Norway)

d) Product registry in Sweden (National Chemicals Inspectorate, Sweden)

3.3. Production processes

All chlorobenzenes are produced by direct chlorination of benzene (in the liquid phase) in the presence of a catalyst (usually ferric oxide) and then by fractionation of the resulting mixture of chlorinated benzenes (89, 101). Separation of mixtures containing the DCB isomers is done by distillation and crystallisation (36); the manufacturing processes of DCBs produce as impurities other chlorobenzenes. Dichlorobenzenes may also be produced by Sandmeyer procedure from appropriate chloroaniline or by chlorination of chlorobenzene (36).

3.4. Use

The world-wide production volumes as well as the use patterns of dichlorobenzenes underline that the *ortho* and *para* forms are of major importance. In Nordic countries the information available on the annual uses of DCBs is shown in Table 3.

o-DCB is principally used as a chemical intermediate for manufacturing agricultural chemicals (pesticides) and dye intermediates. It is a solvent for waxes, gums, resins, tars, rubbers, oils, paints, and asphalts and a degreasing agent for metals, leather, hides, and wool. It is an ingredient of metal polishes, firearm cleaners, rust-preventatives, and upper cylinder lubricants and a heat transfer and a coolant for magnetic coils. It is a cleaning agent and a solvent in formulations for removing paints and a carrier for wood preservatives and repellents. It is used for desulphurization of illuminating gas and for dissolution of pitch on paper making felts (36, 89, 150). It is used as a herbicide, insecticide, and soil fumigant (60). Hydrolysis of *o*-DCB with KOH and NaOH gives *o*-chlorophenol, an intermediate for dyestuffs and initiator for higher chlorinated phenols (102). The major uses of *o*-DCB as evaluated in 1978 were in the US: 70% for organic synthesis of pesticides (mainly 3,4-dichloroaniline herbicides); 15% for

solvent in toluene diisocyanate process; 8% for miscellaneous solvent uses; 4% for dyestuffs; and 3% for miscellaneous use (89).

m-DCB is used as fumigant and insecticide (150), in the production of chlorophenols, and arylene sulphide polymers (102).

p-DCB is used as a space deodorant for toilets and refuse containers and as a fumigant for control of moths, moulds, and mildews. Other major uses are as a general germicide, insecticide; in the manufacture of 2,5-dichloroaniline and dyes; as a chemical intermediate; as an ingredient in pharmaceutical products; in agricultural fumigants. Minor uses of *p*-DCB include uses as a deodorant for restrooms, garbage, and in pig stalls; as an insecticide for control of fruit borers and ants; and as an extreme-pressure lubricant (36, 89, 113, 150, 155). It is used in tobacco seed beds for blue mould control; for the control of peach tree borer; and mildew and mould on leather and fabrics (60). It is used as an additive in resin-bonded abrasive wheels to provide a more open structure, and it vaporises during the curing operation leaving pores and wider grain spacing (100). Hydrolysis of *p*-DCB with cupric salts and hydroxylamine gives the *p*-chlorophenols (102). Nitration of *p*-DCB yields 1,4-dichloro-2-nitrobenzene, an intermediate for dyestuff (103). The reaction of *p*-DCB with sodium sulphide in a polar organic solvent produces poly(phenylene sulphide). An engineering plastic used for surface coatings and model resins (104). The major uses of *p*-DCB as evaluated in 1978 were in the US: space deodorant, 55%; moth control, 35%; and other applications, 10% (89).

4. Occupational Exposure and Uptake

Occupational exposure to DCBs usually results from inhalation of the vapour or particulate matter. Available data describing actual human exposure levels at work place are, however, limited.

A National Occupational Hazard Survey in US conducted between 1972 and 1974 estimated that 697 803 US workers are potentially exposed to *o*-DCB and/or *p*-DCB (85, 87). A US EPA report has estimated that 10 000 workers are potentially exposed during production, processing, and industrial solvent use and 2 million workers are potentially exposed for all occupational activities (89). As to its use as an industrial cleaner it has been estimated in US that 200 workers may be exposed to *o*-DCB fumes in transmission shops alone (89).

o-DCB. Occupational exposure occurs during its manufacture and uses as a chemical intermediate and solvent, probable routes of exposure being inhalation of contaminated air and dermal contact. *o*-DCB levels up to 8.5 ppm (51 mg/m^3) has been detected in the air of a chlorobenzene factory (89). In an other study within *o*-DCB industry, the concentrations in workroom air ranged from 1-44 ppm (average 15 ppm) over prolonged follow-up (84).

p-DCB. Occupational exposure by inhalation and dermal routes probably occurs during its manufacture and use as a chemical intermediate. The common

use as a space deodorant and moth control agent is a potential cause of exposure outside the work place.

In work place atmospheres associated with the manufacture of *p*-DCB, air samples showed *p*-DCB concentrations averaging 204 mg/m^3 (42-288 mg/m^3) near shovelling and centrifuging, and 150 mg/m^3 (108-204 mg/m^3) during pulverising and packaging. No concentrations less than 48 mg/m^3 were found (170). Moreover, *p*-DCB air levels of 33-52 mg/m^3 were found in the work place air of a monochlorobenzene manufacturing plant. A chlorobenzene factory was found to contain levels of 144-204 mg/m^3 of *p*-DCB (89). The air in a factory where moth cakes were made contained 54-150 mg/m^3 and the air in an abrasive wheel facility using *p*-DCB in the manufacturing process contained 48-99 mg/m^3 (89).

A study in the softwood hardwood kraft pulp industry (2 plants), where chlorine-containing compounds were used in different bleaching processes, was undertaken for the monitoring of 40 different organohalogen compounds, including the DCBs, in work place air: *p*-DCB was found at low levels in general air samples (<0.8 μg/m^3) (144).

5. Sampling and Analysis of Work Place Exposure

Analytical methods are available for measuring DCBs in environmental media and biological samples are documented for all three isomers (182), or the *p*-DCB (169). Standard methods for GC analysis are available and have been approved by known organisations (EPA, NIOSH) for determination of DCBs in work place air. The use of more advanced methods (GC-MS) for ambient monitoring of work place air allows specific detection of a variety of volatile chlorine compounds including the DCB isomers at concentrations far below the occupational standards (144).

Analysis of DCBs in air is commonly performed by sampling on solid sorbent tubes packed with materials such as activated charcoal, Tenax, coconut shell charcoal, or Amberlite XAD-2 resin. The sample is then desorbed from the adsorbent with a solvent (carbon tetrachloride; carbon disulphide) or thermally after which vapours pass through a cryogenically cooled trap and subsequently are introduced into a gas chromatograph-mass spectrometer. Analysis with gas chromatography is performed with flame ionisation detector, photoionization detector, or mass spectrometer.

5.1. Established standard methods for ambient monitoring:

o-DCB and p-DCB. In the US NIOSH Manuals of Analytical Methods gas chromatographic determination of *o*- and *p*-DCB are described in methods No. S135 and No. S231, respectively (160). These methods were validated for range levels of 150-629 mg/m^3 of *o*-DCB and 183-777 mg/m^3 of *p*-DCB in air. A known volume (3 litres) of air is drawn (by personal sampling pumps) through a coconut

shell charcoal solid sorbent tube to trap the organic vapours present. The analyte is desorbed from the glass tube with carbon disulphide for analysis of unknown, blanks, and standards in a gas chromatograph with flame ionization detector. The original methods have been combined and further developed, and replaced today by a more advanced but essentially similar US NIOSH Method No. 1003-2 (59). This method was revised 1994 and describes the simultaneous determination of both *o*-DCB and *p*-DCB.

o-DCB and m-DCB. The gas chromatographic method approved by US EPA to measure environmental *o*- and *m*-DCB is based on the original paper of Krost et al. (106). Ambient air is drawn through a bed of Tenax-GC to collect the DCB vapours on the resin. The sample was then thermally desorbed and vapours passed through a cryogenically cooled trap and subsequently introduced into a gas chromatograph-mass spectrometer. Estimated detection limits for *m*-DCB is 0.7 ng/m^3 and for *o*-DCB it is 1.0 ng/m^3 (171).

o-DCB, m-DCB, and p-DCB. Langhorst and Nestrick (107) have described a method for simultaneous determination of *o*-, *m*-, and *p*-DCBs in air and biological samples. Chlorobenzenes in air are sampled (over 4 hours) with a solid sorbent tube packed with Amberlite XAD-2 resin. The adsorbed chlorobenzenes is desorbed with carbon tetrachloride for gas chromatographic analysis using photoionization detector. The method was found valid for determination of DCB range levels between 0.03-90 mg/m^3 in air. The detection limits for different chlorobenzenes were for mono-, di-, tri-, tetra- and pentachlorobenzene 0.003, 0.004, 0.007, 0.009, and 0.015 mg/m^3, respectively (171).

6. Toxicokinetics

6.1. Uptake

No studies were located regarding the rate or amount of absorption of the DCBs by humans or animals after inhalation or dermal exposure. In most of the animal studies the DCBs have been given orally, but the rate or amount of absorption of individual DCBs were usually not investigated. In view of available data it appears that the DCBs are readily absorbed at least through the lung and gastrointestinal tract, and that uptake may occur also through the intact skin (169, 172, 182). Relatively low water solubility and high lipid solubility favour their penetration of most membranes by diffusion, including pulmonary and gastrointestinal epithelia, the brain, hepatic parenchyma, renal tubules, and the placenta (170).

p-DCB is apparently well absorbed by the gastrointestinal tract and from lung but not appreciably through intact skin (51, 110).

After intragastric administration of 1.5 g of *p*-DCB to Chinchillas (11) the unchanged compound could not be detected in the faeces during 6 days, implying that total absorption had occurred. Hawkins et al. (73) reported that in rats, exposed repeatedly to ^{14}C-labelled *p*-DCB through inhalation (1000 ppm 3h/day,

for ten days), or by oral or subcutaneous doses (250 mg/kg/day, for ten days), 91-97% of the radiolabel was excreted in urine and only 2-3% in faeces during 5 days. Absorption of *p*-DCB through the gastrointestinal tract is rapid. Oral doses of 200 or 800 mg/kg to male Wistar rats appeared in the blood, adipose, kidney, liver, lung, heart, and brain tissue in 30 minutes; the liver had 2 times and the adipose tissue 10 times the level found in blood. Tissue uptake was highest 6-12 h after the administration (96).

6.2. Distribution

No experimental studies were located regarding tissue distribution of DCBs in humans after inhalation, oral, or dermal exposure. The data available in humans is related to environmental DCB exposure, showing in general population that *ortho* and *para* isomers can be detected in low amounts in blood (≤68 ng/ml) (7, 12, 26, 93, 96, 122, 123), in adipose tissue (≤146 μg/kg, fat basis) (93, 96, 122, 123), and in breast milk (≤640 μg/kg, fat basis) (48, 93).

o-DCB. Tissue distribution of *o*-DCB was investigated at three dose levels (5, 50, and 250 mg/kg) of radiolabelled compound in the male Wistar rat. Highest concentrations of radioactivity after a low dose were found in fat, liver, and kidney at 6 h after single administration, and then declined rapidly. In blood, the radiolabel was highest at 6-8 h for the low and mid-dose level, and at 24 h for the high-dose level (82). A dose-related accumulation of *o*-DCB in the abdominal and renal adipose tissue of rats occurred following administration of a mixture of organic chemicals including *o*-DCB at doses of 0.4, 0.8, or 2 mg/kg diet per day for 4-12 weeks (92). Experimental data describing the tissue distribution of *o*-DCB have been useful for the development of a physiologically based pharmacokinetic model for *o*-DCB in the rat (81).

m-DCB. There are no exposure studies reporting tissue concentrations of *m*-DCB.

p-DCB. In female rats, the tissue distribution was similar after inhalation, oral and subcutaneous exposure to radiolabelled *p*-DCB as well as after single or repeated modes of administration (73). The concentrations were highest in fat, next highest in kidney and liver, and lowest in lungs, muscle, and in plasma. Uptake of *p*-DCB in fat was relatively high (20- to 70-fold higher than in blood).

The organ distribution (serum, liver, kidney, and fat) of *p*-DCB was compared in male and female rats after inhalation of 500 ppm of *p*-DCB for 24 h in a whole-body chamber. Though no sex differences were observed in the serum levels, the *p*-DCB values were significantly higher in the livers of female than in male rats while the *p*-DCB levels measured in the kidneys were significantly higher in the males. The sex-dependent differences in tissue distribution seem to be associated with the findings that nephrotoxic changes were observed only in male rats and that the appearance of minor hepatotoxic changes was limited to females (167). In another study, only minor differences in the distribution and

excretion of *p*-DCB metabolites were observed between male and female Fisher 344 rats following oral administration of *p*-DCB (105).

The tissue distribution of *p*-DCB and 2,5-dichlorophenol (*2,5*-DCP) content was investigated in male Wistar rats after *p*-DCB administration in the diet for 28 days (see Table 4). *p*-DCB and *2,5*-DCP were detected in the plasma of the high-dose animals in which the concentrations decreased rapidly from days 3 and 7, but thereafter the levels obtained (0.5 and 1.0 µg/ml, respectively) decreased very slowly. No chemical was detected in any tissue at 35 days (16). The tissue distribution was studied also in male rats exposed by inhalation to two concentrations of 451 or 3005 mg/m^3 (75 or 500 ppm) *p*-DCB up to 18 months (15), see Table 5.

Table 4. Tissue concentrations of *p*-DCB and 2,5-DCP in rats fed *p*-DCB for 3, 7, and 28 days

p-DCB in diet (mg/kg diet)	Specimen	Day 3		Day 7		Day 28	
		p-DCB	2,5-DCP	*p*-DCB	2,5-DCP	*p*-DCB	2,5-DCP
0.1	Liver (µg/g)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1		1.3	0.2	0.4	0.1	0.5	0.2
0.1	Kidney (µg/g)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1		0.7	0.9	0.3	0.3	0.3	0.5
0.1	Fat (µg/g)	3	N.D.	2	N.D.	2	N.D.
1		49	N.D.	17	N.D.	19	N.D.

N.D. = not detected

Table 5. Tissue concentrations of *p*-DCB and 2,5-DCP in rats after *p*-DCB inhalation exposure for 6 and 18 months (5 hours/day, 5 days/week)

p-DCB in air (ppm)	Specimen	6 Months		18 Months	
		p-DCB	2,5-DCP	*p*-DCB	2,5-DCP
75	Plasma (µg/ml)	N.D.	1.4	N.D.	0
500		1.3	10.4	0.4	2
75	Liver (µg/g)	N.D.	N.D.	N.D.	N.D.
500		5	2.9	2.7	0.2
75	Fat (µg/g)	29	N.D.	1.9	N.D.
500		831	N.D.	120	N.D.

N.D. = not detected

6.3. Biotransformation and elimination

Dichlorobenzenes are efficiently metabolised and eliminated principally in urine. The biotransformation involves phase I (cytochrome P-450 (P450) metabolism) and phase II (conjugation reactions) as well as phase III reactions (enterohepatic circulation of metabolites and their metabolism by intestinal enzymes). Fig. 1 shows the pathways postulated by den Besten and co-workers (52) for the oxidative metabolism of *o*- and *p*-DCBs in rat liver based on identified metabolites. Fig. 2 shows species differences in the metabolism of *o*-DCB in human and rat liver.

o-DCB and p-DCB. The pathways shown in Fig. 1 were evaluated with special attention for metabolic differences that might contribute to the isomer-specific hepatotoxicity. In the assay, the microsomes were from dexamethasone induced male Wistar rats and the incubation time was 2.5 or 15 min. Major metabolites of *o*-DCB and *p*-DCB were *dichlorophenol*s: 2,3-DCP and 3,4-DCP for the *ortho* isomer and 2,5-DCP for the *para* isomer. Oxidation of primary phenols resulted in the formation of major amounts of *dichlorohydroquinones*: 2,3-DICHQ for the *ortho* and 2,5-DICHQ for the *para* isomer, but in minor amounts of *dichlorocatechols* (DICC). The formation of *polar dihydrodiols* appeared to be a major route for *o*-DCB but not for *p*-DCB. Both dichlorobenzenes were oxidised to metabolites that covalently interacted with protein and only to a small extent with DNA. Reactive benzoquinone metabolites appeared to be responsible for the protein binding. The benzoquinones seemed to be formed in a single P450-mediated oxidation of *para*-substituted dichlorophenols (3,4-DCP and 2,4-DCP) while other dichlorophenols, e.g. 2,3-DCP and 2,5-DCP, were oxidised to hydroquinone derivatives, which need prior oxidation to generate the reactive benzoquinone species. According to the authors, reactive intermediates in the secondary metabolism of *o*-DCB lead to more covalent binding than those derived from *p*-DCB.

The pathways in Fig. 1 are supported, but not in all details, by data reported later by authors from the same laboratory. In the work of Hissink and co-workers (79, 82) the biotransformation and urinary elimination of *o*- and *p*-dichlorobenzenes were investigated in rats. The pathways of *o*-DCB metabolism were identified in liver microsomes from male Wistar, Fischer-344, and Sprague-Dawley rats and in pooled human liver microsomes. The metabolism involving reactive epoxide intermediates appeared to play a more important role than the quinone-related metabolism in terms of toxicity induced by *o*-DCB. Studies with rat and human enzymes, suggested that the cytochrome P450 forms CYP2E1 and CYP2B1/2 are mainly involved, and that detoxification is catalysed by phase II enzymes: epoxide hydrolases, UDP-glucuronosyltransferases, sulphotransferases, glutathione S-transferases, and quinone oxidoreductases. The observed differences in the biotransformation and toxicity of *o*-DCB between rat and man have been thoroughly evaluated by Hissink et al. (80), and in Fig. 2, the main differences are schematically summarised as reported by these authors.

