NICNAS

para-Dichlorobenzene

Priority Existing Chemical Assessment Report No. 13

December 2000

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Preface

This assessment was carried out under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). This Scheme was established by the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act), which came into operation on 17 July 1990.

The principal aim of NICNAS is to aid in the protection of people at work, the public and the environment from the harmful effects of industrial chemicals.

NICNAS assessments are carried out in conjunction with Environment Australia (EA) and the Therapeutic Goods Administration (TGA), which carry out the environmental and public health assessments, respectively.

NICNAS has two major programs: the assessment of the health and environmental effects of new industrial chemicals prior to importation or manufacture; and the other focussing on the assessment of chemicals already in use in Australia in response to specific concerns about their health/or environmental effects.

There is an established mechanism within NICNAS for prioritising and assessing the many thousands of existing chemicals in use in Australia. Chemicals selected for assessment are referred to as Priority Existing Chemicals.

This Priority Existing Chemical report has been prepared by the Director (Chemicals Notification and Assessment) in accordance with the Act. Under the Act manufacturers and importers of Priority Existing Chemicals are required to apply for assessment. Applicants for assessment are given a draft copy of the report and 28 days to advise the Director of any errors. Following the correction of any errors, the Director provides applicants and other interested parties with a copy of the draft assessment report for consideration. This is a period of public comment lasting for 28 days during which requests for variation of the report may be made. Where variations are requested the Director's decision concerning each request is made available to each respondent and to other interested parties (for a further period of 28 days). Notices in relation to public comment and decisions made appear in the *Commonwealth Chemical Gazette.*

In accordance with the Act, publication of this report revokes the declaration of this chemical as a Priority Existing Chemical, therefore manufacturers and importers wishing to introduce this chemical in the future need not apply for assessment. However, manufacturers and importers need to be aware of their duty to provide any new information to NICNAS, as required under section 64 of the Act.

For the purposes of Section 78(1) of the Act, copies of Assessment Reports for New and Existing Chemical assessments may be inspected by the public at the Library, NOHSC, 92-94 Parramatta Road, Camperdown, Sydney, NSW 2050 (between 10 am and 12 noon and 2 pm and 4 pm each weekday). Summary Reports are published in the *Commonwealth Chemical Gazette*, which are also available to the public at the above address.

Copies of this and other Priority Existing Chemical reports are available from NICNAS either by using the prescribed application form at the back of this report, or directly from the following address:

GPO Box 58

Sydney

NSW 2001

AUSTRALIA

Tel: +61 (02) 9577 9437

Fax: +61 (02) 9577 9465 or +61 (02) 9577 9465 9244

Other information about NICNAS (also available on request) includes:

NICNAS Service Charter;

information sheets on NICNAS Company Registration;

information sheets on PEC and New Chemical assessment programs;

subscription details for the NICNAS Handbook for Notifiers; and

subscription details for the Commonwealth Chemical Gazette.

Information on NICNAS, together with other information on the management of workplace chemicals can be found on the NICNAS Web site:

http://www.nicnas.gov.au

Overview

Para-dichlorobenzene (*p*-DCB; CAS No. 106-46-7) was declared a Priority Existing Chemical on 7 April 1998. The declaration of *p*-DCB was in response to concerns relating to possible human health risks and environmental hazards associated with the widespread use of the material in school and public toilet blocks and urinals and as an air freshener.

Up to 1000 tonnes of *p*-DCB are imported and used annually in Australia. *p*-DCB is primarily used as a deodoriser in toilet blocks, in household toilet bowls and as a room freshener. It has some minor uses in the agricultural and pharmaceutical industries.

Occupational exposure to *p*-DCB in Australia is primarily due to operations involved in the handling and processing of imported material. Exposure during such procedures may result from inhalation of dust when opening bags of raw material and of vapour, produced by sublimation or during melting and recasting operations. Exposure of the general public to *p*-DCB is from the use of several consumer products that contain *p*-DCB.

p-DCB is absorbed well by inhalation and oral routes and less well by dermal contact. Target organs for *p*-DCB are adipose tissue, liver, kidneys and lungs. Metabolism of *p*-DCB is by aromatic hydroxylation and, depending on the species involved, results in the formation of epoxides which are converted to their corresponding dichlorophenols. Further metabolism by conjugation with sulfate or glucuronate can occur. The parent compound and its derivatives are rapidly excreted in the urine. There is no evidence that *p*-DCB bioaccumulates in any tissue.

Acute exposure to *p*-DCB vapour within the range of 30 to 60 ppm in air is associated with irritation to the nose, eyes and upper respiratory tract. Exposure to vapour of 80 to 160 ppm results in acute discomfort, painful irritation of the nose and eyes and may induce breathing difficulties. Ingestion of large doses of *p*-DCB have been associated with vomiting, vertigo, disorientation, tiredness and oedema. Chronic exposure to large doses of *p*-DCB may result in headache, nausea, vertigo, ataxia, dysarthria, hyporeflexia, paresthesia, behavioural and haematological changes including anaemia.