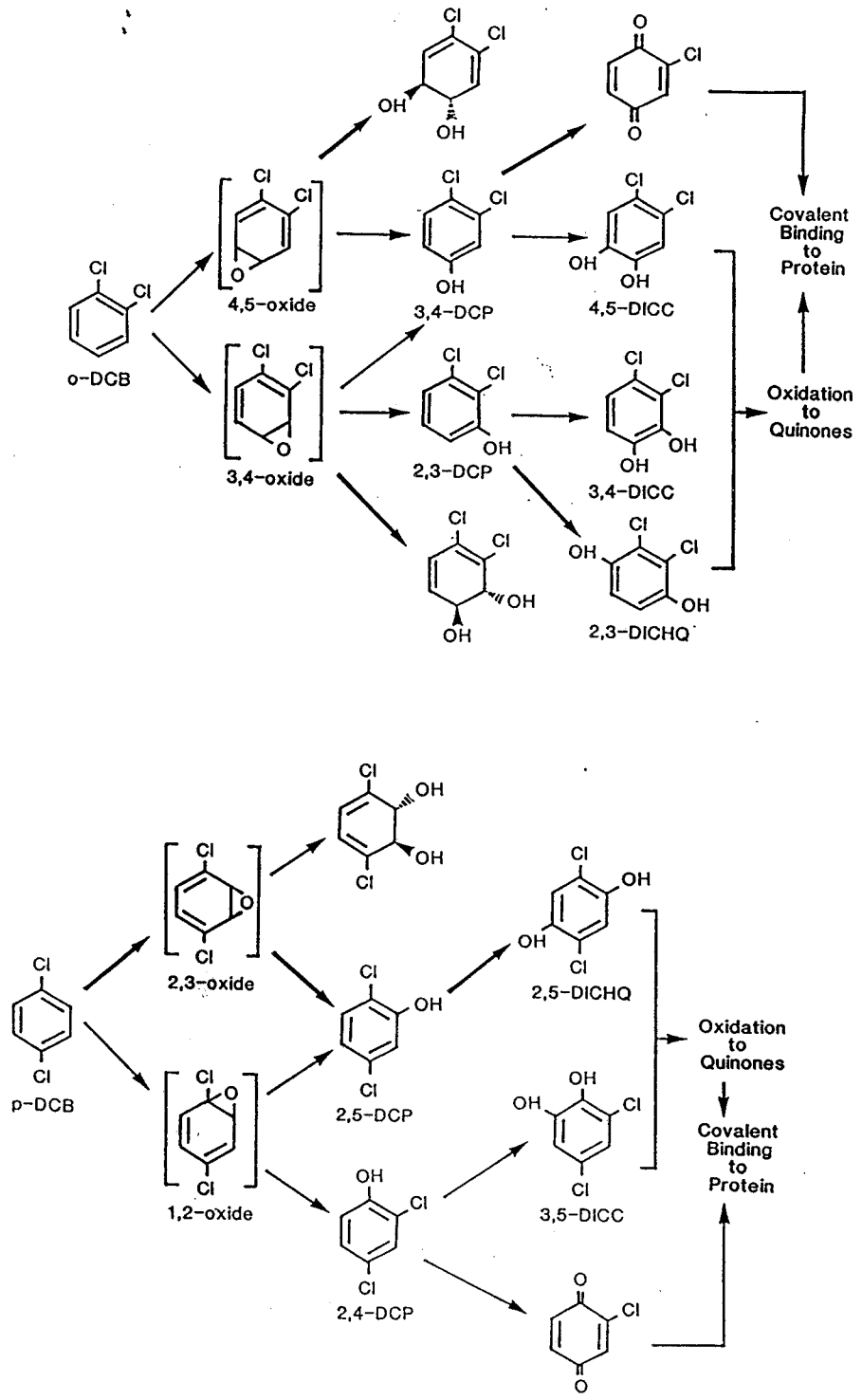


Fig. 1. Pathways of 1,2-dichlorobenzene (*o*-DCB) and 1,4- dichlorobenzene (*p*-DCB) metabolism in rat liver microsomes (52). Thick arrows denote major pathways, thin arrows denote minor pathways. Abbreviations explained in the text.

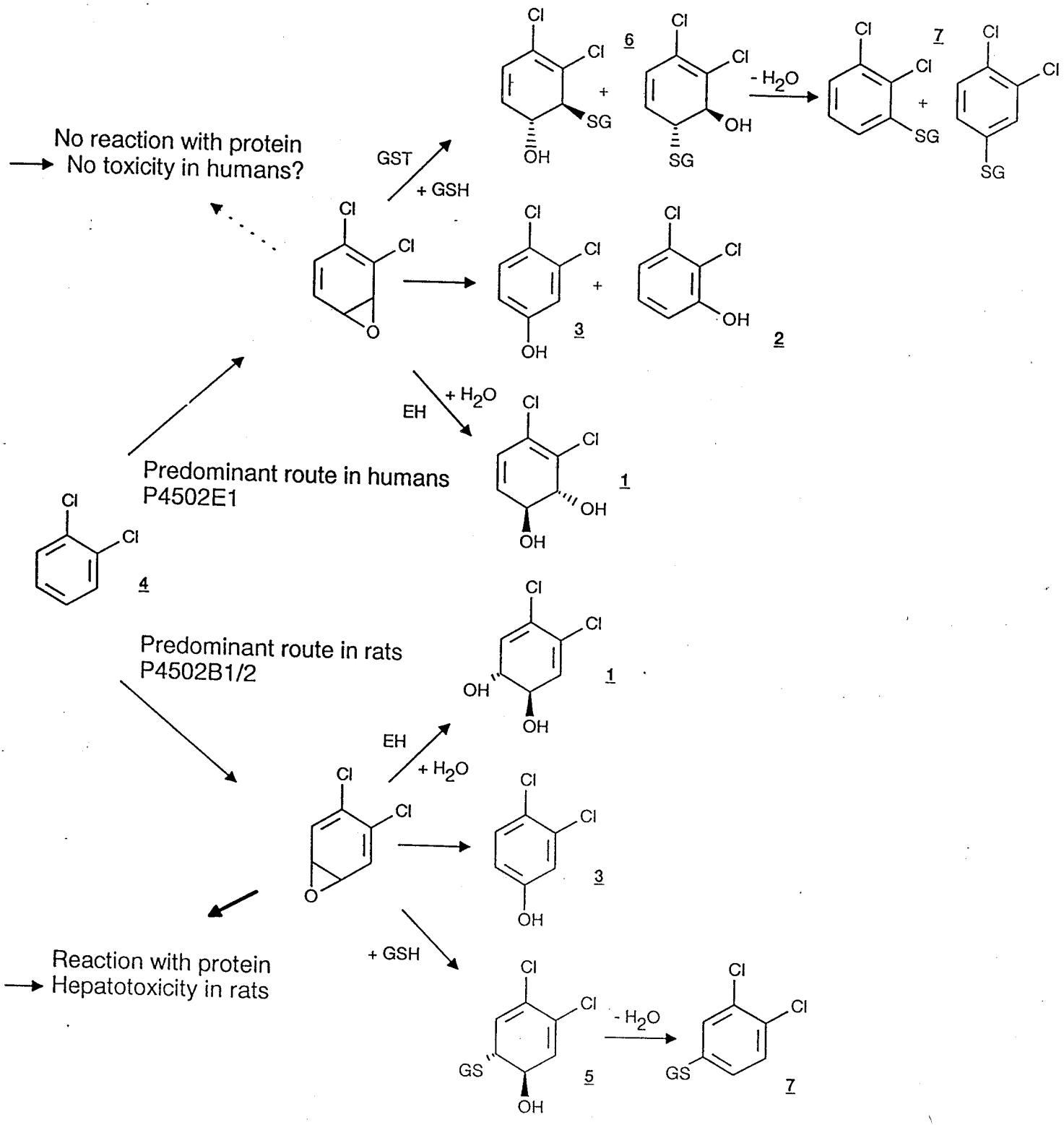


Fig. 2. Pathways of *o*-DCB proposed for the oxidative biotransformation by rat and human liver microsomes. The numbers: 1) Dihydrodiol; 2) 2,3-Dichlorophenol; 3) 3,4-Dichlorophenol; 4) 1,2-Dichlorobenzene; 5, 6 and 7) Glutathione (GSH) conjugates of the epoxides. SG = glutathione derivative, EH = epoxide hydrolase, GST = glutathione S-transferase (80).

o-DCB. The fate of *o*-DCB (radiolabelled) was investigated at different oral dose levels (5, 50, or 250 mg/kg) in the male Wistar rats. The major route of elimination (75-85%) was renal excretion. Faecal excretion ranged from 19% for the low dose to 7% for the high-dose level. Excretion was nearly complete in 24 h for the low and mid-dose level, and within 48 h for the high-dose level. In cannulated rats dosed with *o*-DCB (10 mg/kg), 60% was excreted in bile, 25 % in urine, and < 4 % in faeces, suggesting a considerable enterohepatic circulation in intact rats. The major route of biotransformation was via the glutathione pathway (60 % of the urinary metabolites were mercapturic acids). Other major metabolites (20%) found in urine were sulphate conjugates mostly of 3,4-dichlorophenol and less of 2,3-dichlorophenol. The glutathione-pathway metabolites of bile and urine were, notably, epoxide-derived, whereas no quinone or hydroquinone-derived metabolites were observed (82).

In chinchillas, metabolism of *o*-DCB was studied after a single dose of 500 mg/kg given by stomach tube. *o*-DCB was mainly oxidised to 3,4-dichlorophenol (30%) and 2,3-dichlorophenol (9%), and excreted (primarily in urine) as conjugates of glucuronic and sulphuric acids. Minor metabolites were also excreted as conjugates, including 4,5- and 3,4-dichlorocatechols (3.9%), and 3,4-dichlorophenyl mercapturic acid (5%). The conjugates in the urine were 48% glucuronides, 21% ethereal sulphates and 5% 3,4-dichlorophenyl mercapturic acid. Quinoles were not found. Peak excretion occurred on the first day after dosing. The urinary output of *o*-DCB metabolites was complete in 6 days (10, 11).

m-DCB. Biotransformation of the *m*-DCB in rabbits yields 2,4-dichlorophenol, 3,5-dichlorophenol, and N-acetyl-S-(2,4-dichlorophenyl)-L-cysteine (133) glucuronides (31%), sulphates (11%), mercapturic acid (9%), and catechols (4%) as well as small amounts of 2,4-dichlorophenylmercapturic acid and 3,5-dichlorocatechol (117).

The biotransformation of *m*-DCB into sulphur-containing metabolites (methylsulfonyls) is governed by the mercapturic acid pathway of glutathione conjugates in the liver and by subsequent metabolism in the gastrointestinal tract (enterohepatic circulation) (95, 98, 99). Biosynthesis of the 3,5-dichlorophenyl methyl sulfone (from *m*-DCB) and other methyl sulfones is dependent on the glutathione status in the liver. This has been shown with glutathione depletors (diethylmaleate) which decrease the formation of this metabolite (99).
3,5-Dichlorophenyl methyl sulfone is a potent phenobarbital-like inducer in the rat (97).

The formation of 2,4- and 3,5- dichlorophenyl methyl sulfones has been studied in *m*-DCB exposed rat, as well as their enzyme inducing effect in rat liver. When *m*-DCB was injected intraperitoneally (i.p.) into rats, in which the enterohepatic circulation was interrupted by cannulation of the bile duct, little or no methyl sulfones were detected in blood, liver, kidneys, adipose tissue, or bile. Also in rats, treated with antibiotics and with *m*-DCB, the blood and tissue concentrations of methylsulfonyl metabolites decreased markedly. The formation of methylsulfonyls from *m*-DCB appeared thus to depend largely upon the metabolism by

intestinal microflora. The increasing effects of *m*-DCB administration on aminopyrine and aniline metabolism, and the content of cytochromes P450 and b5 in hepatic microsomes were scarcely observed in the bile duct cannulated and antibiotic pre-treated rats. On the other hand, in rats administered 2,4- or 3,5-dichlorophenyl methyl sulfone, hepatic distribution of each methyl sulfone was similar to that in intact rats, and the degree of increase of the above four parameters was nearly the same as that in the intact rats. The induction of drug metabolising enzymes by *m*-DCB is thus attributable to the action of its methyl-sulfonyl metabolites but not to that of *m*-DCB *per se*. The induction of P450 is associated with an increase in delta-ALA synthetase activity (94, 95).

Dosing rats with *m*-DCB at 1000 mg/kg was porphyrogenic, and at an 800 mg/kg dosage, a biphasic influence on hepatic metabolic activity was noted. This was accompanied by an initial stimulation of delta-amino levulinic acid synthetase activity, and an increased urinary excretion of coproporphyrin, which peaked at one and three days, respectively, and then declined (135).

DCBs may undergo reductive dechlorination resulting in monochlorobenzene production on incubation with intestinal contents of rats. The reductive dechlorination metabolism assayed with the three DCBs was lowest for the *ortho* form. This was consistent with the finding that *o*-DCB tended to accumulate more than the other isomers. The mechanism of the reductive dechlorination is not well understood (164).

p-DCB. In chinchillas, metabolism of *p*-DCB was studied after a single dose of 500 mg/kg given by stomach tube. p-DCB was excreted in the urine as 2,5-dichlorophenol (35% of the dose) and 2,5-dichloroquinol (6%). Metabolites were excreted as glucuronide (36%) and sulphate (27%) conjugates. There was no catechol or mercapturic acid excretion. The urinary excretion peaked on the second day, and it was not complete in 6 days after dosing (11, 133).

Studies in rats following repeated inhalation, oral, or subcutaneous exposure to radiolabelled *p*-DCB showed that 2,5-dichlorophenol (free or as conjugates) was the major metabolite excreted in urine and that 91-97% of the dose was recovered in the urine within 5 days after exposure (73). The metabolism of *p*-DCB proceeds in most animal species probably through microsomal P450-dependent formation of arene oxide intermediates to form the corresponding dichlorophenols (mainly 2,5-DCP; little 2,4-DCP). Dichlorophenols are then readily excreted in urine following conjugation, principally, with glucuronic and sulphuric acids. A competing hepatic reaction to the conjugation of 2,5-DCP may result in minor formation of 2,5-dichlorodihydroquinone which is rapidly conjugated and excreted in urine. Two different mercapturic acids, 2-(N-acetyl-cysteine-S-yl)-1,4-dichlorobenzene and 2-(N-acetyl-cysteine-S-yl)- 2,3-dihydro-3-hydroxy-1,4-dichlorobenzene, have been identified as minor metabolites of *p*-DCB in rat urine. The catechols are minor metabolites in urine. Metabolites detected in the bile are glucuronides and sulphur-containing conjugates (73, 105, 112).

The fate of *p*-DCB was investigated by Hissink and co-workers (79) in male Wistar rats at three different oral dose levels (10, 50, or 250 mg/kg). Excretion

was mainly via the urine (78-85 %) and less via faeces (2-5 %). The major metabolite in urine was 2,5-dichlorophenol, which was excreted as its sulphate (50-60%), glucuronide (20-30%), and in free form (5-10%). Minor metabolites were the N-acetyl-cysteine-S-dihydro-hydroxy-1,4-DCB and N-acetyl-cysteine-S-1,4-DCB (10%). No hydroquinones were found. Biliary excretion ranged from 5% (low dose) to 30% (high dose); the major metabolite was the glucuronide of 2,5-dichlorophenol.

Small amounts (<0.03% of the total dose) of 2,5-dichlorophenyl methyl sulphoxide (M-1), and 2,5-dichlorophenyl methyl sulfone (M-2), have been analysed in 24-h urine following oral administration of *p*-DCB (200 mg/kg) to rats. The level of M-1 in blood was higher than M-2 for 12 h after dosing, but the blood level of M-2 was higher thereafter. The major metabolite of *p*-DCB was 2,5-dichlorophenol in 24-h urine (42% of the dose) (96). DCBs may also be eliminated unchanged in expired air, in urine, and faeces.

2,5-Dichlorophenol has been detected in the urine of workers exposed to *p*-DCB (132) and general population (77, 116).

Comparative studies on DCB isomers. Metabolism has been studied in human and rat liver precision-cut slices which is a cellular test system in which both phase I and phase II enzyme activities are well preserved. Biotransformation rates of *o*-, *m*-, and *p*-DCB to aqueous soluble metabolites (assayed as ^{14}C equivalents) in three human livers showed marked interindividual differences (10-fold) (13). The rank order for DCB-induced toxicity in human liver slices was *m*-DCB > *o*-DCB > *p*-DCB (61). The overall metabolic rate of both the *ortho* and *meta* substituents was clearly greater than that of the *para* isomer in human livers. These findings are similar to those seen in liver slices from Sprague-Dawley rats (62). The metabolic rate assayed with foetal human liver slices displayed a different order: *p*-DCB > *m*-DCB > *o*-DCB. Furthermore, DCB metabolism pathways analysed for glucuronide/sulphate conjugation in comparison with that of GSH/cysteine conjugation suggested that the glutathione-related routes are relatively more important. Significant species differences in the rate of DCB oxidation (and formation of reactive intermediates) were exhibited between rat and man (62, 63).

Human cytochrome P450 (CYP) isoenzymes have been investigated by Bogaards and co-workers (21) *in vitro* to clarify which enzyme and to what extent it is involved in the oxidation of DCBs. The metabolic activity was assayed at a low substrate concentration (100 µM) with microsomes from cell lines expressing one of the human CYP forms: 1A1, 1A2, 3A4, 2E1, or 2D6. CYP2E1 was the most active in production of 3,4-DCP (2180 pmol/min/nmol P450) and 2,3-DCP (980 pmol/min/nmol P450) from *o*-DCB, as well as of 2,5-DCP (335 pmol/min/nmol P450) from *p*-DCB. The 2E1 form was over 10 times more active than 1A2, which was more active than 1A1 and 3A4. The lowest activity was shown by CYP2D6 (<4 pmol/min/nmol P450). When the metabolism of *o*-DCB was measured in microsomes from 22 human livers, up to 5-fold and 8-fold differences were displayed in formation rates of 2,3-DCP (70 to 349 nmol/

min/nmol P450) and of 3,4-DCP (151 to 1210 pmol/min/nmol P450), respectively. The significant correlation shown between *o*-DCB metabolism and the marker activity of CYP2E1 (r = 0.81 or higher) suggested that CYP2E1 is the major enzyme involved in the oxidation of *o*-DCB. Similar findings on enzymes responsible for *o*-DCB metabolism in humans have also been reported by other authors (80).

6.4. Relevant kinetic interactions

In view of the catalytic role of human CYP2E1 in the metabolism of *o*-DCB (80), functional differences in expression of CYP2E1 enzyme in human liver (due to induction or inhibition by other chemicals) may influence the toxicokinetics and thereby individual health risks posed by DCB exposure. Conceivably, heavy alcohol consumption may modulate the metabolic handling of DCBs, either by stimulation (ethanol-induced CYP2E1) or competition (ethanol-mediated inhibition) (141). Studies with human liver slices on DCB-mediated cytotoxicity *in vitro* have more directly suggested interaction by chemicals such as metyrapone or SKF-525A which are inhibitors of cytochrome P450-mediated metabolism (61).