Genotoxicity studies of *p*-DCB have produced negative results. However, *p*-DCB does induce the formation of kidney tumours in male rats and liver tumours in both sexes of mice after prolonged exposure. The formation of kidney tumours in male rats is thought to be due to the presence of the protein, $\alpha_{2\mu}$-globulin. As $\alpha_{2\mu}$-globulin is specific to the mature male rat, *p*-DCB is not considered to present a carcinogenic risk to humans by this mechanism. The tumours observed in mice after prolonged exposure to *p*-DCB are also considered to be irrelevant to humans. There are significant differences in the metabolism of *p*-DCB in the liver of mice and humans and it has been further observed that the mouse strains used demonstrate a high natural rate for liver tumour formation.

Exposure of pregnant rats to *p*-DCB vapour produced no evidence of maternal toxicity or embryo- or foetotoxicity. There have been no teratogenic effects observed in animals or humans as a result of acute or chronic exposure.

Environmental exposure to *p*-DCB can occur due to the use of the product in toilets from which it may be washed into the sewer system or enter the atmosphere by virtue of its volatile nature. *p*-DCB does not persist in air or surface water but accumulates in anaerobic sediments. *p*-DCB has a medium acute toxicity for aquatic life and may impair the reproduction of aquatic life. However, based on current patterns of *p*-DCB use within Australia, the risk to the environment is expected to be low.

The occupational risk assessment for *p*-DCB concluded that, for known Australian work situations, potential atmospheric concentrations of *p*-DCB are unlikely to reach levels likely to cause acute effects, including eye or respiratory irritation. In addition, it is unlikely that workers in these occupations will be at risk from chronic adverse health effects related to *p*-DCB exposure, as margins of exposure are generally high for inhalation exposure. In the absence of any monitoring data for workers involved in the hygiene sector estimates for *p*-DCB exposure were obtained using the UK EASE model. Results from this modelling indicate that the risk to workers is expected to be low.

Recommendations for reducing potential occupational health and safety risks for *p*-DCB include the monitoring of airborne *p*-DCB to be undertaken and a review of the current occupational exposure standard for *p*-DCB by the National Occupational Health and Safety Commission.

The hazard classification should be amended to include the follows safety phrases, S23 (Do not breath vapour), S24 (Avoid contact with skin), S25 (Avoid contact with eyes) and S51 (Use only in well ventilated areas).

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Acronyms and Abbreviations

8-oxodG	8-oxo-7,8-dihydro-2'-deoxyguanosine
ACGIH	American Conference of Governmental Industrial Hygienists
ADE	aldrin epoxidase
ADG	Australian Dangerous Goods
AICS	Australian Inventory of Chemical Substances
ALT	alanine aminotransferase
APHA	American Public Health Association
AST	aspartate aminotransferase
ASTM	American Standard Test Method
BCF	bioconcentration factor
BOD	biochemical oxygen demand
BrdU	5-bromo-2'-deoxyuridine
BSO	buthionine sulfoximine
BUA	Beratergremium für Umweltrelevante Altstoffe
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstracts Service
cDNA	complementary deoxyribonucleic acid
CNS	central nervous system
CYP	cytochrome P450
DCB	dichlorobenzene
DCBQ	dichlorobenzoquinone
DCP	dichlorophenol
DNA	deoxyribonucleic acid
dw	dry weight
EA	Environment Australia
EASE	Estimation and Assessment of Substance Exposure
$E_\beta C_{50}$	concentration leading to a 50% reduction in biomass
EC	European Commission
EC_{50}	concentration at which an effect is produced in 50% of test organisms
ECD	electron capture detection
ECOD	7-ethoxycoumarin *O*-deethylase
EH	epoxide hydrolase
EINECS	European Inventory of Existing Commercial Chemical Substances
ELS	early life stage
$E\mu C_{50}$	concentration leading to a 50% reduction in growth rate
EPA	Environmental Protection Agency (USA)
EROD	7-ethoxyresorufin *O*-deethylase
EU	European Union
F344	Fisher-344
FID	flame ionisation detection
GC	gas chromatography
GLP	good laboratory practice
GLT	glucuronyl transferase
γ-GT	γ-glutamyl transferase

GSH	glutathione
GST	glutathione S-transferase
HPLC	high performance liquid chromatography
IUCLID	International Uniform Chemical Information Database
IARC	International Agency for Research on Cancer
IUPAC	International Union of Pure and Applied Chemistry
i.p.	intraperitoneal
i.v.	intravenous
LBB	lethal body burden
LC	lethal concentration
LD	lethal dose
LDH	lactate dehydrogenase
LOAEL	lowest observed adverse effect level
m-DCB	*meta*-dichlorobenzene (1,3-dichlorobenzene)
MLD	minimum lethal dose
MOE	margin of exposure
MS	mass spectroscopy
MSDS	Material Safety Data Sheet
NADH	nicotinamide adenine dinucleotide (reduced)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NDPSC	National Drugs and Poisons Schedule Committee
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NIOSH	National Institute for Occupational Safety and Health (USA)
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOHSC	National Occupational Health and Safety Commission
NTP	National Toxicology Program (USA)
OECD	Organization for Economic Cooperation and Development
o-	CB *ortho*-dichlorobenzene (1,2-dichlorobenzene)
p-DCB	*para*-dichlorobenzene (1,4-dichlorobenzene)
PEC	predicted environmental concentration
PID	photoionization detection
PNEC	predicted no effect concentration
PPE	Personal protective equipment
ppm	parts per million
QSAR	Quantitative Structure-Activity Relationship
RNA	ribonucleic acid
RTECS	Registry of Toxic Effects of Chemical Substances
S-D	Sprague-Dawley
SDH	sorbitol dehydrogenase
STEL	short-term exposure limit
STP	sewage treatment plant
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
TGA	Therapeutic Goods Administration
TOC	total oxygen concentration
TWA	time-weighted average
UDS	unscheduled DNA synthesis
UK	United Kingdom
UN	United Nations

1. Introduction

1.1 Declaration

The chemical *para*-dichlorobenzene (*p*-DCB), Chemical Abstracts Service (CAS) number 106-46-7, was declared a priority existing chemical for full assessment under the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act), as amended, by notice in the *Chemical Gazette* on 7 April 1998.

The declaration was made on the basis that there were reasonable grounds for believing that the handling and use of *p*-DCB may give rise to a risk of adverse health effects. In summary, these grounds were:

- the potential for occupational and environmental exposure and potential adverse health effects and in particular, possible carcinogenic effects;
- lack of publicly available information on the health and environmental effects of *p*-DCB which is of concern in view of their widespread use and potential for exposure; and
- a need for characterisation of exposure and associated health and environmental risks.

In accordance with the Act, persons who wished to manufacture or import *p*-DCB into Australia were required to apply for assessment whilst *p*-DCB remained a priority existing chemical. As *p*-DCB is not manufactured in Australia, applications were limited to importers. A list of applicants is included in Section 2.

1.2 Objectives

The objectives of this assessment were to:

- characterise the hazards of *p*-DCB to human health (particularly carcinogenicity) and the environment;
- characterise current and potential occupational, public and environmental exposure to *p*-DCB;
- characterise the risk of adverse effects resulting from exposure to workers, the general public, and the environment; and
- make appropriate recommendations to control exposures and/or reduce potential health and environmental risks.

1.3 Australian perspective

The primary use of *p*-DCB in Australia is as a deodoriser for use in public toilet facilities. Domestically, *p*-DCB is used in households as a deodorant and a room

freshener. It has some minor use as a moth repellent and mildew control agent and in the agricultural and pharmaceutical industries.

Due to the widespread use of *p*-DCB in Australia there has been concern over possible health effects, including eye and upper respiratory tract irritation and concern that it may be a possible human carcinogen.

The declaration of *p*-DCB as a Priority Existing Chemical (*Chemical Gazette 7 April 1998*) indicated that it was to be assessed with *ortho*-dichlorobenzene. While under investigation it became apparent that the use and toxicological profiles of these two chemicals were substantially different. Consequently, the two chemicals have been assessed and published separately. The *ortho*-dichlorobenzene report may be obtained from NICNAS.

1.4 International perspective

Historically, *p*-DCB has been manufactured and used internationally on a large scale for several decades. *p*-DCB has been used extensively in industrial and domestic situations as a space deodorant, toilet deodoriser, moth repellent and mildew control agent. It has been used as an insecticide for the control of termites and other organisms in soil and as a fungicide agent for the control of plant diseases. Other uses of *p*-DCB have included the manufacture of dyes, polyphenylene sulfide resin and 1,2,4-trichlorobenzene and in the pharmaceutical and agricultural industries.

1.5 Sources of information

Information required for assessment was supplied by applicants and notifiers and located through comprehensive database and literature searches.

Reviews on health effects of *p*-DCB by other national or international organisations have been carried out by Environment Canada (1993), the UK Health and Safety Executive (1994), the United States Department of Health and Human Services (1998) and the OECD (SIAR, 1999).