Pre-treatment of male rats with phenobarbital, an inducer of CYP2B1, potentiated the hepatotoxicity of both *o*- and *m*-DCB. In control rats a low dose (30µl/180g rat) of *o*-DCB or *m*-DCB elicited only minimal hepatotoxicity whereas in phenobarbital rats it caused extensive or massive centrolobular liver necrosis. The *para* isomer did not produce liver lesions in control or in phenobarbital-treated rats (27). Similar findings on phenobarbital-dependent potentiation of isomer-specific hepatotoxicity have been reported also by other authors (71, 72, 80, 158). Inhibitors of cytochrome P450 (as SKF-525A, CCl_4, piperonyl butoxide) prevent the hepatotoxicity of absorbed *o*-DCB probably by decreasing its bioactivation and by enhancing the elimination via exhaled air. Notably, Reid et al. reported that *o*-DCB-induced covalent binding and bronchiolar necrosis in rat lung was considerable reduced in animals pre-treated with phenobarbital (137).

The role of cytochrome P450 enzymes has been studied for *o*-DCB mediated toxicity. Enzyme induction of the ethanol-inducible form CYP2E1 (by pyridine) increased hepatotoxicity in male Fischer rats more dramatically than the pre-treatment with phenobarbital (induction CYP2B1 form). Potentiation due to induction of PAH-inducible CYP1A1 isoform (by β-naphthoflavone) was rather small compared with the effects mediated by pretreatments causing induction of CYP2E1 or CYP2B1. Pre-treatment with piperonyl butoxide (inhibits P450 activity) resulted in decreased hepatic and renal toxicity of *o*-DCB (177).

The interactive effects of a previous 4-h inhalation exposure (on first day) to acetone, methylethylketone, methylisobutylketone, or cyclohexanone on DCB-induced liver toxicity in rats was studied after a subsequent 4-h inhalation exposure to *o*-DCB (380 ppm) on the next day. The hepatotoxicity of *o*-DCB was

enhanced by all ketones except acetone which interacted as follows: acetone pre-exposure potentiated (at 4785 ppm), reduced (at 10670 ppm) or suppressed (at 14790 ppm) *o*-DCB-induced hepatotoxicity (28). The biphasic action of acetone may be explained by (i) stimulation of *o*-DCB metabolism (increased toxicity) due to CYP2E1 induction, and by (ii) inhibition of *o*-DCB metabolism (decreased toxicity) in the presence of acetone. Studies with human liver microsomes have shown that acetone inhibits in a dose-dependent manner the formation of 2,3-DCP and 3,4-DCP metabolites from *o*-DCB (21).

Stine et al. (158) studied modulation of DCB isomer-induced hepatotoxicity in male rats. Equimolar doses of *o*- and *m*-DCB depleted intrahepatic glutathione, while *p*-DCB had no effect. Prior depletion of hepatic glutathione with *phorone* markedly potentiated the hepatotoxicity of *o*- and *m*-DCB, while the toxicity of *p*-DCB increased to a far lesser degree. A comparison between Fisher 344 and Sprague-Dawley male rats revealed that the latter strain was relatively refractive to the acute hepatotoxicity of *o*-DCB following i.p. administration of 1.8 or 5.4 mmol/kg.

The toxicity of *o*- and *m*-DCB was influenced by cytochrome P450 enzyme inducers and inhibitors implicating pathways that give rise to electrophilic reactants. The influence by modulators of glutathione metabolism implicate pathways that are required for the detoxification and elimination of electrophilic intermediates and reactive oxygen species formed during the metabolism of *o*- and *m*-dichlorobenzenes.

7. Biological Monitoring

Biological monitoring based on urinary metabolite excretion is recommended in the US (169). The assay of 2,5-dichlorophenol (2,5-DCP) in urine is known as a useful index of exposure to *p*-DCB. However, the colorimetric analysis method used by Pagnotto and Walkley (132) to determine 2,5-DCP should be replaced by more specific procedures. Baselt (14) describes a procedure for gas chromatography with electron-capture detection (ECD). In this method 5 ml urine sample is treated with concentrated acid to hydrolyse conjugated metabolites and then the free 2,5-DCP is extracted into a solvent for direct analysis with ECD gas chromatography. The sensitivity of this method is 5 mg/l. Highly sensitive methods that may be applicable in biological monitoring have been reported with low detection limits (0.5 µg/l) for analysis of DCBs in rat blood with ECD gas chromatography (79, 157).

Pagnotto and Walkley (132) reported that workers exposed to *p*-DCB air concentrations ranging from 7-49 ppm had 10-233 mg/l of 2,5-DCP in the urine at the end of the work shift, and that excretion started very shortly after the exposure had begun, and rose to a maximum at the end of the exposure period. The presence of 2,5-DCP in urine was revealed also by its distinctive odour, noticeable at 100mg/l or even at lower levels. A satisfactory correlation was reported between postexposure urinary 2,5-DCP values and the average air values

of *p*-DCB; about 90-100 mg/l of 2,5-DCP was found in urine at *p*-DCB air concentrations of 33 ppm (198 mg/m^3).

For the biological monitoring of exposure to *o*-DCB, no standard method was located for metabolite screening in human urine. However, there are methods describing simultaneous ECD gas chromatographic separation and quantification of dichlorophenol acetate ester derivatives of 2,3-DCP and 3,4-DCP (the main metabolites of *o*-DCB) as well as of 2,5-DCP (the main metabolite of *p*-DCB) as developed by Bogaars et al. (21) for the assay of dichlorobenzene metabolite formation in human liver microsomes. The applicability of this method in biological monitoring of DCB exposure should be validated as the analytical assay is both specific and sensitive (limit of detection was 0.2 µg/l).

Measurement of the three dichlorobenzenes (ppb levels) in human urine and blood samples by gas chromatography with photoionization detection can be used as a method for simultaneous exposure monitoring of all three isomers (107).

Data from the monitoring of *p*-DCB exposure among 4 men for 5 days in a chemical factory showed a significant correlation (r=0.64, p=0.01) between the increase in *p*-DCB concentration in worker's urine over the whole work shift (ranging from 17.5 to 55.9 µg/l) and the time weighted levels of *p*-DCB in personal air samples (ranging from 24.9 to 77.8 mg/m^3). The authors proposed a biological exposure index (B.E.I.) of 250 µg/l for exposure to *p*-DCB at a daily environmental vapour level of 450 mg/m^3 (75 ppm) (66).

DCB concentrations in human blood (≤68 µg/l), urine (≤39 µg/l), exhaled air (≤5µg/m^3), and adipose tissue (≤11.7 mg/kg) of the general population have shown that non-occupational exposure is not uncommon and may even be relatively high (12, 26, 122, 123). Exposure to DCBs probably occurs through consumption of contaminated drinking water and food (particularly fish) or through inhalation of contaminated ambient air (179, 180); exposure to *p*-DCB products (mothballs, toilet deodorants), commonly used in homes and public restrooms, results in measurable levels of 2,5-dichlorophenol metabolite excretion in human urine (77, 169). In a recent study of 1000 adults living throughout the United States, 98 % had detectable levels of 2,5-dichlorophenol in their urine, and 96% had detectable levels of p-dichlorobenzene in their blood. Urinary 2,5-dichlorophenol concentrations ranged up to 8700 µg/l (median and mean concentrations of 30 µg/l and 200 µg/l, respectively) (78).

8. Mechanisms of Toxicity

According to structure-activity comparison studies, the differences in toxicity are much greater within the group of mono- to hexachlorobenzene congeners than within the dichlorosubstituted benzenes (182). To predict dichlorobenzene exposure related health risks in humans, the understanding of mechanistic differences in isomer-specific and species-specific toxicity is of importance.

8.1 Cellular energy metabolism

Dichlorobenzene studies in rat liver mitochondria preparations suggest that the isomers may act in a decreasing order of potency *o*-DCB > *m*-DCB > *p*-DCB as uncouplers of the mitochondrial oxidative phosphorylation, and that this effect is paralleled by K^+ release from the mitochondria (131). DCBs may function as agents blocking mitochondrial respiration, and may thereby cause hypermetabolism which in turn could explained DCB-induced weight losses observed in animals in spite of increased food and water consumption (115).

8.2. Tissue lesions due to metabolic activation and molecular binding

8.2.1. Thyroid

The most severe organ effects caused by dichlorobenzenes have been observed on the thyroid, the liver, and the kidney (53, 115). In male Wistar rats, plasma thyroid hormone levels (thyroxine and triiodothyronine) decreased clearly more after a single exposure to *o*-DCB than to the *p*-DCB (1 or 2 mmol/kg, i.p.). These findings were explained by the formation of reactive metabolites formed probably in the liver from dichlorobenzenes (probably phenols) that displace thyroxine from its binding sites on transthyretin, the major plasma transport protein in the rat, and by isomer-specific biotransformation that renders the *ortho* form more toxic than the *para*. It is unclear whether the reduction of plasma thyroid hormone is also due to some alterations in hepatic thyroxine metabolism (53).

8.2.2. Liver

o-DCB and m-DCB. Biotransformation appears to play a key role in the initiation of acute hepatic injury induced by *o*-DCB or *m*-DCB administration. Centrolobular hepatic necrosis in rat and mouse is associated with covalent binding of high amounts of radiolabel from C^{14}-DCB (probably mediated by active metabolites) and depletion of tissue *glutathione* in the liver (27-30, 53, 84, 138). Strain differences occur in *o*-DCB-induced hepatotoxicity; male Fisher 344 rats are much more susceptible than Sprague-Dawley rats (158). Deficient detoxication capacity due to low epoxide hydrolase enzyme activity in Fisher rats is one of the likely explanations (81). Differences in strain-specific metabolism of *o*-DCB are studied to explain differences in hepatotoxicity (63, 72, 158). Reactive oxygen species released from Kupffer cells seem to play a major role in the progression of *o*-DCB hepatotoxicity in the Fischer 344 rat (71).

o-DCB and p-DCB. Microsomal oxidation (phase I) of DCBs to reactants responsible for tissue toxicity is mediated by arene epoxides and benzoquinones. Isomer-specific toxicity, tested *in vitro,* appears to be better explained by benzoquinones than by arene oxides (see Fig. 1). Both *o*- and *p*-DCB were oxidised *in vitro* to metabolites that covalently interacted with protein but only to a small extent with DNA. Protein binding was inhibited by ascorbic acid which converted reactive benzoquinone metabolites into nonreactive hydroquinones and catechols. In the presence of ascorbic acid, a substantial amount of protein-bound meta-

bolites of *o*-DCB was still observed in contrast to *p*-DCB which showed nearly no binding. This was explained, in the case of *o*-DCB, by a direct formation of reactive benzoquinone metabolites in a single P450-mediated oxidation of *para*-substituted dichlorophenols (such as 3,4-DCP). In contrast, the major phenol isomer derived from *p*-DCB (i.e. 2,5-DCP) is oxidised to its hydroquinone derivative, from which the reactive benzoquinone species can be generated only by further oxidation. Residual protein binding in the presence of ascorbic acid could also indicate involvement of reactive arene oxides in the protein binding of *o*-DCB, but not of *p*-DCB. However, molecular orbital computer calculations did not provide indications for differences in chemical reactivity and/or stability of the various arene oxide/oxepin tautomers that can be formed from either *o*-DCB or *p*-DCB. Reactive intermediates in the secondary metabolism of *o*-DCB lead to more covalent binding than those derived from *p*-DCB, which correlates with their reported hepatotoxic potency (52). It should be underlined that the urine and bile metabolite profile studies by Hissink et al. (79, 82) implicated epoxides rather than quinones/hydroquinones as the active intermediates formed *in vivo* in the rat.

When hepatic and renal toxicity mechanisms were examined in male Wistar rats after a single i.p. administration of 1, 2, or 4 mmol/kg *o*-DCB or *p*-DCB (53), tissue glutathione status was strongly decreased, but only in the liver and only after *o*-DCB administration. Severe hepatotoxicity was observed only after exposure to *o*-DCB but not to *p*-DCB. Glutathione depletion in the liver occurred before the elevation of plasma ALT levels. Changes in the kidney (the target organ for *p*-DCB in male rats) showed as only effect protein droplets in the tubular epithelial cells at 72 h after *p*-DCB administration. In light of this study, the hazardousness by *o*-DCB (hepatotoxicity) was relatively greater than by the *p*-DCB (nephrotoxicity).

p-DCB. The lack of acute hepatotoxicity of *p*-DCB seems to result from its limited biotransformation and formation of low amounts of reactive metabolites (52, 79, 158). Covalent binding of radiolabelled *p*-DCB to liver macromolecules (as compared to that of the hepatotoxic *ortho* isomer) has been low in different rat strains, Sprague-Dawley (138), Fischer 344 (158), and Wistar (109).

Modulation of phase I (cytochrome P450) and phase II (mainly glutathione) metabolism has resulted in observations suggesting that toxicity evoked by dichlorobenzenes is determined by their metabolism. For example, treatment of mice with P450 enzyme inhibitors, carbon disulphide, metyrapone or piperonyl butoxide, prevents *p*-DCB-induced hepatotoxicity. Peroral (p.o.) administration of *p*-DCB (100-400 mg/kg) to mice pre-treated with buthionine sulfoximine (an inhibitor of glutathione synthesis) resulted in dose-dependent hepatotoxicity as judged by increased serum ALT activities and liver calcium concentrations and histological examination, whereas *p*-DCB administration alone (up to 1200 mg/kg) resulted in no hepatotoxicity. These results suggested that enhanced hepatotoxicity is caused by cytochrome P450-dependent activation and inadequate rates of detoxification (119).

8.2.3 Kidney

Although the *o*-DCB is recognised primarily as a hepatotoxicant, also nephrotoxicity is observed in rats at high dosage levels. In general, *ortho* substitution enhanced hepatic and renal toxicity, the liver being more sensitive than the kidney. *o*-DCB-induced kidney lesions are not believed to be mediated by the same mechanism as those ascribed for the *p*-DCB (41, 176).

The biotransformation of *m*-DCB that involves enterohepatic handling has been implicated in the production of sulphur-containing metabolites (3,5-dichlorophenyl methyl sulphones) which can be identified in α_{2u}-globulin nephropathy as methylsulphone- α_{2u}-globulin complexes in rat kidney (108).

8.3. Binding to α_{2u}-globulin

Exposure to *p*-DCB (but not to *o*-DCB) produce the male rat specific α_{2u}-globulin nephropathy, which has been associated with the carcinogenic effects of *p*-DCB in male rat kidney. This syndrome involves cellular exfoliation and restorative cell proliferation (22, 41, 108, 126, 147, 166, 167). The α_{2u}-globulin is a soluble protein produced in large quantity in livers of young adult males, secreted into the blood, filtered at the glomerulus, excreted into urine or reabsorbed in the kidney. *p*-DCB and/or its metabolite (2,5-dichlorophenol) binds to α_{2u}-globulin and causes accumulation of hyaline droplets in the lysosomes of kidney proximal tubule epithelial cells. The *p*-DCB-induced hyaline droplets contain a DCB(derivative)-α_{2u}-globulin complex that is resistant to catabolism by the lysosomal proteases. Accumulation of protein droplets filled with this complex leads to cell death and subsequent cell proliferation. Lack of nephropathy and renal tumours in male rats after long-term (76-week) inhalation of *p*-DCB at a vapour level of 500 ppm (112) provide evidence that there is a threshold for renal effects even in male rat, and that effects obtained at high doses in the rat are not predictive for man.

8.4. DCB-induced cell proliferation

o-DCB and m-DCB. Acute hepatotoxicity studies which involved hepatocyte replication measurements in addition to assessment of serum ALT activity and hepatic histology were carried out in male B6C3F1 mice after oral administration of DCB isomers. The results from this work suggested that the hepatocyte proliferation induced by *o*-DCB or *m*-DCB is compensatory regeneration while that induced by *p*-DCB is a response to mitogenic stimulation (165).

p-DCB. Carcinogenicity of *p*-DCB reported in male and female mouse liver and male rat kidney (130) has stimulated a number of mechanistic work. At the dosage levels used in the cancer bioassay, cell proliferation activity is greatly enhanced in the male rat kidney relative to controls, indicating that *p*-DCB may induce kidney tumours by a non-genotoxic/cytotoxic mode of action that is mediated by *p*-DCB(derivative)-α_{2u}-globulin complex (57, 168).

Eldridge and co-workers (56) studied the relationship between *p*-DCB induced hepatocellular proliferation activity and its tumour formation activity in the liver. Single doses of *p*-DCB given by gavage to mice (600 mg/kg/day) and to rats (300 mg/kg/day) produced a burst of cell proliferation and an increase in liver weight. However, no necrosis or releases of liver-associated enzymes into the serum were seen at studied doses. In a 90-day cell proliferation study, no liver necrosis was seen, either. Nevertheless, there was a dramatic induction of cell proliferation and increase in weight in the mouse liver during the first week of exposure at the doses, which produced cancer. These experiments indicated that *p*-DCB may not operate through a cytotoxic mode of action in the formation of mouse liver tumours. The data suggested that *p*-DCB produces a mitogenic induction of liver cell proliferation. *p*-DCB also induced cell proliferation in the female rat liver, even though no induced rat liver tumours were seen. From this it was concluded that induced proliferation may be viewed as a necessary, but not sufficient, event for tumour formation (37, 55).

9. Effects In Animals and in Vitro Studies

9.1. Irritation and sensitisation

o-DCB. Dichlorobenzenes may be regarded as chemicals acting primarily as irritants of the upper airways. This has been generally observed in animal experiments (see Table 6). Irritation has been evaluated for *o*-DCB also by means of a standard method based on the mouse oronasal 15-min exposure test for determination of the concentration that produces a 50% decrease in the respiratory rate (RD_{50}). The RD_{50} value of *o*-DCB was 1088 mg/m^3 (181 ppm). The lowest test level was 697 mg/m^3 (116 ppm) which decreased significantly (26% below normal) the respiratory rate. As the *o*-DCB exposure concentration for RD_{50} was one quarter of that required to produce centrilobular liver cell injury (indexed at a 50% decrease in liver glucose-6-phosphatase activity), the authors concluded that *o*-DCB acts primarily as an irritant (49).

Signs (slight to moderate) that were interpreted as pain as well as conjunctival irritation were observed in rabbit eye following administration of two drops of *o*-DCB (ca 100 mg for 30 s before rinsing the eye with water); recovery was complete within 7 days. Moreover, it was shown that a single 7-h vapour exposure to 3239 mg/m^3 (539 ppm) *o*-DCB caused eye irritation in rats (84).

p-DCB. Guinea pigs were exposed to 50 ppm (300 mg/m^3) *p*-DCB for 12 weeks to test sensitisation. Two weeks post-exposure, an intravenous injection of 0.5 ml of a mixture of *p*-DCB and serum albumin to guinea pigs did not provoke an anaphylactic reaction (159).

Table 6. Effects of dichlorobenzenes in animals after single or short-term exposures

Species (m, male f, female)	Route of administration	Exposure data	Effect	Reference
o-DCB				
rat (m)	inhalation	9207 mg/m^3 (6 h)	LC_{50} (hypotonia, somnolence, lachrymation)	23
mouse (f)	inhalation	7428 mg/m^3 (6 h)	LC_{50}	23
guinea pig	inhalation	6000 mg/m^3 (20 h)	All died; narcotic; liver and kidney injury	32
rat (m)	inhalation	5872 mg/m^3 (7 h)	Lethal to most animals (in 3 days); eye irritation; difficulty to breath; anaesthesia; liver (centrolobular necrosis); kidney (cloudy swelling of tubular epithelium)	84
rat (m)	inhalation	5872 mg/m^3 (2 h)	All survived	84
rat, mouse, guinea pig	inhalation	4808 mg/m^3	Irritation (eyes, nose), drowsiness, coma, some deaths	32
rat (m)	inhalation	3239 mg/m^3 (539 ppm): 3 h or 7 h	All survived. Irritation (eye). Increased weight of liver (centrilobular necrosis) and kidney (cloudy swelling of tubular epithelium)	84
mouse (m)	inhalation	2356 mg/m^3 (4 h)	Centrilobular liver injury (mild)	49
rat (m)	inhalation	2218 mg/m^3 (4 h)	Increased serum enzymes (glutamate and sorbitol dehydrogenase) and liver cell injury (mild). NOAEL =1478 mg/kg	29
rat (m)	inhalation	1833 mg/m^3 (6 h/d, 2-4 days)	Increased serum levels of liver enzymes	30
mouse (m)	oronasal	1088 mg/m^3 (15 min)	50% decrease of respiration rate (RD_{50})	49

Cont.

Table 6. Cont.

Species (m, male f, female)	Route of administration	Exposure data	Effect	Reference
mouse (m)	intraperitoneal	1228 mg/kg	LD_{50}	120
mouse (m)	intraperitoneal	1014 mg/kg	Bronchial epithelial cell necrosis	137
rat (m)	intraperitoneal	50, 100, 250, 300, 800 mg/kg	Sperm abnormalities (dose-dependent effect)	124
rat (m)	intraperitoneal	735 mg/kg	Increased bile duct-pancreatic fluid flow and low fluid protein concn. Serum ALT normal.	183
rat (m)	intraperitoneal	588 mg/kg	Kidney histopathology normal	53
mouse (m)	intraperitoneal	375 mg/kg/day (2 days)	Bone marrow clastogenicity (similar response level for all isomers)	120
rat (m)	intraperitoneal	147 mg/kg	Increased plasma ALT/AST; decreased plasma thyroid hormone	53
rat (m)	intraperitoneal	220 mg/kg	Glycogen loss, minimal liver necrosis	27
guinea pig	oral	3375 mg/kg	LD_{50}	51
rat	oral	2138 mg/kg	LD_{50}	51
guinea pig (m, f)	oral	2000 mg/kg	All died	84
mouse	oral	2000 mg/kg	LD_{50}	51
rabbit	oral	1875 mg/kg	LD_{50}	51

Cont.

Table 6. Cont.

Species (m, male f, female)	Route of administration	Exposure data	Effect	Reference
rat (m)	oral	1784 mg/kg	All survived, liver necrosis, little hepatobiliary damage	4
mouse (m)	oral	300 mg/kg	Liver necrosis, increased liver weight and serum ALT	165
rat (m)	oral	172 mg/kg	Liver necrosis, increased serum ALT/AST	4
rat (m)	oral	98 mg/kg	Liver degeneration (early centrilobular lesions)	4
rat (m)	oral	75 mg/kg	Cytochrome P450 destruction	4
guinea pig (m, f)	oral	800 mg/kg	All survived. Transient loss of body weight	84
rabbit	subcutaneous	653 mg/kg (3 doses)	Fall in white cell count, leucopenia, agranulocytosis	32
m-DCB				
mouse (m)	intraperitoneal	1061 mg/kg	LD_{50}	120
mouse (m)	intraperitoneal	263 mg/kg/day (2 days)	Bone marrow clastogenicity (similar response level for all isomers)	120
rat (m)	intraperitoneal	220 mg/kg	Normal to minimal liver necrosis (rarely)	27
rat (m)	oral	1481 mg/kg	All survived, liver necrosis, little hepatobiliary damage	4
rat (m)	oral	450 mg/kg	Liver necrosis, increased serum ALT/AST	4

Cont.

Table 6. Cont.

Species (m, male f, female)	Route of administration	Exposure data	Effect	Reference
mouse (m)	oral	300 mg/kg	Liver necrosis, increased liver weight and serum ALT	165
rat (m)	oral	129 mg/kg	Liver degeneration (early centrilobular lesions)	4
mouse (m)	oral	300 mg/kg	Liver necrosis, increased liver weight and serum ALT	165
p-DCB				
rat (f)	inhalation	6010 mg/m^3 (4h)	maximum tolerated level for repeated daily exposure	73
mouse (m)	intraperitoneal	4557 mg/kg	No histopathologic changes in the lung	137
mouse (m)	intraperitoneal	2000 mg/kg	LD_{50}	120
rat (m)	intraperitoneal	800 mg/kg	Sperm abnormalities	125
rat (m)	intraperitoneal	735 mg/kg	NOAEL (Bile duct-pancreatic fluid flow and fluid protein. Serum ALT)	183
rat (m)	intraperitoneal	588 mg/kg	Increased plasma ALT/AST	53
rat (m)	intraperitoneal	560 mg/kg	Little or no liver necrosis	27
mouse (m)	intraperitoneal	533 mg/kg/day (2 days)	Bone marrow clastogenicity (similar response level for all isomers)	120
rat (m)	intraperitoneal	294 mg/kg	Decrease in plasma thyroxine	53
rat (m)	intraperitoneal	147 mg/kg	Protein droplets in renal tubular epithelial cells	53
guinea pig	oral	7595 mg/kg	LD_{50}	51

Cont.

Table 6. Cont.

Species (m, male f, female)	Route of administration	Exposure data	Effect	Reference
rat (m)	oral	3863 mg/kg	LD_{50}	64
rat (f)	oral	3790 mg/kg	LD_{50}	64
mouse	oral	3220 mg/kg	LD_{50}	51
rabbit	oral	2812 mg/kg	LD_{50}	51
rat (m)	oral	2790 mg/kg	All survived, no liver necrosis, no increased serum ALT/AST, no hepatobiliary damage	4
rat	oral	2512 mg/kg	LD_{50}	51
mouse (m)	oral	1800 mg/kg	Hepatocyte proliferation (mitogenic stimulation).	165
guinea pig (m, f)	oral	1600 mg/kg	All survived. (LD_{100}=2.8 g/kg)	83
rat (m, f)	oral	1000 mg/kg	All survived. (LD_{100}=4.0 g/kg)	83
rat (m)	oral	475 mg/kg (24h)	Liver degeneration (early centrilobular lesions)	4
rat (m)	oral	220 mg/kg (7 days)	α_{2u}-globulin in urine and kidney; hyaline droplets in renal tubular epithelial cells	148
rat (m, f)	dermal	>6000 mg/kg	LD_{50}	64

The sensitising potential of *p*-DCB was studied (25) in the guinea pig maximisation test (GPMT) according to Magnusson and Kligman (114) and in conformance with the EC and OECD guidelines. Twenty-four female guinea pigs were used as test subjects and another 24 females made up the control group. Five of the animals in the *p*-DCB treated group and one animal in the control group had positive skin reactions after challenge exposure to 25% *p*-DCB in petrolatum. According to the authors, 16.7% of the animals in the test group should be regarded as sensitised (25), *p*-DCB was therefore classified as a mild (grade II) sensitiser according to the Magnusson and Kligman classification (114).

9.2. Single exposure toxicity

Dose-response relationship data reported for acute effects of dichlorobenzene isomers in animal experiments are summarised in Table 6.

o-DCB. Dichlorobenzene animal inhalation studies have mostly been carried out with the *ortho* isomer and at relatively high vapour concentrations. Available data on lethal/narcotic concentrations in rat, mouse, guinea pig, and rabbit, appear not conclusive for evaluation of species differences in acute toxicity. Hollingsworth et al. (84) exposed altogether 95 male rats for single periods (1, 2, 7, or 10 h) to *o*-DCB vapour at different concentrations (539 to 977 ppm), thereby showing consistent dose-dependent effects. Rats survived for 2 h at the highest concentration (977 ppm) but succumbed to an exposure of 7 h or 10 h. However, 7 h exposure to 3239 mg/m^3 (539 ppm) caused no deaths. During the exposure rats exhibited drowsiness, unsteadiness, eye irritation, difficulty in breathing, and anaesthesia. In male Swiss mouse (10/group) inhalation exposure to 7 different vapour levels in the range 2356-5865 mg/m^3 (392-976 ppm) *o*-DCB for 4 h was not lethal (49). Nominal concentration of 1000 ppm of *o*-DCB was lethal to guinea pigs after 20 h (narcosis, injury to the liver and kidney) while effects of a similar exposure on rabbits were less lethal (32).

m-DCB. No inhalation exposure data was found for the *meta* isomer.

p-DCB. Groups of female rats (four/group) were exposed for 4 h by inhalation to different air concentrations of *p*-DCB and observed for adverse reactions. In these experiments, a concentration of 1000 ppm (6000mg/m^3) was found to be the ’maximum’ tolerated level for repeated daily exposure of rats by inhalation (73). A single 24-h inhalation exposure to 500 ppm of *p*-DCB was well tolerated by male and female rats (167). It may be summarised that inhalation of dichlorobenzene vapour concentrations around 1000 ppm (6000 mg/m^3) are lethal in rodents.

9.2.1. Acute liver toxicity

o-DCB. Liver damage has been investigated in male Sprague-Dawley rats after inhalation exposure to different sublethal concentrations. In the first study serum enzyme activities showed dose-dependent increases when measured 24 h after a single 4-h exposure to *o*-DCB (204, 305, 426, 607, or 774 ppm). At 1854 mg/m^3 (305 ppm), serum glutamate dehydrogenase (GLDH) and sorbitol dehydrogenase

(SDH) increased over 3-fold while the glutamic pyruvic transaminase (ALT) and glutamic oxaloacetic transaminase (AST) activities increased only 1.3-fold or less. Serum GLDH and SDH were more sensitive indicators of toxicity than the aminotransferases. A single 4-h exposure caused, however, a greater increase in enzyme activities than a 2 or 4 times repeated 6-h-per-day exposure at 309 ppm (30). In the second study, rats were examined for acute liver toxicity after a single 4-h exposure to *o*-DCB (245, 369, 610, or 739 ppm). The findings (at 369 ppm or higher) showed increases in serum GLDH and SDH activities and decreases in centrolobular liver cell glucose-6-phosphatase (G-6-P) staining intensity. Exposure levels showed a linear correlation with the logarithmic values of blood enzyme activities while the relationship between the logarithmic values of blood enzyme activities and the liver G-6-P staining intensity showed a linear inverse correlation (29). In a later study, Brondeau et al. (28) reported that groups of rats exposed to 374-386 ppm *o*-DCB for 4 h exhibited 24 h postexposure at average 7- to 13-fold increased serum GLDH activities, slightly increased liver glutathione S-transferase activities but no effect on liver cytochrome P450 protein content. Also groups of OF1 mice were examined for G-6-P activity (48 h after being) exposed to *o*-DCB at 244-263 ppm for 4 h; no constant effect on liver G-6-P staining intensity was observed. A single 7-h vapour exposure of rats to 3239 mg/m^3 (539 ppm) of *o*-DCB caused central lobular necrosis in liver (84).

m-DCB. Centrolobular hepatic necrosis is an acute toxic effect reported to be commonly observed in rodents both after *o*- and *m*-DCB exposure, to be less severe in rats (but not in mice) after administration of the *meta* isomer, and to be associated with covalent binding and depletion of tissue glutathione in the liver, and with leakage of liver enzymes into serum (4, 27, 99, 158). Experiments in male B6C3F1 mice have shown that m-DCB was more hepatotoxic than o-DCB after a 200 mg/kg single dose and that cell proliferation induced by exposure was compensatory to centrilobular hepatocellular injury (165).

p-DCB. No hepatotoxicity could be observed in male ddY mice administered *p*-DCB (up to 1200 mg/kg, p.o.) as examined 30 h after dosing for plasma ALT activities, liver calcium concentrations, and by liver histology (119).

Comparative studies on DCB isomers. Stine et al. (158) studied DCB isomer-induced hepatotoxicity in Fisher-344 rats pre-treated with known modulators (phenobarbital, SKF-525A, phorone) of liver metabolism (see section 6.4.). Severe centrilobular hepatic necrosis and high plasma ALT levels were observed after single doses of 1.8-5.4 mmol/kg of *o*-DCB but not after *p*-DCB administration. The *m*-DCB produced intermediate hepatic injury and moderate increases in plasma ALT levels following doses at 2.7-5.4 mmol/kg. Equimolar doses of *o*- and *m*-DCB depleted intrahepatic glutathione, while *p*-DCB had no effect. Comparisons between Fisher 344 and Sprague-Dawley male rats revealed that the latter strain was relatively refractive to the acute hepatotoxicity of *o*-DCB following i.p. administration of 1.8 or 5.4 mmol/kg. The toxicity of DCBs seems to be strongly dependent on isomer-specific metabolism and also readily influenced by metabolic modifiers in studied animals.

Female Wistar rats treated with each isomer of DCB in an oral dose of 250 mg/kg once daily for 3 days showed an increase in liver weights. *m*- and *o*-DCB significantly enhanced cytochrome P450-dependent hepatic activities of aminopyrene demethylase and/or aniline hydroxylase. The activity of delta-aminolevulinic acid synthetase was increased by all DCB isomers (8).

Administration of a single i.p. dose of *o*- or *p*-DCB (=<4 mmol/kg) produced only in *o*-DCB-treated male Wistar rats severe hepatotoxic effects as indicated by glutathione depletion, increased plasma ALT, and centrilobular hyperthrophy and hepatocellular degeneration (53). When DCB-induced liver toxicity was studied in male Fischer rats after a single i.p. dose (=<4 mmol/kg), plasma ALT activity was increased by *o*-DCB as function of the dose. Centrilobular necrosis was observed in rats treated with *o*-DCB while in rats treated with *meta* or *para* isomer the morphology was relatively normal as also plasma ALT levels (176).

In the study of Allis et al. (4), Fisher 344 rats were gavaged with, in up to 25 different dosages, of DCBs (<2800mg/kg) and then evaluated 24 h later. Centri-lobular hepatic necrosis and elevations in serum ALT and AST proved that *o*-DCB was the most toxic in terms of both earliest onset and degree of response at higher dosages whereas *m*-DCB was clearly less toxic. *p*-DCB did not cause above-mentioned changes, but it caused centrilobular hepatic degenerative changes with doses from 475 mg/kg and upwards. There was little indication of hepatobiliary damage as the measurements of serum cholesterol, alkaline phosphatase, and total bilirubin showed no clear changes. *o*-DCB appeared to be a suicide substrate for liver cytochrome P450 which decreased dose-dependently, beginning at dosages (75 mg/kg) lower than the onset of necrosis (172 mg/kg). The *m*-DCB treatment increased P450 content at lower dosages (75-230 mg/kg) but decreased it at higher dosages; the decline in P450 preceded the onset of hepatocyte death (450 mg/kg). Treatment with *p*-DCB increased P450 content, beginning at 380 mg/kg (4).

In conclusion, the differences observed in the hepatotoxic potential within the isomers of dichlorobenzenes suggest as evidenced by past and recent reports that *o*-DCB is the most potent hepatotoxin, *m*-DCB is a mild hepatotoxin, and *p*-DCB is not producing hepatotoxicity (27, 52, 53, 71, 138, 158, 166, 176).

9.2.2. Acute kidney effects

o-DCB. A single vapour exposure to 3239 mg/m^3 *o*-DCB (539 ppm for 3 h or 6.5 h) caused cloudy swelling of tubular epithelium in the kidneys of male rats. Increases in the average weights of the kidneys (and the livers) were also found (84).

p-DCB. Nephrotoxicity has been studied and compared in male and female rats after exposure to 3005 mg/m^3 (500 ppm) of *p*-DCB for 24 h by the inhalation route, and after administration of 200 mg/kg by the oral route, but being only found in male rats (166-168).

9.2.3. Acute pulmonary effects

o-DCB and p-DCB. Necrosis of bronchiolar and bronchial epithelial cells was reproducibly elicited in mice (after 36 h) by a single intraperitoneal injection of 6.9 mmol of *o*-DCB per kg but not after *p*-DCB in doses up to 31.0 mmol/kg (no histopathologic changes in the lung).

The amount of covalent binding of radioactive material was studied and compared in rat lung after administration of an equimolar dose of *o*-DCB-^{14}C and of *p*-DCB-^{14}C. The binding preceded the onset of histopathological changes. The necrogenic *o*-DCB produced considerably more binding than did the nontoxic *para* isomer. A semiquantitative relationship existed between the amount of binding in lung and the extent of pulmonary necrosis (137). In animal experiments using radiolabelled trace amount doses of DCB (0.5 mg/kg) it was shown that o-DCB (46) was bound to tissue DNA of the liver, lung, kidney, and stomach of rat and mouse, and that the binding of p-DCB (109) was only detectable in mouse tissues. The *para* isomer showed a lower index of covalent binding to DNA than the *meta* isomer in all other tissues except in the murine lungs.

9.3. Short- and medium term exposure

o-DCB. No adverse effects on gross appearance, behaviour, growth, mortality, organ weight, haematology, urine tests, or tissue histopathology were observed in rats, guinea pigs, rabbits, or monkeys which were exposed for 6 to 7 months to *o*-DCB vapours at an air level of 93 (85-98) ppm (7 h/day, 5 days/week), or in rats, guinea pigs, or mice which were treated similarly but at an air level of 49 (46-52) ppm (84).