Due to the availability of a number of overseas assessment reports, not all primary sources of data were evaluated. All critical studies, that is, those which contribute significantly to an understanding of the metabolism, toxicity, clinical effects and hazard evaluation of *p*-DCB, have been evaluated for this report and their content have been taken into consideration in making the recommendations stemming from this assessment. Sources referred to but not sighted are acknowledged in the body of this report and in the Reference Section. All relevant studies published since these reports became available have been evaluated. The last literature search for this assessment was conducted on 14 March 2000.

In addition, surveys were conducted by NICNAS. Questionnaires were designed and sent to importers, re-sellers, re-packers, formulators, and end users of *p*-DCB to obtain information on amounts of *p*-DCB imported, uses, formulation process, Material Safety Data Sheets (MSDS), labels, worker and environmental exposure,

and control measures. Workplace site visits were also carried out to obtain information to assist in the assessment.

1.6 Peer-review

During all stages of preparation, this report has been subject to internal peer review by NICNAS, Environment Australia and the Therapeutic Goods Administration. The toxicology/hazard assessment component of this report was peer reviewed by Dr Michael Davies of the Heart Research Institute, Camperdown, NSW, Australia. Dr George Nossar of Royal Prince Alfred Hospital provided advice on respiratory irritation and Dr Gene McConnell of ToxPath Inc. provided advice on carcinogenicity.

2. Applicants

2.1 List of applicants

Following the declaration of *p*-DCB as a Priority Existing Chemical, 7 companies responded as either importers or potential importers of *p*-DCB into Australia for use as air freshener/deodorant blocks or research use. The applicants supplied information on the properties, import quantities and uses of the chemical. In accordance with the *Industrial Chemicals (Notification and Assessment) Act 1989*, NICNAS provided the applicants with a draft copy of the report for comment during the corrections/variation phase of the assessment. Data for the assessment were also provided by 4 notifiers, that is, companies which purchase *p*-DCB in Australia and formulate it into various products.

The applicants were, as follows:

Amtrade International P/L
PO Box 6421
St. Kilda Road Central PO
Melbourne
Victoria 3004

Bio-Scientific Pty Ltd
PO Box 78
Gymea
NSW 2227

Crown Scientific Pty Ltd
144 Moorebank Avenue
Private Mail Bag 4
Moorebank
NSW 2170

Recochem Inc
PO Box 478
Wynnum
Queensland 4178

Redox Chemicals P/L
30-32 Redfern Street
Locked Bag No. 60
Wetherill Park
NSW 2164

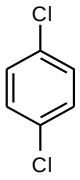
Sigma-Aldrich
PO Box 970
Castle Hill
NSW 2154

Unipuns International Pty Ltd
PO Box 1109
Ferntree Gully
Victoria 3128

3. Chemical Identity and Composition

3.1 Chemical identity

Table 1 - Chemical identity

Chemical name (IUPAC)	1,4-Dichlorobenzene
Other names	*para*-dichlorobenzene *p*-Dichlorobenzene *p*-DCB 1,4-DCB
CAS Number	106-46-7
EINECS Number	203-400-5
RTECS Number	CZ4550000
Empirical formula	$C_6H_4Cl_2$
Structural formula	
Molecular weight	147.00

Trade and product names are listed at Appendix 1.

3.2 Chemical composition

Commercially available *p*-DCB in Australia is typically more than 99.8% pure and contains the following impurities:

≤ 0.1%	1,2- and 1,3-dichlorobenzene
≤ 0.05%	chlorobenzene and trichlorobenzenes

4. Physical and Chemical Properties

4.1 Physical properties

p-DCB is a volatile solid, present as colourless or white crystals at ambient temperature and pressure (Merck, 1989). It has an aromatic, camphor-like odour with an odour threshold in air of 0.18 ppm and an odour threshold in water of 0.011 mg/L (Amoore and Hautala, 1983).

Conversion factors

1 ppm = 6.01 mg/m^3 and 1 mg/m^3 = 0.17 ppm (at 20°C and 1013 hPa; Verschueren, 1996).

The physical properties of *p*-DCB are given in Table 2.

Table 2 - Physical properties

Property	Value	Reference
Melting point	53.1°C	Lide, 1994
Boiling point	174.55°C	Lide, 1994
Flash point (closed cup)	66 °C	NFPA 1994
Ignition Temperature	> 500°C	Rathjen, 1975
Density (20°C)	1.2475 g/cm^3	Lide, 1994
Vapour pressure (20°C)	0.84 hPa	Verschueren, 1983
Water solubility (25°C)	79 mg/l	Verschueren, 1996
Solubility in organic solvents	Soluble in alcohol, ether, acetone, benzene	Lide, 1994
Henry's Law constant (25°C)	321.1 Pa.m^3/mol	BUA, 1994
Partition coefficient (25°C)	Log P_{ow} = 3.37	Banerjee *et al*., 1980 Miller *et al*., 1985

4.2 Chemical properties

Hydrolysis

Hydrolysis of *p*-DCB to *p*-chlorophenol and hydroquinone proceeds only under drastic conditions (days at > 200^{o}C).