Rats were fed *o*-DCB by stomach tube, 5 days/wk for 138 doses in 192 days at three dose levels of 18.8, 188, or 376 mg/kg/day. At the high dosage, the liver and kidney weights were increased, the weight of the spleen was slightly decreased, and slight histopathological changes were observed in liver. After middle dosage, only slight increases in the liver and kidney weights occurred. The level of no adverse effect lied between 18.8 and 188 mg/kg (84). Similar results were obtained from ten and 90-day toxicity studies which were conducted in Sprague Dawley rats administered up to 300 mg/kg (10-day study) or 400 mg/kg (90-day study) of *o*-DCB by gavage (142). When *o*-DCB was given orally to rats for 60 to 120 days, it increased liver weights and triglyceride levels in treated rats. The level of hepatic ATP was lower than that in the liver of control rats. *o*-DCB decreased state 3 respiration, but increased state 4 respiration (121).

Rodent toxicity studies of o-DCB (129). Survival and mean body and organ weights were studied in rats administered *o*-DCB doses up to 1000 mg/kg and in mice administered doses up to 4000 mg/kg by gavage for 14 days. To evaluate the toxicity of *o*-DCB and to set the maximum tolerated dose for the 2-year cancer study, 13-week studies were conducted in rats and mice administered doses up to 500 mg/kg by gavage for 13 weeks. The main findings are presented in Table 8.

m-DCB. Male and female Sprague-Dawley rats (100 rats) received in a 10-day gavage study *m*-DCB at doses of 0, 37, 147, 368, or 735 mg/kg/day. Body weights were depressed at the high dose, liver weights and also serum chlolesterol were increased in both sexes at 368 and 735 mg/kg. Centrolobular hepatocellular degeneration was observed at 368 mg/kg in males and at 735 mg/kg in females, and thyroid lesion at 37 mg/kg and above. In a subsequent 90-day gavage study the animals (100 rats) received *m*-DCB at doses of 0, 9, 37, 147, or 588 mg/kg/day. Body weights were depressed at 588 mg/kg; liver weights were increased in both sexes at 147 and 588 mg/kg; relative kidney weights were elevated in males at 147 mg/kg and in both sexes at 588 mg/kg. Serum cholesterol and calcium levels were elevated in females at 37, 147, and 588 mg/kg, and in males at 9 mg/kg and doses above. Histopathology at 147 and 588 mg/kg revealed liver and thyroid lesions in both sexes, and pituitary and kidney lesions in males. According to the authors a NOAEL was not firmly established for *m*-DCB (115). Centrolobular hepatic necrosis is a common finding reported in rats after *m*-DCB administration (4, 27, 158).

p-DCB. Rodent toxicity studies of p-DCB (130). Survival and mean body and organ weights were studied in rats after *p*-DCB doses up to 8000 mg/kg and in mice after doses up to 4000 mg/kg given by gavage for 14 days. The 13-week animal studies were conducted in rats administered *p*-DCB doses up to 1500 mg/kg and in mice administered doses up to 1800 mg/kg by gavage for 13 weeks in order to characterise the toxicity and to set doses for the 2-year studies. Clinical chemistry and haematological studies were performed during the 13-week studies to assess effects of *p*-DCB on the liver, kidney, and hematopoietic system and occurrence of hepatic porphyria. Two 13-week studies were performed in rats. Because nephrotoxicity was observed in male rats at all doses (300-1500 mg/kg) of the first study, a second study was performed at doses of 38-600 mg/kg. In the 13-week studies, survival was decreased in males given 1200 or 1500 mg/kg and in females given 1500 mg/kg. Doses of 1200 or 1500 mg/kg produced degeneration and necrosis of hepatocytes, hypoplasia of the bone marrow, lymphoid depletion of the spleen and thymus, and epithelial necrosis of the nasal turbinates in male and female rats. Renal tubular cell degeneration was observed in male rats receiving 300 mg/kg or more in the first study, but only slight changes were seen at 300 mg/kg in the second study. Liver weight to brain weight ratio was increased in male rats receiving doses of 600 mg/kg or more. Administration of *p*-DCB to rats for 13 weeks produced slight but statistically significant decreases in the hematocrit, red blood cell count, and haemoglobin level in all males receiving doses of 300-1200 mg/kg. No clear haematology changes were observed in female rats. *p*-DCB produced minimal changes in clinical chemistry parameters in the 13-week studies. Serum cholesterol levels were increased by doses of 600 mg/kg or more in male rats and 900 mg/kg or more in female rats. Doses of 300 mg/kg or more in male rats reduced serum triglycerides. The blood urea nitrogen level was increased slightly in male rats dosed with 900 mg/kg or more. Urinary porphyrins were increased slightly in

male rats administered 1200 or 1500 mg/kg and females receiving 1200 mg/kg. These increases were modest and indicative of a mild porphyrinuria rather than hepatic porphyria. Liver porphyrins were not increased at any dose. The main findings are presented in Table 8.

Nephrotoxicity of *p*-DCB was investigated in a subchronic study. Fischer 344 rats of both sexes were dosed with 0, 75, 150, 300, or 600 mg/kg/day, 7 d/week, by gavage for 4 or 13 weeks. There was no indication of a nephrotoxic action in female rats. Dose-dependent effects typical for 'light hydrocarbon nephropathy' were observed exclusively in the male rats. At dosages of 75 mg/kg and above, analysis of urinary enzymes showed that renal leakage and urinary output of cytosolic and lysosomal enzymes (lactate hydrogenase, LDH; β-N-acetylglucosaminidase, NAG) were pathologically enhanced. Increased incidence of gram-positive hyaline droplets in cortical tubular epithelia without other alterations were seen in 75 mg/kg group. At 150 mg/kg and above tubular single cell necrosis, hyaline droplet accumulation, signs of partial tubular epithelial regeneration, and of chronic nephropathy were observed (22).

9.4. Long-term exposure/carcinogenicity

The tumour promoting activity of *o*-DCB and *m*-DCB were tested together with 18 other halogenated benzenes by using the rat liver foci bioassay as an indicator of carcinogenicity. After administration of the liver cancer-initiating chemical (diethylnitrosamine) to male and female Sprague-Dawley rats, the animals were observed for increased incidence of diethylnitrosamine-initiated gamma-glutamyl-transpeptidase-positive foci in the liver. Of the compounds tested, only 1,2,4,5-tetrachlorobenzene and hexachlorobenzene were positive. Thus, *o*-DCB and *m*-DCB did not possess tumour-promoting activity in rat liver (76). Based on the carcinogenicity bioassay of the *p*-DCB isomer, it has been suggested that *para* isomer may act as a promoter of liver tumours in mice (130).

o-DCB. In the 2-year NTP-carcinogenicity study (129), *o*-DCB was administered in corn oil by gavage at dosages of 60 or 120 mg/kg per day for 5 days per week to groups of 50 Fischer 344 rats and 50 B6C3F1 mice of each sex. There were corresponding vehicle and untreated control groups of 50 rats and 50 mice of each sex. Only the survival of high dose male rats was significantly shorter than that of controls. As gavage error may have contributed to the deaths in this group the lower survival of high dose males does not necessarily mean that the maximum tolerated dose (MTD) was exceeded. The only compound-related, non-neoplastic, histological lesion that was observed in the 2-year study was a dose-related increase in the tubular regeneration in the kidney of male mice (control, 17%; low dose, 24%; high dose, 35%). No evidence of carcinogenicity of *o*-DCB was detected in either sex of the mice or rats under the conditions of this 2-year study; however, according to critical opinions the MTD was probably not used in this study.

m-DCB. There are no studies available on carcinogenicity.

p-DCB. In the 2-year NTP-carcinogenicity study (130), *p*-DCB was administered in corn oil by gavage (5 days per week) to male F344/N rats at doses of 0, 150, or 300 mg/kg and to female F344/N rats and male and female B6C3F1 mice at doses of 0, 300, or 600 mg/kg per day for 2 years (50 animals per group). Survival of high dose male rats was significantly lower than that of vehicle controls after week 97 while the survival in the low dose female rats and in mice groups of both doses and sexes was similar to that of vehicle controls. Clear evidence of carcinogenicity for male rats was shown by a significantly increased incidence of renal tubular cell adenocarcinomas (controls, 2%, low dose 6%; high dose 14%). There was no evidence of carcinogenicity for female rats. Whereas clear evidence of carcinogenicity for both male and female mice was shown by increased incidences of hepatocellular carcinomas (in males: control, 28%; low dose, 22%; high dose, 64% and in females: control, 10%; low dose, 10%; high dose, 38%) and hepatocellular adenomas (in males: control, 10%; low dose, 26%; high dose, 32%; and in females: control, 20%; low dose, 13%; high dose, 42%). Marginal increases were observed in the incidences of pheochromocytomas of the adrenal gland in male mice. Nonneoplastic effects in the kidney of male and female rats, in the liver of male and female mice, and in the thyroid gland and adrenal gland of male mice were also associated with the administration of *p*-DCB (130).

Loeser and Litchfield (112) reported long term inhalation toxicity studies on *p*-DCB. Groups of 76-79 (SPF, Alderly Park Wistar) rats of both sexes and 75 female (SPF Swiss) mice were exposed for 5 h/day on 5 days/week at 0, 451, or 3005 mg/m^3 (0, 75, or 500 ppm) for a total period of 76 weeks (rats) or 57 weeks (mice) followed by 36 weeks (rats) or 19 weeks (mice) without *p*-DCB exposure. No overt signs of toxicity were seen nor were there any treatment-related effects on the biochemical determinations, urine analysis, or haematological parameters. Slightly elevated urinary coproporphyrin excretion and increased liver and kidney weights were regarded as treatment related effects in the 500 ppm exposure group of rats. The non-tumour and tumour pathology did not indicate any treatment-related effect in any group of either species. Notably, no renal tumours or nephrotoxicity was observed in the male rats.

9.5. Mutagenicity and genotoxicity

All dichlorobenzene isomers have been extensively studied for mutagenicity, but found in most experiments negative, and thus DCBs may all be classified as putatively non-genotoxic. Data from dichlorobenzenes up to 1982 have been reviewed by IARC (89) and summarised by Brusick (33).

9.5.1. In vitro studies

o-DCB. Genotoxicity of the non-carcinogenic isomer *o*-DCB has been screened in four common *in vitro* short-term tests, which were Ames Salmonella/microsome mutagenesis assay, and mouse lymphoma L5178Y (MOLY) cells mutagenesis assay, and the assay for chromosome aberration (ABS) and sister chromatid

exchanges (SCE) induction in Chinese hamster ovary cells. In these tests *o*-DCB was reported negative in two (1st and 3rd) and positive in two (2nd and 4rth) (9, 161).

Effects of *o*-DCB were examined in the rat hepatocyte primary culture/DNA repair assay. Based on preliminary toxicity tests, *o*-DCB (diluted with DMSO) was tested at 8 concentrations ranging from $1x10^{-7}$ to 1.0 % (v/v). Cultures were exposed to *o*-DCB for 18 h and 20 nuclei were randomly counted due to no obviously positive cells being observed upon scanning of the slides. Concentration of 0.01% and above was cytotoxic (151). Based on preliminary toxicity determinations, *o*-DCB was further tested at 5 different concentrations ranging from $1x10^{-7}$ to $1x10^{-3}$% (v/v). None of the test concentrations caused a significant increase in the unscheduled DNA synthesis over the solvent control, and these concentrations were not genotoxic to the hepatocytes (151).

The ability of *o*-DCB to induce chromosome aberrations in cultured Chinese Hamster ovary cells was evaluated in the presence and absence of added metabolic activation by Aroclor-induced rat liver S-9 fraction. The maximum dose selected for both nonactivated and activated cultures was the solubility of *o*-DCB in water (140 μg/ml). Although isolated test points with *o*-DCB cause a statistically significant increase in the frequency of the chromosomal aberrations with and without metabolic activation compared to the negative control (DMSO), the assays were considered negative due to lower than average frequency of chromosomal breaks in the negative control (19).

No induction of unscheduled DNA synthesis (increased thymidine uptake) was induced with the DCB isomers in cultivated human lymphocytes. In this test system and in the absence of a rat liver metabolising system (S-9 mix), the dichlorobenzenes (1 mM) exerted clear cytotoxic (cell viability) and genotoxic (decreased thymidine uptake) effects in the order, *m*-DCB > *o*-DCB > *p*-DCB. In the presence of S-9-mix these effects disappeared in lymphocytes incubated with the *p*-DCB isomer (134).

m-DCB. Mutagenicity was assayed with *Salmonella typhimurium* strains TA100, TA98, UTH8414, and UTH8413. None of the three DCBs was mutagenic in any strain with or without S-9 from Aroclor treated rats (47).

p-DCB. The carcinogenic isomer *p*-DCB was not found to be genotoxic in a variety of short-term *in vitro* tests (130, 169, 182) while the *o*-DCB was positive in some assays.

The ability of *p*-DCB to induce sister-chromatid exchanges in cultured human lymphocytes (of two donors) was investigated at three concentrations (0-0.2 μg/ml). The authors concluded that *p*-DCB was able to induce a cytotoxic effect, measured as a decrease in third and second metaphases, together with an increase of SCEs (39).

p-DCB increased the frequency of reverse mutations in *Aspergillus nidulans* (136). Dichlorobenzene isomers were, however, all non-mutagenic in the Ames test for mutagenesis in *Salmonella typhimurium* using different strains (TA98, TA100; TA1535, TA1537, TA1358) with and without metabolic activation by

Aroclor 1254-induced rat liver S-9 fraction (74, 152). Negative results were also reported for *p*-DCB *in vivo* in rodent bone-marrow (cytogenetic assay) and dominant lethal assays. In the latter assay, *p*-DCB was not mutagenic at any maturation stage of the 8-week spermatogenetic cycle in mice exposed to 75, 225, or 450 ppm for 6 h/day for 5 days (112).

The frequency of forward mutations was determined at the HGPRT locus in Chinese Hamster Ovary cells exposed *in vitro* to *p*-DCB with and without metabolic activation by Aroclor-induced rat liver S-9 fraction. No mutagenicity was observed at concentrations ranging from 25 to 250 μg/ml in the presence or absence of activation. In preliminary cytotoxicity assays, the percent survivors parameter ranged from 0.03 to 2.1% at the high dose level under all conditions of exposure (20).

p-DCB showed no chromosomal activity when studied *in vitro* for chromosomal aberrations and sister chromatic exchange in Chinese hamster ovary cells (65). Using mouse lymphoma cells to test forward mutations in L5178Y/TK+/-, *p*-DCB was negative when assayed without and with metabolic activation (S9 from Aroclor 1254-induced rat liver) (130).

The ability of *p*-DCB to cause chromosome aberrations in Chinese hamster ovary cells was evaluated with and without added metabolic activation by Aroclor 1254-induced rat liver S-9 fraction. Both nonactivated and S-9-activated cultures were treated with 3 doses of *p*-DCB. Nonactivated cultures were incubated with the test material (50, 100, or 150 μg/ml) for 12 hours and activated cultures were incubated for 2 hours with test material (25, 50, and 100 μg/ml) and then incubated for 10 hours with replaced normal medium. The *p*-DCB-treated cultures did not show any significantly greater increase in the frequencies of chromosomal aberrations relative to their negative control (DMSO) in the absence or presence of S-9 (130).

9.5.2. In vivo studies

The ability of *o*-DCB to cause chromosome aberrations was evaluated in bone marrow cells of male Charles River rats (30/group) receiving subcutaneous doses of 0.04, 0.2, and 1 g/kg/day for 16 days. Six animals per dose were killed after 1, 2, 4, 8, and 16 days of treatment. Toxicity was indicated in the high dose animals by increased mortality and decreased body weight gain compared with control animals. No differences were observed between treatment groups and controls for mean red blood cell counts, mean haemoglobin concentrations, or mean hematocrit values. No significant increase was found in the number of chromosome aberrations observed at any of the dose levels tested relative to the control (143).

The ability of *o*-DCB to cause chromosome aberrations was evaluated in bone marrow cells of Sprague-Dawley rats (3/sex/treatment) killed at 6, 12, and 24 hours following a single i.p. injection and analysis of 100 metaphases per animal. *o*-DCB did not cause a significant increase in the frequency of chromosomal breaks or aberrations at any of the dose levels (135-600 mg/kg) tested relative to the controls (DMSO) (18).

The first evidence of genotoxicity of *o*- and *p*-DCB in mammalian cells has been obtained using fully comparable experimental procedures. Colacci et al. (46) investigated the ^{14}C binding of radiolabelled *o*-DCB (0.5 mg/kg) 22 h after i.p. injection into male Wistar rats and BALB/c mice. *o*-DCB was covalently bound to DNA, RNA, and proteins of liver, kidney, lung, and stomach in both species. The covalent binding index to liver DNA was typical of carcinogens classified as weak initiators. The enzyme-mediated *in vitro* interaction of *o*-DCB with calf thymus DNA or synthetic polyribonucleotides was carried out by a microsomal mixed-function oxidase system, with or without cytosolic GSH-transferases, which seemed to play, except in the lungs, a minor role in *o*-DCB bioactivation in rat and mouse tissues. Lattanzi et al. (109) studied the ^{14}C binding of radiolabelled *p*-DCB (0.5 mg/kg) 22 h after i.p. injection into male Wistar rats and BALB/c mice. *p*-DCB was covalently bound to DNA (0.6 pmol/mg or lower) from lung, liver, kidney, and stomach of mice but not in rats. The covalent binding index to mouse liver DNA was typical of carcinogens classified as weak initiators. The *in vivo* and *in vitro* covalent binding index showed that *p*-DCB interacted with DNA and proteins to lesser extent than o-DCB.

The DCB isomers were tested for acute toxicity (LD_{50}) and clastogenicity in 8-week-old NMRI mice by i.p. administration. Four doses of each isomer (up to 70% of LD_{50}) were tested for clastogenic activity. Increased dose-dependent formation of micronucleated polychromatic erythrocytes, observed in femoral bone marrow 30 hr after the injection, was considered to be due to the clastogenic activity. All DCBs were clastogenic ($p<0.01$), inducing a 3-5-fold greater number of micronuclei than the micronuclei count in control animals; the greatest effect (a 7-fold increase) was induced by benzene (120).

The mutagenic potential of *o*- and *p*-DCB was evaluated in the germ cells (Sex-Linked Recessive Lethal Mutation Assay) of Drosophila males exposed by inhalation. Male flies in the various test groups were exposed in sealed hypovials to *o*-DCB (nominal concentrations up to 17000 ppm/h) or to *p*-DCB (nominal concentrations up to 15600 ppm/h). The mortality during exposure and premating in experimental groups was in a range of 3.4 - 34%. None of the treatments resulted in mutant frequencies significantly greater than the negative controls (air only) (6).

9.6. Reproductive and developmental toxicity

o-DCB. In the teratology study, pregnant Fischer 344 rats and inseminated New Zealand white rabbits were exposed at nominal air concentrations of 0, 100, 200, or 400 ppm (0, 606, 1212, or 2424 mg/m^3) *o*-DCB. At each concentration, 30 or 32 bred rats and 28 or 30 inseminated rabbits were exposed for 6 h/day on days 6-15 of gestation (rats) and on days 6-18 of gestation (rabbits). Maternal toxicity of *o*-DCB was reflected by a reduced rate of body weight gain in all groups of the treated rats. Liver weights were increased at 400 ppm. *o*-DCB was not teratogenic nor fetotoxic in rats. Rabbits exposed at 400 ppm *o*-DCB showed a decreased rate

of body weight gain over the first 3 exposure days. No evidence of major malformations among the foetuses of either test species was observed (75).

Sperm abnormalities and ultrastructural changes in Sprague-Dawley rat testes were observed 10 days after intraperitoneal administration of *o*-DCB (0, 50, 100, 250, 300, or 800 mg/kg). Abnormal sperm such as excessive curvature, banana- and wedge-shaped heads as well as twisting and curling tails were observed. For sperm head and tail abnormalities, the effects seemed to be dose-dependent. *o*-DCB interfered with spermatogenesis (124).

m-DCB. The *meta* isomer was administered by gavage at dosages of 50, 100, and 200 mg/kg to pregnant Sprague-Dawley rats on day 6-15 of gestation (controls: no data specified). No teratological effect was found as examined for foetal changes by measurement of litter size, foetal weight, deciduoma, skeleton, and visceral examination, residue analysis and microscopy (145).

p-DCB. In the teratology study, inseminated New Zealand white rabbits were exposed at nominal air concentrations of 0, 100, 300, or 800 ppm *p*-DCB. At each concentration, 28 or 30 inseminated rabbits were exposed for 6 h/day on days 6-18 of gestation. A slight decrease in maternal body weight gain was observed at 800 ppm during the first 3 days of the study, but no other adverse effects were seen in either the maternal or foetal rabbits (75).

The latter results are supported by an other inhalation teratology study (2) in which rats (Alderley Park) were exposed 6 h/day at concentrations of 0, 75, 200, or 500 ppm (0, 449, 1200, or 3055 mg/m^3) of *p*-DCB on day 6-15 of pregnancy. The authors concluded that there was no evidence of embryo- or fetotoxicity, nor any indication of a teratogenic effect at atmospheric levels of *p*-DCB up to 500 ppm (112).

Neeper-Bradley et al. (128) conducted a two-generation reproduction toxicity study of *p*-DCB inhalation at 0, 66, 211, or 538 ppm (0, 397, 1268, or 3233 mg/m^3, 6 h/day) in two generation of Sprague-Dawley rats involving 28/sex/group of weanling rats (F0) exposed for 10 weeks and mated. Selected weanlings (F1) were similarly exposed for 11 weeks and mated. There were no F0 or F1 reproductive effects. F1 and F2 litters exhibited reduced body weights and increased perinatal deaths at 538 ppm with no gross lesions observed. In spite of observed adult toxicity, no effects on reproduction were observed as studied in three generations.

In another two-generation reproduction toxicity study of Sprague-Dawley rats (24/sex/group), *p*-DCB was administered orally in olive oil at concentrations 30, 90, or 270 mg/kg body weight. The rats of F0 generation were exposed 11 weeks (males) and 2 weeks (females) before mated, and thereafter only the females. F1 generation was exposed from day 21 and onwards. In the mid and/or high dose groups, *p*-DCB had adverse effects: on the number of stillborn pups, pups deceased during lactation (days 1-4, 5-21), and pups with retarded body weights, on erection of ears and opening of eyes, pups with positive draw up tests (reduced), alteration of the skin (dry, squamous) and ring tails in pups, and on organ weights of adult males (livers and kidneys: increased; spleens: decreased).

No effects on the weights of female livers, kidneys, or spleens were found. The authors conclusion was that the low dose showed no effects on the fertility parameters of rats, but already the mid dose of 90 mg/kg caused massive damage in pups (24).

Giavini et al. (67) investigated the teratogenic potential of *p*-DCB in pregnant (Charles River) rats dosed on day 6-15 with 0, 250, 500, 750, or 1000 mg/kg by gavage. *p*-DCB induced maternal weight retardation only at 500 mg/kg and higher; no effects on liver weights were seen. Mean foetal weight was reduced only at the highest dose level. The occurrence of visceral and skeletal malformations in foetuses of *p*-DCB-treated dams was no different from that of controls; an increase in the number of skeletal variations was observed at 750 and 1000 mg/kg; a dose related increase in the frequency of extra ribs was recorded starting at 500 mg/kg. No differences were observed in comparison with control values in the degrees of ossification of selected areas. A reduction in foetal weight was observed at the 1000 mg/kg dose level. The embryotoxic effects observed were concluded to be a consequence of maternal suffering (reduced food intake) rather than a direct effect of the chemical on the embryonic development, therefore *p*-DCB was concluded not teratogenic in the rat.

p-DCB was administered by gavage at dosages 50, 100, and 200 mg/kg to pregnant Sprague-Dawley rats on day 6-15 of gestation (controls: no data specified). No teratological effect was found as examined for foetal changes by measurement of litter size, foetal weight, deciduoma, skeleton, and visceral examination, residue analysis and microscopy (145).

Sperm abnormalities and ultrastructural changes in Sprague-Dawley rat testes were observed 10 days after intraperitoneal administration *p*-DCB (800 mg/kg). Increased levels of abnormal sperm such as excessive curvature, banana- and wedge-shaped heads as well as twisting and curling tails were observed. *p*-DCB interfered with spermatogenesis (125).

9.7. Other studies

o-DCB. Brondeau et al. (31) investigated the haematological consequences of acute exposure to airborne irritants assuming that *o*-DCB might act as a stressor in experimental animals. The effects of a single 4-h exposure to *o*-DCB vapours at irritant levels were examined on blood cell counts in male Sprague-Dawley rats. Since stimulation of the hypophysis-adrenal axis seems to be a common denominator that may account for stressor-induced physiological effects, experiments were performed in non-operated and in adrenalectomized rats. Leucopenia without any change in red blood cell or leukocyte differential counts was observed in a dose-dependent manner in rats exposed to 10, 16, or 29 ppm for 4 h. The leucopenic effect was adrenal dependent, and assumed to result from glucucorticoid-induced transient temporary transfer of leukocytes from plasma to other compartments (e.g. to bone marrow), and so regarded as an associative response to sensory irritation by *o*-DCB.

Ribonucleic acid and protein synthesis was found to be strongly inhibited in HeLa cells exposed for 30 minutes to 350 μg/ml of *o*-DCB. A possible mechanism that may lead to such inhibition is the uncoupling of oxidative phosphorylation (127).

p-DCB. A suspicion that *p*-DCB is cataractogenic led to thorough studies in mice, rabbits, monkeys, guinea pigs, and ducks. With high exposures to high vapour concentrations or by feeding the material dissolved in olive oil to rabbits (0.5 to 1 g/kg/day for 260 days in a year) has failed in all instances to induce cataracts. Rabbits exposed repeatedly to 798 ppm (770-880 ppm) in air for 8 h/day (for 62 days) developed transient oedema of cornea, and as much as 3-5 diopters of oedema of optic disc, oedema of neighbouring retina, and congestion of retinal veins, but no haemorrhages or exudates; eyes returned to normal in 17 days after discontinuing exposure. In addition to rabbits, also rats (up to 69 days) and guinea pigs (up to 23 days) were exposed to *p*-DCB inhalation under the same circumstances as the rabbits; lens changes were not observed. In no instance have cataracts been reported in animals after exposure to *p*-DCB which was assuredly pure (69, 83).

Comparative studies on DCB isomers. DCBs (0.24 mM) studied in rat liver mitochondrial preparations exhibited marked decreases in the respiratory control index due to uncoupling of the mitochondrial oxidative phosphorylation showing an order of decreasing toxicity: *ortho* > *meta* > *para* (131).

DCB isomers were incubated with human liver precision-cut slices to clarify their hepatotoxic potential in man, and to evaluate isomer-specific effects as well as to compare results from humans with rats. The DCBs produced no significant differences from control when incubated at 1 mM concentration. At cytotoxic concentrations (2 mM) the isomers exhibited the following rank order *m*-DCB > *o*-DCB > *p*-DCB. Metyrapone blocked the cytotoxicity of *o*-DCB, and SKF 525 blocked the toxicity of *m*-DCB but neither one of these P450 inhibitors could block the toxicity of *p*-DCB (61). *o*- and *m*-DCBs were metabolised in liver slices from humans and rats at a greater rate than the *p*-DCB. In human livers, the individual rates of DCB metabolism varied less for *p*-DCB than for the other two isomers. The metabolism of *p*-DCB is thus not likely to be governed by the same enzymes as the metabolism of the other isomers (62).

Toxicity studies in a dynamic liver culture system using short term viable tissue culture of rat liver slices showed, by measurements of potassium ion content, protein synthesis, and release of lactic dehydrogenase, that isomer toxicity decreased in a similar order as shown *in vivo*, i.e *o*-DCB > *m*-DCB > *p*-DCB. The DCBs were less toxic in slices from Sprague-Dawley rats than in Fischer rats, a finding also confirmed by studies *in vivo* (154).

10. Observations in Man

Observation in man are mainly based on case reports among the general population after accidental exposure to, or misuse of products containing DCB.

Many reports from the early literature contain poor information on the purity and the actual dose/time relationship. Intoxications in adults and children resulting from non-occupational exposure have been numerous due to wide uses of DCBs (usually *para*) as space deodorant and moth repellent. Symptoms and signs that have been described include, weakness, dizziness, acute illness with nausea, asthenia, anaemia, granulocytopenia, leukocytosis, periorbital swelling, intense headache, profuse rhinitis, vomiting, weight loss, numbness, clumsiness, burning sensation in the legs, subacute yellow atrophy of the liver and jaundice, and chronic illness with pulmonary granulomatosis. Compiled data on case reports exist (110, 169, 172).

10.1. Acute effects by contact and systemic distribution

o-DCB. Inhalation of *o*-DCB vapour at a concentration of 100 ppm (601 mg/m^3) may cause irritation to eyes and respiratory passages without other effects (58). No eye or nasal irritation has been observed in industrial workers manufacturing and handling *o*-DCB in workrooms in which measured air levels ranged from 1 - 44 ppm (average 15 ppm = 90 mg/m^3) or in unacclimated persons exposed to undefined low concentrations of *o*-DCB (detected by its odour) around an inhalation chamber in which rats were exposed to 50 ppm (300 mg/m^3) (84).

The use of *o*-DCB for pest control in a laboratory building resulted in accidental exposure of 26 workers to vapours of *o*-DCB at least for 4 work days (8 h/day) at air concentrations that were defined by a characteristic strong odour. Most individuals had eye, nose, and throat irritation and ten persons had, in addition, severe headache, fatigue, nausea, and dizziness, and one had partial facial oedema. Only 4 subjects reported no symptoms (184).

Intense erythema and oedema appeared promptly when *o*-DCB was applied locally to the skin of one arm of a 47-year-old glazier who had worked with *o*-DCB (hand contact) and developed eczematous dermatitis of the hands, arms, and face (110). A burning sensation was reported in two subjects following application of an unspecified amount of *o*-DCB liquid to the skin for 15 min. The response became more intense during a 1-hour exposure and disappeared when the liquid was removed. A diffuse redness of the treated area developed that progressed to a darker red colour with blister formation by 24 h. A brown pigment formed at the site which was apparent 3 months postexposure (140).

m-DCB. No data is available on effects specific for *m*-DCB in humans.

p-DCB. Exposure to *p*-DCB at vapour concentrations from 15 ppm (90 mg/m^3) upwards is recognised by its odour. Inhalation of *p*-DCB vapour is irritating (eyes, nose) to most people in concentrations between 50 and 80 ppm (300 and 480 mg/m^3) and discomfort becomes quite severe at 160 ppm (83).

Solid *p*-DCB is painful in the eyes, but has negligible irritating action on the intact, uncovered skin. It does produce burning sensation when held in close contact for excessive periods of time. In general, it presents no skin irritation problem unless exposures are unusually severe and prolonged (83).

o-DCB. Industrial inhalation exposure of a young man to nonreported levels of Orthosol (containing 95% *o*-DCB and 5% *p*-DCB) for 6 months resulted in severe pallor, exhaustion, and vomiting, with intense gastric pain, and headache. He got also a rapidly developing haemolytic anaemia. Rapid and complete recovery followed cessation of exposure. As 13 co-workers, similarly exposed, suffered no injury, this report may imply that individual susceptibility to *o*-DCB exposure may vary greatly among the workers (110).

Intermittent exposure of workmen handling *o*-DCB at different plant operations in the manufacturing industry is known to cause irritation to eyes and upper respiratory system when the vapour concentrations rise above 100 ppm in work-room air. In the studied company, the workers who had been exposed to relatively high vapour concentrations and for times of many years were described to show greater tolerance than unacclimated persons. High vapour concentrations cause burning of eyes and flow of tears, coughing, difficult breathing, and nausea, whereas exposure to vapour levels 1- 44 ppm showed no ill effects. All men employed by the *o*-DCB manufacturing company mentioned above had received periodic medical examinations that included blood counts (RBC, WBC, and differential), determination of haemoglobin, blood urea, nitrogen, hematocrit, mean corpuscular volume, sedimentation rate, and urine analysis. But no evidence of adverse effects caused by exposure to *o*-DCB could be found in these workmen (84).

Ambient concentrations of *o*-DCB up to 100 ppm in a wool-handling operation induced sporadic irritation of the respiratory passages and eyes without other ill effects (58).

m-DCB. No data is available on effects specific to *m*-DCB in humans.

p-DCB. There are case reports suggesting that long-term exposure following continued extensive use of moth balls of *p*-DCB in homes (at concentrations defined by a strong odour) may potentially be toxic to the CNS and cause reversible brain stem injury. Intoxication following an apparently rather high exposure to *p*-DCB for 6 years (due to use of moth balls) has been reported in a 25 year old woman who had symptoms that had gradually and slowly developed into severe cerebellar ataxia, dysarthria, moderate weakness of limbs, and hyporeflexia. After cessation of exposure her symptoms gradually disappeared during a time period of several (6-8) months (118). Encephalopathy with severe cerebellar and neuropsychological symptoms (visual disturbances, ataxia, tremor, dysarthria, asthenia, adynamia, somnolence) developed in a young woman who admitted a habit of intentional exposure to *p*-DCB that had continued for several months by inhalation of vapours from *p*-DCB blocks. The abuse of *p*-DCB was revealed in urine by a characteristic aromatic odour and excretion of the *p*-DCB metabolite 2,5-dichlorophenol. After hospitalisation and discontinued exposure the patient showed a rapid and favourable improvement (139).

Neural disorders including enhanced muscular reflexes, mild clonus of the ankle, and tremors of the fingers developed in eight workers employed in the

production of *p*-DCB-based (major ingredient) moth proofing agents for 1 to 7 months. The workers developed also irritation of the mucous membranes of the eyes and throat, methaemoglobinemia, and loss of appetite and body weight, as well as blood changes (lymphocytosis, thrombocytopenia, granulocytopenia). The *p*-DCB-specific effects are uncertain as exposure to compounds other than *p*-DCB may have occurred in the workplace (110, 181).

According to an early report by Berliner (17) the eyes of a 27-year-old woman became completely cataractous twelve to fourteen months after an attack of hepatic enlargement, jaundice, and loss of weight which was ascribed to excessive exposure to vapours of *p*-DCB in her home; the exposure had been discontinued for one year before development of cataracts. A second woman, aged 25, had monocular, immature, anterior peripheral cortical cataract with a history of jaundice and weight loss six months earlier. It was suspected that she had been poisoned by vapours from 2 cans of *p*-DCB which were kept in a closet in which the patient spent considerable time sewing in the previous year. The identity, source, and purity of the materials (described as "moth-repelling chemical" or "deodorising agent") involved in the prolonged exposure of these two cases were not established. Concern of possible lens effects led later to studies with exposure to assuredly pure *p*-DCB. However, no cataractogenic effects have been reported in animals or humans (69).

According to an early industrial company report (2), repeated skin contact with *p*-DCB resulted in dermatitis when workers handled cakes of pure chemical. But in general, this material has not caused skin problems to the workers (83).

Hollinsworth et al. (83) have reported on the industrial use of *p*-DCB. In their report, 58 men had worked continually or intermittently in operations involving the handling of *p*-DCB for periods of 8 months to 25 years. In the first survey, air levels ranged from 10 to 550 ppm (average 85 ppm). A faint odour was noted at 15-30 ppm, it became strong at 30-60 ppm. Painful irritation of the eyes and nose was recorded at 50-160 ppm, and breathing was difficult at air levels over 160 ppm. In a second survey, ambient exposure levels of 100-725 ppm (average 380 ppm) were experienced irrespirable even by acclimated persons whereas concentration 5-275 ppm (average 90) in air were acceptable to the workmen. In a 3rd survey, after major changes in operations, the workmen complained of eye and nose irritation only in areas with air concentrations 50-170 ppm (average 105 ppm), but they had no complaints in areas where the concentrations ranged from 15 to 85 ppm (average 45 ppm). In spite of thorough periodic medical examinations of the exposed men there was no evidence of organic injury, haematologic effects, or eye changes (cataracts).

10.3. Genotoxic effects

In general, data on genotoxicity in mammals is very limited (33). The only *in vivo* human genotoxicity data reported refer to accidental inhalation exposure to *o*-DCB in 26 individuals. The exposure was so high (strong odour) that it caused clinical symptoms in 10 of the exposed individuals. Karyotype analysis of

cultured peripheral blood leukocytes from the 8 male and 18 female workers of the laboratory where exposure to *o*-DCB occurred for 4 working days revealed that approximately 8.9% of cells from the exposed subjects showed clastogenic chromosomal alterations compared with 2.0% of cells from ten control subjects. The significantly increased aberration levels of exposed group returned to control level after several months (184).