Combustion products

Oxides of carbon, hydrogen chloride gas and some phosgene may form on combustion of *p*-DCB.

Reactivity

p-DCB may react vigorously with oxidising materials (Sax, 1996). *p*-DCB may react with some plastics, particularly styrene, acrylonitrile, and acrylonitrile-butadiene-styrene based plastics, rubber and coatings.

Polymerisation

p-DCB does not polymerise.

Explosivity

p-DCB does not form explosive mixtures with air.

5. Methods of Detection and Analysis

5.1 Identification

The isomers of dichlorobenzene are quantitatively determined by gas chromatography (GC) and, if required for definitive analysis, gas chromatography/mass spectroscopy (GC-MS). Sample preparation is based on concentration techniques utilising adsorption onto porous resins or activated charcoal with thermal or extractive desorption techniques followed by electron capture (ECD), flame-ionisation (FID) or photoionization (PID) detection. The use of capillary columns has been found to provide better resolution and sensitivity than packed columns (Washall and Wampler, 1988).

The analytical accuracy for *p*-DCB can be influenced by several factors such as sampling flow rates, temperature and humidity. These factors can influence the adsorption of *p*-DCB onto various sorbants (APHA, 1995). Separation of all isomers of dichlorobenzene can be achieved using Carbowax 20M coated glass capillary columns with isothermal or temperature gradient methods (Korhonen, 1983).

The purity of bulk dichlorobenzenes can be determined by melting point analysis.

5.2 Atmospheric monitoring

NIOSH method 1003, for the determination of occupational airborne *p*-DCB (NIOSH, 1994) entails the passage of 10 litres of sample volume over activated charcoal at a rate of 50 to 200 mL/min. Desorption is achieved with carbon disulfide followed by GC-FID. The detection limit is 0.1 mg/m^3.

The determination of dichlorobenzenes in ambient air can be achieved by either passive or active means. Passive sampling involves adsorption onto activated charcoal with desorption by carbon disulfide followed by capillary GC. Active methods include adsorption onto Tenax (a porous polymer) using an air flow rate of 23 L/hr. Thermal desorption at 200^o to 250^{o}C is followed by GC/MS (Wallace, 1987). The limit of detection is within the ng/m^3 range.

Information provided by an industry survey indicated that, within Australia, detection of airborne *p*-DCB at one workplace is achieved by the use of Dräger short term sampling tubes (type: chlorobenzene 5/a; qualitative). The method is not specific for *p*-DCB and the range and accuracy of the tube was stated to be 5 to 200 ppm ± 15%.

5.3 Water monitoring

Determination of dichlorobenzenes in water samples can be achieved using several methods. Older methods utilise liquid/liquid partitioning. The UK Department of the Environment (1986) method requires a 2-litre sample of water to be extracted with

hexane and an aliquot subjected to capillary column analysis with GC-ECD or GC-FID. If wastewater is to be analysed clean up of the sample will be required. The detection limit for *p*-DCB is stated to be in the range of 9 ng/mL for surface water and 420 ng/mL for wastewater.

Recent techniques depend on purging the sample with an inert gas, usually helium or nitrogen, which is then passed through a sorbent. Final analysis is achieved by thermal desorption followed by GC or GC/MS techniques. The detection limit varies depending on the quality of water sampled and can range from 0.1 to 100 ng/mL. For wastewater, an intermediate clean-up procedure is required.

5.4 Soil and sediments analysis

The determination of *p*-DCB in soil or sediment samples according to the method of Oliver and Bothen (1982) involves the extraction of 30 g wet weight (or 10 to 15 g dry weight) of sample with hexane/acetone. GC-ECD is proceeded by extraction and column chromatography. The detection limit is given as 5 μg/kg.

5.5 Biological monitoring

The presence of dichlorobenzenes in biological samples including urine, blood, tissues, milk and breath can be detected by various techniques, however, many of the techniques have not been validated. Generally, extraction of dichlorobenzenes from biological samples requires liquid/liquid partitioning or, for blood, urine and human milk, purge techniques using an inert gas can be used.

The presence of *p*-DCB in breath can be detected without the need for prior clean-up by the use of a spirometer to provide pure air and an adsorbent cartridge or canister for collection of the breath samples (Thomas *et al.*, 1991). Analytes are then concentrated or thermally desorbed prior to detection by GC/MS. The detection limit using Tenax cartridges is approximately 1 $\mu g/m^3$.

6. Manufacture, Importation and Use

6.1 Manufacture and importation

6.1.1 Manufacture

The manufacture of *p*-DCB does not occur in Australia but is undertaken in the USA, Canada, Europe and Japan. In 1989, it was estimated that the world-wide production capacity (excluding the former USSR) for *p*-DCB was 165,000 tonnes while world-wide consumption for the same year was estimated to be 113,000 tonnes (BUA, 1994).