10.4. Carcinogenic effects

Human data on DCB-related cancer is inadequate. Four cases involving cancer and exposure to DCB(s) have been reported. In the two male subjects with chronic lymphoid leukemia, one had been exposed to glue containing 2% *o*-DCB from 1945-1961, and the other had been exposed from 1940-1950 to solvent containing *ortho*- (80%), *meta*- (2%) and *para*- (15%) DCBs. One of the two female cases of acute myeloblastic leukemia had been exposed to the same solvent mixture of dichlorobenzenes while cleaning of clothes (2 l per year for several years); the other case was a 15-year old girl who had for some time removed stains from her own clothes with a product containing 37% *o*-DCB (68). Myeloblastic leukemia was reported in a 40-year-old man who had been exposed for 22 years to *o*-DCB in the preparation of dyestuffs (163). In spite of these cancer cases with a probable long-term exposure history of exposure to DCB(s), the cause and effect relationships have not been assessed with any certainty.

10.5. Reproductive and developmental effects

A pregnant 21-year-old woman developed a pica for *p*-DCB. She consumed throughout pregnancy one or two blocks of toilet air freshener per week, which were composed primarily of *p*-DCB. She developed a severe hypochromic, microcytic anaemia, with excessive polychromasia, and marginal nuclear hypersegmentation of the neutrophils. She recovered completely after withdrawal of the chemicals. Neonatal examination of the child revealed no abnormalities (38).

11. Dose-Effect and Dose-Response Relationships

In Table 6, acute effects of dichlorobenzenes in experimental animals are presented in relation to doses/concentrations and routes of exposure. Data on inhalation experiments are available only from *o*-DCB and *p*-DCB studies whereas administration by other routes allows dose-effect comparisons between all the three isomers. In Table 7, short-term effects of dichlorobenzenes in experimental animals are presented by data from inhalation studies. In Table 8, short-term effects of oral administration of DCBs are summarised for comparison of the dose relationships shown by the rodent bioassay data originating from *o*- and *p*-DCB toxicity studies. There is no long-term exposure data available for *m*-DCB. In Table 9, data are shown on dose-effects and dose-responses in humans.

o-DCB (Table 9). The odour thresholds reported for *o*-DCB are from 2 ppm (low) to 50 ppm (high), and the irritating concentration 25 ppm (146). According to industrial experience, the odour becomes strong and irritation noticeable at concentration around 100 ppm. The 'good' warning properties of *o*-DCB frequently mentioned in old literature thus refer to rather high airborne concentrations (84). No evidence of organic injury or untoward haematological effects could be found in industrial workers who had been exposed to air containing *o*-DCB at concentrations ranging from 1-44 ppm (average 15 ppm) for many years (84). There are no more recent studies on long-term human follow-up.

m-DCB. There are no studies available on the dose-effect or dose-response in humans. For toxicity in rats, dose-effects have been reported in 10-day and 90-day gavage studies by McCauley et al. (115), see data in Table 7. A NOAEL was not firmly established in these rats in spite of exposure starting at test dose levels as low as 9 or 37 mg/kg/day.

p-DCB (Table 9). The odour thresholds reported for *p*-DCB levels are from 15 ppm (low) to 30 ppm (high), and the irritating concentration 40 ppm (146). According to industrial experience, men working with *p*-DCB continuously or intermittently at air concentrations of 15-85 ppm (average 45 ppm) had no complaints of irritation. At 50-170 ppm (average 105 ppm) the workmen complained of eye and nose irritation (83). Above 160 ppm the odour is intolerable to any person who is not adapted to it. While the odour and irritating effects are considered as 'good' warnings that prevent from any severe overexposure to *p*-DCB, it should be recognised that a person may become sufficiently accustomed to odour to tolerate high exposure (83).

Table 7. Effects of dichlorobenzenes in animals after repeated exposures

Species (m, male f, female)	Route of administration	Exposure data	Effect	Reference
o-DCB				
rabbit	inhalation	6912 mg/m^3 (8 h/day) 2 months	No effects	162
rabbit	inhalation	4808 mg/m^3 (8 h/day) 2 months	LC_{10}	162
rabbit (pregnant)	inhalation	2424 mg/m^3 (6 h/d), days 6 through 18	Retarded weight gain	75
rat (pregnant)	inhalation	2424 mg/m^3 (6 h/d), days 6 through 15	Retarded weight gain, increased liver weights	75
rabbit	inhalation	2043 mg/m^3 (8 h/day) 2 months	Histopatological changes in lungs	162
rat (m, f); guinea pig (m, f); rabbit (m, f); monkey (f)	inhalation	559 mg/m^3 (85-98 ppm): 7 h/day, 5 d/week, 6-7 months	Decreased spleen weight (normal histology; guinea pig). NOAEL (behaviour; growth; mortality; organ weights; histopathology of lungs, heart, liver, kidney, testes; qualitative urine tests). Haematology data normal (rabbit and monkey)	84
rat (m, f); guinea pig (m, f); mice (f)	inhalation	294 mg/m^3 (46-52 ppm): 7 h/day, 5 d/week, 6.5 months	NOAEL (behaviour; growth; mortality; organ weights; histopathology of lungs, heart, liver, kidney, spleen, testes)	84
rat (f)	oral	18.8, 188, or 376 mg/kg/day (5 d/week, 27 weeks)	Increased liver and kidney weights; decreased spleen weight; liver (cloudy swelling). NOAEL =18.8 mg/kg	84

Cont.

Table 7. Cont.

Species (m, male f, female)	Route of administration	Exposure data	Effect	Reference
rat (m, f)	oral	300 mg/kg/day, for 10 days	Weight loss. Decreased organ weights (heart, kidney, spleen, testes and thymus). Increased weight of liver (slight necrotic lesions). Serum cholesterol increased	142
rat (m, f)	oral	38, 75 or 150 mg/kg/day, for 10 days	Liver weight increased. Elevated serum cholesterol	142
rat (m, f)	oral	25, 100 or 400 mg/kg/day, for 90 days	Weight loss. Increased organ weights (liver, kidney). Hepatocellular degeneration/hypertrophy/ single cell necrosis. Elevated serum ALT, BUN, and total bilirubin. NOAEL = 25 mg/kg	142
rats	sub-cutaneous	40, 200, 1000 mg/kg/day x 16 days	No chromosome aberration effects	143
m-DCB				
rat (m, f)	oral	368 or 735 mg/kg/day, for 10 days	Weight loss. Decreased organ weights (brain, spleen, thymus, heart, testes and lungs). Centrolobular hepatocellular degeneration. Thymus atrophy. Elevated serum cholesterol, alkaline phosphatase and calcium	115
rat (m, f)	oral	37, 147 mg/kg/day, for 10 days	Liver weight increased; minimal necrotic lesions. Elevated serum cholesterol and calcium	115

Cont.

Table 7. Cont.

Species (m, male f, female)	Route of administration	Exposure data	Effect	Reference
rat (m, f)	oral	147 or 588 mg/kg/day, for 90 days	Weight loss. Increased weight of liver, kidney, brain testes. Thyroid and pituitary lesions. Hepatocellular degeneration/necrotic cell foci. Elevated serum cholesterol and calcium.	115
rat (m, f)	oral	9 or 37 mg/kg/day, for 90 days	Thyroid and pituitary lesions. Minimal hepatocellular lesions. Elevated serum cholesterol and calcium.	115
p-DCB				
rat (m, f); rabbit (m, f); guinea pig (m, f)	inhalation	4796 mg/m^3 (732-848 ppm), 8 h/day, 5 d/week: rat <69 days; rabbit <62 days; guinea pig <23 days.	Tremors, weakness, weight loss, eye irritation, unkempt appearance, anaesthesia, some deaths. Liver (cloudy swelling, necrosis); kidney (cloudy swelling/ tubular epithelium); lung (congestion, emphysema). No lens changes.	83
rabbit (pregnant)	inhalation	4808 mg/m^3 (6 h/d); days 6 through 18	Retarded maternal weight gains, no feto-toxicity	75
mouse (f)	inhalation	451 or 3005 mg/m^3: 5 h/day, 5 d/week, 57 weeks	No overt sign of toxicity, no treatment related tumours	112

Cont.

Table 7. Cont.

Species (m, male f, female)	Route of administration	Exposure data	Effect	Reference
rat (m, f)	inhalation	451 or 3005 mg/m^3: 5 h/day, 5 d/week, 76 weeks	No overt sign of toxicity. Increased liver and kidney weight. Histopathological examination of most tissues (all normal). No treatment related tumours. NOAEL = 451 mg/kg	112
rat (pregnant)	inhalation	451, 1202, or 3055 mg/m^3 (6 h/d) for days: 6 through 15 of pregnancy	No embryo/fetotoxicity or teratogenicity	112
rat (m); guinea pig (m, f)	inhalation	2049 mg/m^3 (334-351 ppm): 7 h/day, 5 d/week, 6 months	Reduced growth and slight histological changes in liver (male guinea pig). Increased liver and kidney weight (male rat)	83
rat (m, f) ; guinea pig (m, f); rabbit (m, f); mice (m); monkey (f)	inhalation	950 mg/m^3 (140-180 ppm): 7 h/day, 5 d/week, 5-7 months	Slightly reduced growth (male guinea pig). Increased weight of liver (rat, female guinea pig) and kidney (male rat)	83
rat (m, f); guinea pig (m, f); rabbit (m, f)	inhalation	1040 mg/m^3 (151-188 ppm): 7 h/day, 5 d/week, 16 days	Lung histology: slight changes (oedema, congestion, alveolar haemorrhage). Increased weight of liver (granular degeneration) and kidney (rat). Decreased spleen weight (guinea pig)	83

Cont.

Table 7. Cont.

Species (m, male f, female)	Route of administration	Exposure data	Effect	Reference
rat (m, f); guinea pig (m, f); rabbit (m, f); monkey (f)	inhalation	577 mg/m^3 (85-110 ppm): 7 h/day, 5 d/week, 6-7 months	NOAEL (behaviour; growth; mortality; organ weights; histopathology of lungs, heart, liver, kidney, testes; qualitative urine tests). Haematology data normal (rat and rabbit)	83
guinea pig (m)	inhalation	300 mg/m^3 (12 weeks)	No sensitisation, no allergy enhancing effect	159
rabbit (m, f)	oral	500 or 1000 mg/kg/day (5 d/week), 12 months	Tremors, weakness, weight loss. Liver (cloudy swelling, little focal necrosis). No cataracts	83
rat (m, f)	oral	0, 75, 150, 300 or 600 mg/kg/day (7 d/wk) for 13 weeks	Nephrotoxic in male (hyaline droplet accumulation)	22
rat (m)	oral	10, 100, or 500 mg/kg/day (5 d/week), 4 weeks	Liver (cloudy swelling, centrolobular necrosis). Renal tubular epithelium (cloudy swelling, cast formation). NOAEL =10 and 100 mg/kg	83
rat (f)	oral	18.8, 188, or 376 mg/kg/day (5 d/week), 20-27 weeks	Increased liver and kidney weights; decreased spleen weight. Liver (slight cirrhosis, focal necrosis). NOAEL =18.8 mg/kg	83
duck	oral (diet)	0.5% in diet for 35 days	Retarded growth, deaths (3/10), no cataracts	83

Table 8. Effects of dichlorobenzenes observed in toxicity/carcinogenicity bioassays

Species	Exposure	Dose LOEL	NOAEL	Effect
o-DCB (NTP 1985 study)				
mouse(male)	gavage (2 y)	120 mg/kg		Kidney/tubular regeneration
mouse	gavage (13 w)	500 mg/kg		Increased liver weight (relative)
rat (male)	gavage (13 w)	500 mg/kg	250 mg/kg	Renal tubular lesions, decreased thymus weight
rat	gavage (13 w)	500 mg/kg	250 mg/kg	Kidney Increased weight
rat (male), mouse	gavage (13 w)	500 mg/kg	250 mg/kg	Thymic lesions (lymphoid depletion)
mouse	gavage (13 w)	500 mg/kg	250 mg/kg	Spleen lymphoid depletion
mouse(female)	gavage (13 w)	30 mg/kg		Decreased spleen weights (relative)
rat (female)	gavage (13 w)	250/500 mg/kg	500 mg/kg	Increased total liver porphyrin
mouse (male)	gavage (13 w)	250 mg/kg		Increased serum levels of liver enzymes or changes in alkaline phosphatase
mouse (female)	gavage (13 w)	250 mg/kg	125 mg/kg	Increased total liver porphyrin
mouse (male)	gavage (13 w)	250 mg/kg	125 mg/kg	Hepatocellular degeneration/ necrosis
rat	gavage (13 w)	125/250 mg/kg	60 mg/kg	Liver lesions
rats	gavage (13 w)	125 mg/kg	60 mg/kg	Increased liver weights (relative)
rat	gavage (13 w)	-	500 mg/kg	Increased serum levels of liver enzymes or changes in alkaline phosphatase
p-DCB (NTP 1987 study)				
mouse	gavage (2 y)	600 mg/kg		Thyroid hyperplacia
rat	gavage (2 y)	300mg/kg (male) 600 mg/kg (female)		Nephropathy
mouse	gavage (2 y)	300 mg/kg 600 mg/kg		Liver tumors (adenomas, carcinomas)
rat	gavage (2 y)	300 mg/kg (male)	600 mg/kg (female)	Mononuclear cell leukemia
rat	gavage (2 y)	300 mg/kg (male)	600 mg/kg (female)	Parathyroid hyperplacia

Cont.

Table 8. Cont.

Species	Exposure	Dose LOEL	NOAEL	Effect
mouse	gavage (2 y)	300 mg/kg		Nephropathy
mouse	gavage (2 y)	300 mg/kg		Hyperplacia in adrenal gland
mouse	gavage (2 y)	300 mg/kg		Hepatocellular damage/necrosis
rat (male)	gavage (2 y)	150/300 mg/kg	600 mg/kg (female)	Kidney tumors (tubular cell adenocarcinomas)
mouse	gavage (13 w)	1500 mg/kg		Thymic lesions; spleen lymphoid or myeloid depletion; bone marrow myeloid depletion
rat (female)	gavage (13 w)	1200 mg/kg		Hepatocellular damage/necrosis
mouse	gavage (13 w)	1000 mg/kg		Increased total liver porphyrin
mouse	gavage (13 w)	900 mg/kg		Increased liver weights (relative)
mouse (female)	gavage (13 w)	900 mg/kg		Increased thymus weight
rat	gavage (13 w)	600 mg/kg		Increased liver weights (relative)
mouse	gavage (13 w)	600 mg/kg	337.5 mg/kg	Hepatocellular damage/necrosis
rat (male)	gavage (13 w)	600 mg/kg		Kidney Increased weight
rat	gavage (13 w)	300 mg/kg (male)	1500 mg/kg (female)	Nephropathy
rat	gavage (13 w)	300 mg/kg (male) 1200 mg/kg (female)		Increased serum levels of liver enzymes or changes in alkaline phosphatase
mouse	gavage (13 w)	250 mg/kg		Reduced spleen weight; reduced white blood cell count
mouse	gavage (13 w)		11000 mg/kg	Increased serum levels of liver enzymes or changes in alkaline phosphatase

Table 9. Effects of dichlorobenzenes in humans

Study	Exposure	Exp. concn.	Effects	Medical examinations	Subjects	Reference
o-DCB						
Accident report	inhalation (8 h/day) 4 work days	'high' concns. (=strong odour)	Irritation (eyes, nose, throat), severe headache, fatique, nausea, dizziness	Clinical symptoms (10 subjects); Increased chromosomal aberrations in blood lymphocytes	8 men and 18 women	184
Industrial survey	inhalation	65-100 ppm	Irritation (eyes, respiratory tract)		workers	58
Industrial plant study	inhalation (8 h/day, 5 d/wk) all plant operations	15 (1 - 44) ppm	No apparent odour	No evidence of organic injury or of untowarded haematological effects	workmen	84
Case report	dermal	handling a mixture containing o-DCB	Had developed eczematoid dermatis (hands, arms, face)	Sensitisation shown (patch test) when applied to skin of arm (intense erythema and oedema)	47 year old glazier	54
Case report	dermal	applied to skin for 15-60 min	Burning sensation intensified in 60min; redness, dark colour and blisters seen in 24h		2 subjects	140

Cont.

Table 9. Cont.

Study	Exposure	Exp. concn.	Effects	Medical examinations	Subjects	Reference
p-DCB						
Industrial plant study:	inhalation (8 h/day, 5 d/wk, 0.7 to 25 years)	continuous/inter-mittent		No evidence of organic injury or of untowarded haemato-logical effects were seen in	58 workmen	83
1st Survey	all operations	15 - 30 ppm	Faint odour	any of the three surveys. Lens		
		30 - 60 ppm	Strong odour	changes never seen		
		50 - 80 ppm	Painful irritation by non-acclimated men (eyes/nose)			
		80-160 ppm	Painful irritation by acclimated men			
		160-550 ppm	Irrespirable concentrations			
2nd Survey	ordinary jobs	90 (5 - 275) ppm	Acceptable concentrations			
	high-exp. jobs	380 (100 -725) ppm	Irrespirable concentrations			
3rd Survey	all operations	45 (15 - 85) ppm	No irritation			
	all operations	105 (50 - 170) ppm	Complained eye and nose irritation			
Industrial report	dermal	solid particles	Negligible irritation of intact skin; painful to eyes			83
Case report	ingestion	unknown amounts	Haematological disturbances			38

12. Previous Evaluations by (Inter)National Bodies

An IPCS Environmental Health Criteria Document was published by WHO (182) on all three DCB isomers. The main conclusions were that ‘if good industrial practices are followed, the risks associated with occupational exposure to chlorobenzenes are considered to be minimal’ and that ‘current concentrations of chlorobenzenes in the environment pose a minimal risk for humans’. It was also recommended that the database for human health risk evaluation should be improved. For *p*-DCB it was concluded that it may act as a non-genotoxic carcinogen in rodent liver.

The ACGIH documentations on dichlorobenzenes were revised 1991 and 1996. The critical effects are as reported for *o*-DCB: the human eye and upper respiratory irritation (at 100 ppm) and liver damage in animals (at 50 ppm), and for *p*-DCB: human eye irritation (at 17 ppm), renal toxicity in rats (at 25 ppm)¸ and carcinogenicity in animals. The occupational standards for *o*-DCB (TLV-TWA, 25 ppm; TLV-STEL, 50 ppm) (1) and for *p*-DCB (TLV-TWA, 10 ppm; 3A) (2) are recommended without any skin notation. *p*-DCB is classified as an animal carcinogen (3A) in the TLV/BEI Booklet 1995-1996 (3). In the BUA Report published in Germany 1987 on *m*-Dichlorobenzene (34) it was stated that despite the potential hepatotoxicity of *m*-DCB as indicated by acute toxicity studies in rodents, no data were located on its subchronic or chronic effects (carcinogenicity), and no toxicity data were located on effects in humans, either.