The manufacture of *p*-DCB is accomplished by the fluid phase chlorination of benzene in the presence of a catalyst, usually ferric chloride, aluminium chloride or stannous tetrachloride, at atmospheric pressure between 20 to 80^{o}C (Beck, 1986). By adjusting the temperature of the process and the molar ratio of benzene to chlorine, the percentage of final chlorinated products can be determined. The process typically yields a mixture of chlorobenzene, isomers of dichlorobenzene and small quantities of higher chlorinated benzenes. Subsequent purification is achieved by distillation and fractional crystallisation. The resulting *p*-DCB contains less than 0.5% each of 1,2- and 1,3-dichlorobenzene and less than 0.1% monochlorobenzene and trichlorobenzene.

6.1.2 Importation

Based on Customs import data and industry information, for the past 5 years the amount of *p*-DCB imported into Australia typically ranges between 500 to 1000 tonnes per annum.

In 1998, five companies imported *p*-DCB into Australia. Imported raw material arrives in shipping containers, packaged in 4 ply paper bags each containing 25 kg net weight of *p*-DCB. Imported material is typically 99.8% pure. Only one company was identified as an importer of a finished product (a pharmaceutical) containing *p*-DCB. The imported raw material is used in the formulation of air freshener and toilet deodorant blocks and, to a lesser extent, insect repellent blocks and veterinary products.

6.2 Use

Within Australia, *p*-DCB is used almost exclusively for the production of air freshener and toilet deodorant blocks, where the chemical acts mainly to disguise odours.

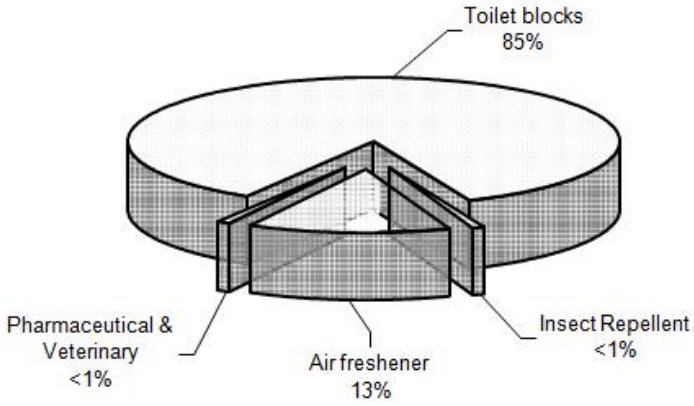
A survey of the handling and uses of dichlorobenzenes was undertaken by NICNAS. The survey identified the following areas and sectors of industry in which *p*-DCB is regularly used, mostly as an air freshener/deodoriser and predominantly in toilet

facilities:

- State Government (public buildings, police stations, correctional institutions);
- Council buildings (public toilet facilities);
- Schools (public and private);
- Motels/Inns/Caravan Parks/Resorts;
- Hotels/Leagues Clubs/Service Clubs/Night Clubs;
- Sporting Clubs and Sporting Facilities (e.g. bowls clubs);
- Industry (company toilets, transport, packaging, automotive and marine sector);
- Cleaning industry (associated with cleaning the facilities provided in the above areas); and
- Household use (as an air freshener/deodorant and moth/silverfish repellent).

To use an air freshener/deodoriser or repellent disk, the cellophane wrapping is punctured and the disk is left in a suitable location. The *p*-DCB undergoes sublimation and the vapour disperses. Where use in urinals is required, the blocks or disks are unwrapped and placed directly in the urinal. A breakdown of the uses of *p*-DCB is presented in Figure 1.

Figure 1 - Uses of *p*-DCB in Australia



An estimated 5 tonnes per year (less than 1%) of *p*-DCB are used in the agricultural sector. The National Registration Authority for Agricultural and Veterinary Chemicals has 7 products registered with *p*-DCB listed as an active constituent. Three are for moth repellency for home use, e.g. in candles or coils. The concentration of *p*-DCB in these products is 990 to 1000 g/kg. The other

formulations are classified as veterinary products. These are topical dressings and are registered for use as blowfly strike treatments (*p*-DCB present at 50 to 400 g/kg) or mulesing fluids (*p*-DCB present at 10 g/kg).

A small quantity, approximately 30 kg, is imported as a finished product for pharmaceutical use.

Further consideration of non-industrial uses (agricultural/veterinary and pharmaceutical) is not included in this assessment.

Approximately 10 kg of analytical grade material was identified for use in Australia for teaching and research purposes in schools and universities.

6.3 Manufacture of air freshener/deodoriser blocks

The major process involved in the manufacture of air freshener and toilet deodorant blocks is the addition of dye and perfume to *p*-DCB and then compression of flaked or granular *p*-DCB into disks or blocks. Due to a tendency for the imported material to become fused whilst in transit prior processing of the material is required, either melting/recrystallising and flaking or milling. The *p*-DCB is added to a hopper from which it enters an enclosed tank and reduced to a molten state by heating to 60^{o}C. A small quantity of dye and perfume are added prior to spreading the liquefied material onto a stainless steel conveyer belt which results in the formation of a thin layer of blended material suitable for flaking. Alternatively, the solid material is milled and then mixed with dye and perfume. The next step involves the pressing of the blended material into blocks of the required weight, typically 25, 50 or 100 g blocks. Subsequently, the blocks are wrapped in cellophane, labelled and boxed for distribution.

One importer also supplies blended *p*-DCB (containing dye and perfume) in flake form in 600 kg bags for sale to other processors who press and wrap the product.

The amount of *p*-DCB in finished commercially available air freshener/deodorant products ranges from 988 to 992 g/kg.

6.4 Export

A small quantity of *p*-DCB products (air freshener and toilet deodorant blocks) for export was identified. The amount of material exported accounted for less than 1% of all raw material imported into Australia.

7. Environmental Exposure

7.1 Environmental exposure

No studies with respect to environmental fate and toxicity were provided by applicants. As such, international reports and summary data have been relied upon. Reports available include the German BUA Report (BUA, 1994), the Canadian Environmental Protection Act report (1993), and the OECD draft report (SIAR, 1999). Data within these reports are largely the same and appears in the IUCLID datasheet (International Uniform Chemical Information Database), which has also been used where appropriate, predominantly to obtain fate and toxicity data. Results from this datasheet are non-confidential data supplied to the European Commission by European industry. Where IUCLID results appear in the SIAR (1999), original reports have been validated by the French authorities.

7.1.1 Release

Less than 1000 tonnes/year are used in the formulation and end use of air freshener/toilet blocks.

Reformulation

The manufacture/reformulation of *p*-DCB into air freshener/deodoriser blocks is described above in Section 6.3. There are no actual figures on release estimates during this process, but melting of the imported product is conducted in an enclosed tank, where releases to air and water are expected to be minimal. Generally, it would be expected that, compared to the end use of the product, release to water will be negligible through this process, with any release being predominantly to the atmosphere. While no specific data on releases to the atmosphere are available, a default release factor of 1% will be used based on the Technical Guidance Document (European Commission, 1996). Up to 10 tonnes may be expected to be released annually. Assuming reformulation processes are conducted on 200 days of the year, this results in estimated release to the atmosphere of up to 50 kg per day.

End use

Figure 1 shows in the order of 13% *p*-DCB used in air fresheners and 85% used in toilet blocks.

When used in air fresheners, release is assumed to be primarily to air, while use in toilet blocks will see release to both the atmospheric and aquatic compartments.

In the SIAR report (1999) on *p*-DCB, it was estimated based on a literature report that 67% of the toilet block evaporated during use. In the absence of other information, for the purposes of this assessment, this estimation will also be used,

with the remainder assumed to go to the sewer system. Therefore, annual releases of *p*-DCB when used in toilet blocks will consist of up to 570 tonnes evaporating to air, and up to 280 tonnes per annum being released to sewer.

Releases of *p*-DCB are summarised in Table 3. These are worst case calculations based on a maximum use of 1000 tonnes per annum.

Table 3 - Worst case environmental release estimates

	Quantity of use (kg)	Release (%)	Annual release (kg)	Days per year	Daily release (kg)		
					Air	Water	Soil
Reformulation	1000000	1	10000	200	50	-	-
End use							
Air freshener	130000	100	130000	365	356	-	-
Toilet Blocks	850000						
- Air		67	570000	365	1560	-	-
- Water		33	280000	365	-	770	-
Total Daily Releases					**1966**	**770**	**0**

7.1.2 Fate

Modelling predicts that at equilibrium, in the order of 99% of this chemical will partition to air, with 0.22% and 0.66% partitioning to water and soil respectively (Trent University, 1998). Negligible amounts are expected to partition to sediments, suspended sediment, biota and aerosols.

Equilibrium was determined using the Australian Capital Territory as a model inland environment with Lake Burley Griffin as the water body, and using environmental inputs for volumes of air, soil, water etc. as described in Connell and Hawker (1986).

Atmospheric fate

p-DCB is expected to partition predominantly to the atmosphere at equilibrium. The chemical absorbs radiation weakly at wavelengths greater than 300 nm, so direct photolysis in the atmosphere is not likely (Government of Canada, 1993). However, reaction with photochemically produced hydroxyl radicals in the atmosphere will occur. Howard *et al.* (1991) has estimated the photo-oxidation half-life to range between 8.4 to 83.6 days.