In the BUA Report from 1990 on *o*-Dichlorobenzene (35) the main concluding statements were on the concern about the organ toxicity revealed by acute, subchronic or chronic toxicity studies in animals; besides its (*o*-DCB) potential toxicity to the liver and kidneys also effects on other target organs (spleen and thymus) were pointed out. Although periodic medical examinations have not revealed any organ or haematological effects in exposed humans, there are case reports on mixed chemical exposure and adverse health effects in humans such as leukaemia/anaemia, with alleged but not proven associations with *o*-DCB exposure.

Compiled data of the health hazard, safety health and handling, and toxicology of dichlorobenzenes have been published (42-44), and also the NTP database obtained from the toxicology and carcinogenesis studies in US of *o*-DCB (129) and *p*-DCB (130).

The Agency for Toxic Substances and Disease Registry (ATSDR) has published 1993 (based on peer reviewed data) a Toxicological profile for 1,4-dichlorobenzene (169). This document describes levels of significant human exposure and health effects, and for the protection of public health the following Minimal Risk Levels (MRL) were derived: Inhalation MRL of 0.2 ppm (for intermediate-duration inhalation exposure to *p*-DCB). Oral MRL of 0.1 mg/kg/day (for intermediate-duration exposure to *p*-DCB).

CEC has reviewed the data on *p*-DCB (40).

12.1. Carcinogenicity classification

Carcinogenicity evaluation made by IARC (88-90):

- For *o*-DCB (Group 3). 1) Evidence in humans: inadequate; 2) evidence in animals: inadequate. Overall summary evaluation of carcinogenic risk to humans is group 3: The agent is not classifiable as to its carcinogenicity to humans.
- For *p*-DCB (Group 2B). 1) Evidence in humans: inadequate; 2) evidence in animals: sufficient. Overall summary evaluation of carcinogenic risk to humans is group 2B: The agent is possibly carcinogenic to humans.

US EPA Classifications for *o*-DCB (D); not classifiable as to human carcinogenicity. (Basis for classification: Based on no human data and evidence of both negative and positive trends for carcinogenic responses in rats and mice. Human carcinogenicity data: None. Animal carcinogenicity data: Inadequate (173)).

US EPA Classification for *m*-DCB (D); not classifiable as to human carcinogenicity. (Basis for classification: Based on no human data, no animal data and limited genetic data. Human carcinogenicity data: None. Animal carcinogenicity data: None (174))

NIOSH has recommended that *p*-DCB be treated as a potential human carcinogen (175).

13. Evaluation of Human Health Risks

13.1. Groups at extra risk

The wide use of dichlorobenzenes (*para*) as space deodorants and moth repellents may be a source of environmental and general population exposure that may significantly add to occupational uptake and the risk of toxicity.

Marked interindividual variation may occur in propensity to the ill effects associated with human exposure to dichlorobenzene(s). The reasons underlying enhanced susceptibility are unknown in humans. It seems possible that individual differences as regards activation/detoxification/elimination of DCBs play a key role in toxicity, and that variability in DCB biotransformation is probably of greater concern for exposure to the *ortho* (and *meta*) than to the *para* isomer.

13.2. Assessment of health risks

The data on occupational and non-occupational exposure are sufficient to predict the main dose-related health risks from exposure to *o*-DCB and *p*-DCBs in workers, whereas the effects of low-level, long-term intermittent exposure are more difficult to assess. In spite of the lack of human data on *m*-DCB specific toxicity, certain health effects may be predicted in humans for all the three DCB isomers. In general, inhalation of vapour or sprays or dust of DCBs is irritating to the eyes, nose, and throat. If swallowed, DCB isomers cause burning pain in the stomach, nausea, vomiting, and diarrhoea. Haemoglobin may change to methaemoglobin and result in impaired transport of oxygen. In repeated exposure,

the toxicological effect is injury primarily to the liver and secondarily to the kidneys. Depression of the central nervous system will occur at concentrations that are extremely objectionable to the eyes and nose (110, 113, 162).

o-DCB. Using the mouse respiratory irritancy model, the RD_{50} concentration (181 ppm) obtained for the acute irritative effect of *o*-DCB inhalation was much lower than the 4-h exposure concentration (598 ppm) required to produce hepatotoxicity. *o*-DCB thus seems to act primarily as an irritant. In humans, irritation of the eyes and respiratory passages has been reported in industrial workers at ambient concentrations of 100 ppm (601 mg/m^3), while the NOEL value observed in workers was 1 - 44 ppm (average 15 ppm = 90 mg/m^3) (58, 84). Acclimated industrial workers showed greater tolerance to *o*-DCB vapour exposure than unacclimated persons. The risk of being sensitised by direct dermal exposure to *o*-DCB does not seem to be high in humans; there is only one case report of a glazier who had worked with *o*-DCB and had developed severe eczematoid dermatitis (54).

Great differences in individual susceptibility to the ill effects of *o*-DCB vapour inhalation (severe pallor, exhaustion, and vomiting, with intense gastric pain, headache, and haemolytic anaemia) have been observed among workers exposed to nonreported levels of Orthosol (containing 95% *o*-DCB, 5% *p*-DCB) for 6 months (110).

The only report of *in vivo* human genotoxicity describes an accidental 4-day inhalation exposure of 26 individuals to *o*-DCB at unknown levels (high enough to cause clinical symptoms in 10 subjects of 26). The exposed subjects had a higher rate (8.9%) of chromosomal lesions in cultured peripheral blood leukocytes than the control subjects (2.0%) (184). However, based on overall results from extensive testing for mutagenicity, *o*-DCB is regarded as putatively non-genotoxic.

The toxicity data accumulated so far from comparative studies show consistently that the potency of *o*-DCB to cause liver and other tissue damage is manifold compared to the *para* isomer. This has been mechanistically explained by clear differences in the extent of harmful biotransformation indicated by covalent binding and depletion of tissue glutathione. No overt signs of toxicity or organ damage could be found by Hollingsworth et al. (84) in two different low-level inhalation exposure experiments on rodents and monkeys at a vapour concentration of 93 ppm in air (7 h/d, 5 d/w, for 6-7 months), and on rats, guinea pigs and mice at a vapour concentration of 49 ppm (7 h/d, 5 d/w, for 6.5 months). Liver toxicity was observed in rats at dose levels of 98 mg/kg upwards (4, 53, 129) and in mice from dose levels of 250 mg/kg upwards (129, 165). The possible risk for toxicity (liver, kidney, thyroid, spleen, lung) from long-term occupational exposure should be considered also in view of individual susceptibility, because several of the enzymes responsible for *o*-DCB metabolism are known to be polymorphically expressed in man. Experiments on pooled microsomes from 5 human livers, and rat liver microsomes from different strains, led Hissink et al. (80, 81) to conclude that humans are possibly less susceptible to *o*-DCB-induced

acute hepatotoxicity than the rat. However, before any conclusions can be drawn the metabolism of *o*-DCB needs to be analysed in a larger number of human liver samples.

No evidence of carcinogenicity of *o*-DCB was found in a rodent cancer bioassay (129). Because *o*-DCB is more toxic than the *para* isomer, the dosage levels used for the *o*-DCB study were thus lower than those used in the cancer study of *p*-DCB (130). In humans, *o*-DCB exposure has been connected with 5 cases of leukaemia (88, 163), but no conclusions on carcinogenicity can be drawn from these data.

m-DCB. In view of the data accumulated on *m*-DCB, it seems that the potential toxicity of this isomer resembles more that of *o*-DCB than of *p*-DCB.

p-DCB. The handling of solid compound has caused dermatitis in workers, although generally no skin problems seem to have occurred (2, 83). No irritation of the eyes and nose occurred in workers at 15-30 ppm (90-180 mg/m^3). Vapour concentrations of 50-80 ppm (300-480 mg/m^3), which in most people would probably cause considerable irritation, were tolerated 'without complaints' by acclimated industrial plant workers (83). Sensitisation studies in guinea pigs based on inhalation exposure (50 ppm, for 12 weeks) were negative (159). In the guinea pig maximisation test (GPMT) *p*-DCB was classified as a mild (grade II) contact sensitiser (25).

Neonatal examination of a child whose mother consumed, throughout her pregnancy, 1-2 blocks (mostly *p*-DCB) of toilet air freshener per week, showed no abnormalities (38). A two-generation reproduction toxicity study done with Sprague-Dawley rats (24) showed that the daily dose level of 90 mg/kg (by gavage) produced 'massive damage' in the pups (NOEL 30 mg/kg, this dose was calculated to be comparable to vapour inhalation exposure at 75 ppm, 8 h/day, 5 days/week). The study findings are not in line with the negative findings from another two-generation reproduction toxicity study also using SD rats (128), nor with the negative findings from rodent teratology studies based on dosing by inhalation (2, 75) or by gavage (67, 145).

Toxicology and carcinogenesis studies have shown that *p*-DCB is a non-genotoxic animal carcinogen, and that in comparison with the *ortho* (and *meta*) form, it is only a weak hepatotoxin. Also its pulmonary toxicity is clearly less when compared to the *ortho* isomer (137).

p-DCB induces kidney tumours in male Fisher-344/N rats (at dose levels 150 and 300 mg/kg/day by gavage, in a 2-year cancer bioassay) by a mechanism associated with tubular accumulation of α_{2u}-globulin which, according to current understanding, is not relevant to humans (130). The finding that *p*-DCB (at dose levels 300 and 600 mg/kg/day by gavage) caused liver tumours both in male and female B6C3F1 mice (130) may be of relevance to human risk assessment. Mutagenicity and cell proliferation studies (37, 55, 57) suggest that the mechanism leading to the formation of mouse liver tumours is based on mitogenic activity (causing cell proliferation in the liver and increased liver weight) but not on genotoxic activity by *p*-DCB. *p*-DCB is a mitogen that stimulates dose-

dependently cell proliferation also in the female rat liver. The lack of a tumorigenic response in rats showed that induced cell proliferation may be viewed as a necessary, but not sufficient, event for tumour formation, and that mice may be more sensitive than rats to nongenotoxic carcinogens.

13.3. Scientific basis for an occupational exposure limit

The database for setting occupational exposure limits for *o*- and *p*-DCB is rather comprehensive. However, it should be underlined that the current occupational exposure limits, as evaluated for example by ACGIH, are principally based on ambient occupational monitoring data obtained long ago (83, 84), or accidental exposure reports lacking any reliable information on sources (chemical exposure profile) and levels of airborne exposure. Also animal studies conducted by inhalation have been far too few. Overall, the data available on low-level inhalation exposure as well as on mixed exposures are scanty.

The critical effects of DCB isomers are:

o-DCB: Irritation to mucous membranes of the eyes and airways (ca. 100 ppm), NOEL < 44 ppm. Due to the limited data, it is not possible to draw firm conclusions on the potential risk from *o*-DCB as a sensitiser, or on its organ toxicity (liver, kidney, thyroid, lungs) in humans. *o*-DCB caused no overt toxicity or organ toxicity in rodents exposed by inhalation at the vapour level of 49 ppm (7 h/d, 5 d/w, 6-7 months) (84).

m-DCB: The database is inadequate, but based on chemical analogy, irritation to the skin and respiratory passages can be predicted. *m*-DCB is a mild (rat) to moderate (mouse) hepatotoxin with adverse dose-dependent effects on the thyroid (both sexes), pituitary (male) and kidney function (male) in the rat (a 90-day gavage study; LOEL 9 mg/kg/day).

p-DCB: Irritation to mucous membranes of the eyes and airways (ca. 50 ppm). Carcinogenicity of *p*-DCB in rats and mice by nongenotoxic mechanisms cannot, at the present time, be adequately assessed in terms of human health risk.

14. Research Needs

Occupational surveys are needed to clarify the current levels of dichlorobenzene(s) in work place air and to quantify the potential health hazards in view of the work places and workers involved. Knowledge of the actual ambient exposure levels will help to judge the need for individual control by means of biological monitoring. Molecular toxicomechanistic (isomer-specific and species-specific) studies both on acute and long-term effects of dichlorobenzenes would greatly contribute to the assessment of possible occupational health risk. For example, identification of the presumed host (metabolic) factors that may account for the profound individual differences reported in human susceptibility to *o*-DCB-induced toxicity are of great interest. Animal toxicokinetic studies based on intermittent low-level inhalation exposure, in line with occupational modes of

DCB uptake, are needed for the risk evaluation of dose-response effects of *o*-, *m*-, and *p*-dichlorobenzenes. The risks of enhanced target organ toxicity, e.g. due to *o*-DCB-mediated liver damage and its potentiation by metabolic modulators, have not been studied in animals exposed by the inhalation mode. The effects of DCB vapours on pulmonary drug-metabolism and lesion in lung tissue need to be further studied. The carcinogenic mechanisms of *p*-DCB need to be further explored to enhance human health risk assessment.

15. Summary

Elovaara Eivor. 122. Dichlorobenzenes. Nordic Expert group for Criteria Documentation of Health Risks from Chemicals. *Arbete och Hälsa* 1998;4:1-76.

Ortho- and *para*-dichlorobenzenes are more widely used than the *meta* isomer. The use of *p*-DCB in space deodorants and moth repellents may result in non-occupational exposure.

o-DCB is a skin irritant, and may cause sensitisation. Vapours cause strong irritation of the eyes and the airways without other ill effects in industrial workers. However, particular individual susceptibility to *o*-DCB, resulting in the development of systemic symptoms and haemolytic anaemia, has been described in one case report. *o*-DCB induces organ toxicity (liver, kidney, thyroid, lung) in animals. *Critical effect*: Mucous membrane irritation.

m-DCB is irritating to the eyes and the upper airways; it is organotoxic like the *ortho* isomer, and induces α_{2u}-globulin nephropathy like the *para* isomer.

p-DCB crystals seldom cause skin irritation, whereas the vapours are irritating. High exposure may cause haematological effects, organ damage (liver, kidney, spleen), and symptoms of the central nervous system. *p*-DCB is carcinogenic in male rats (renal tubular cell adenocarcinomas) and in male and female mice (hepatocellular adenomas and carcinomas) by mechanisms that are believed to be non-genotoxic. Kidney tumours likely bear no relevance to human risk assessment, as the tumorigenic (α_{2u}-globulin-linked) mechanism is unique to male rats. Carcinogenicity data in humans is insufficient. *Critical effect*: Mucous membrane irritation. Animal carcinogenicity cannot be presently assessed in terms of human health risk.

Keywords: Carcinogenicity, health effects, hepatotoxicity, individual susceptibility, isomer-specific toxicity, nephrotoxicity, occupational exposure limits, sensitisation.

16. Summary in Swedish

Elovaara Eivor. 122. Diklorobensener. Nordiska Expertgruppen för kriteriedokumentation av kemiska hälsorisker. *Arbete och Hälsa* 1998;4:1-76.

Av de tre isomera formerna av diklorobensen (DKB) används orto- och paraisomeren i betydligt större utsträckning än metaisomeren. Användningen av p-DKB som luktborttagningsmedel på toaletter och i malkulor kan orsaka icke arbetsmiljörelaterad exponering.

o-DKB är en hudirritant och kan orsaka sensibilisering. Ångorna ger upphov till kraftig irritation i ögon och luftvägar hos industriarbetare utan andra påtagliga hälsoeffekter. Individuell känslighet har emellertid beskrivits i en fallrapport där o-DKB hade orsakat systemiska symptom och hemolytisk anemi. o-DKB är organtoxiskt (lever, njure, sköldkörtel, lungor) i djur. Kritisk effekt: Slemhinneirritation.

m-DKB är irriterande för ögonen och de övre luftvägarna; den är organtoxisk som ortoisomeren och inducerar, liksom paraisomeren, α_{2u}-globulin nefropati.

p-DKB kristaller orsakar sällan hudirritation men ångorna är irriterande. Höga exponeringsnivåer kan ge hematologiska effekter, organskada (lever, njure, mjälte) samt symptom från centrala nervsystemet. p-DKB är carcinogent i hanråttor (njurtubulicellsadenocarcinom) och i möss av båda könen (levercellsadenom och carcinom) genom mekanismer som anses vara icke-genotoxiska. Njurtumörerna har troligen ingen relevans i riskbedömning eftersom mekanismen för tumöruppkomsten (α_{2u}-globulin kopplad) är unik för hanråttor. Carcinogenisitetsdata för människa är otillräckliga. Kritisk effekt: Slemhinneirritation. Den konstaterade carcinogena effekten på försöksdjur kan i dag inte bedömas med avseende på hälsoeffekter hos människa.

Nyckelord: Carcinogenicitet, hälsoeffekter, levertoxicitet, individuell känslighet, isomerspecific toxicitet, njurtoxicitet, hygieniskt gränsvärde, sensibilisering.

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18. Data bases used in search for literature

In the search for literature the following data bases were used:

-NIOSHTIC
-Medline
-Toxline
-RTECS
-HSDB
-IUCLID

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Appendix 1

Occupational exposure limits for Dichlorobenzenes in air.

Country	ppm	mg/m^3	Comments	Year	Ref.
Denmark	25	150	ortho-	1994	1
	25	150	para- *		
Finland	50	300	ortho-	1996	2
	75	460	15 min short term		
	75	460	para-		
	120	730	15 min short term		
Germany	50	300	Skin, ortho-	1996	3
	100	600	30 min short term		
	50	300	para-		
	100	600	30 min short term		
Iceland	50	300	ceiling, ortho-	1989	4
	75	450	para-		
	110	700	15 min short term		
Netherlands	25	150	ortho-	1996	5
	50	301	15 min short term		
	25	150	para-		
	50	300	15 min short term		
Norway	25	150	ceiling, ortho-	1995	6
	40	240	para-		
Sweden	50	300	ceiling, ortho-	1996	7
	75	450	para-		
	110	700	15 min short term		
USA (ACGIH)	25	150	ortho-	1997	8
	50	301	15 min short term		
	10	60	para-, animal carcinogen		
(NIOSH)	50	300	ortho-	1994	9
	-	-	para-, carcinogen		
(OSHA)	50	300	ortho-	1994	9
	75	450	para-		

* intended change to 10 ppm

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