The hydroxyl radical reaction rate constant for *p*-DCB in the atmosphere was determined experimentally at 27^0C as 4.8 x 10^{-13} cm^3/molecule/s, and at 22^0C as 3.2 x 10^{-13} cm^3/molecule/s using different methods (BUA, 1994). Assuming a global mean hydroxyl radical concentration of $5x10^5$ molecule/cm^3, this corresponds to half-lives of 33 and 50 days, which is within the range reported above.

The presence of *p*-DCB in rainwater indicates that it persists long enough to be returned to the earth's surface by atmospheric wash out (Government of Canada, 1993).

Aquatic fate

Experimental Henry's Law Constants have been reported in BUA (1994), and range from 214.8 $Pa.m^3/mol$ (10^oC) to 394.1 $Pa.m^3/mol$ (30^oC). The experimental result at 25^oC was 321.1 $Pa.m^3/mol$. According to the scale of Mensink (1995), these results suggest *p*-DCB is readily volatile from aqueous solution. Wang and Jones (1994) state that volatilisation has been found to be the predominant removal process for chlorobenzenes from lakes and coastal seawater.

Howard (1989) reported an estimated half-life of 4.3 hours from a model river one metre deep flowing at 1 m/s with a wind velocity of 3 m/s at 20^0C. Other volatilisation half-lives ranging between <1 and 31 days have been reported (Government of Canada, 1993).

An experiment conducted to investigate the fate of *p*-DCB in coastal water by means of a mesocosmic plant is described in BUA (1994). In open water tanks, with a height of 5.5 m and diameter of 1.8 m, environmentally relevant concentrations of a mixture of test substances were added to 13 m^3 of unfiltered seawater (no sediment was present). The water was stirred four times per day for two hours, to simulate tides and water currents. The experiment was conducted in spring, summer and winter, and reported half lives of 18, 10 and 13 days respectively. Further, in summer, the experiment was conducted with and without addition of the microbicide $HgCl_2$, and resulted in similar half lives of 10.6 and 11 days respectively. This ruled out the possibility that biodegradation by plankton and microorganisms may contribute to total elimination.

In contrast to modelling predictions, monitoring data conducted in the Great Lakes area of North America indicate that adsorption to sediment is a major environmental fate process. Its detection in Lake Ontario sediment cores indicates that the chemical has persisted in these sediments for decades. Adsorption to sediment in water will attenuate volatilisation. *p*-DCB may biodegrade in aerobic water after microbial adaptation; however it is not expected to biodegrade under anaerobic conditions which may exist in lake sediments or various ground waters (Howard, 1989). Similarly, extensive monitoring data tabulated in the SIAR (1999) report shows sediment readings from rivers in Germany, France, the Netherlands, Denmark and Japan. Sediment measurements tended to greatly exceed those found in the surface waters, with concentrations in sediments ranging from <0.01 to $>10^3$ μg/kg dw. Reported concentrations in surface waters ranged from <0.01 to 4.05 μg/L. For a more detailed discussion of monitoring data, see Section 7.2.5.

If groundwater is exposed to *p*-DCB, due to the low bacterial density, low oxygen content, and generally very low organic matter content, a long residence time is expected which may last for decades (BUA, 1994).

Terrestrial fate

The IUCLID data sheet provides several results (with test reports validated in the SIAR (1999) though conditions are not available in detail for all the tests) in a wide range of soils and sediment with Koc values ranging from 155 to 1375 (mean 584). The highest Koc of 1375 was reported for a sediment and no other characteristics are available.

The SIAR (1999) states that some tests were performed with very low contents of organic carbon thereby increasing the possible error of the result. The OECD Test Guideline 106 suggests an organic carbon content of 0.6 to 3.5%. Results obtained from soils with an organic carbon content in this range are reported in Table 4.

Table 4 - Soil characteristics[1]

Soil	% Sand	% Silt	% Clay	% OC[2]	pH	Koc
Sandy soil	90	8	2	2.6	4	155
Silty loam	9	68	21	1.1	-	273
Dormont	2	38	60	1.2	4.2	280
Sandy agricultural soil	86.5	7.5	1.4	2.2	4.8	364
Podsol	81.5	10	7.2	3.56	3.88	744
Rendzina	8.5	68.3	20.6	1.11	7.9	748

[1] (data from SIAR 1999); [2] OC = organic carbon.

These results are suggestive of medium to low mobility. From these data, Koc appears independent of the various soil characteristics including pH. The less reliable results obtained with very low contents of organic carbon (<0.76% and not reported in the above table) which are typical of many parts of Australia, tended to show higher Koc values (>595).

Leaching from hazardous waste disposal areas in Niagara Falls to adjacent surface waters has been reported and the detection of *p*-DCB in ground water indicates that leaching can occur (Howard, 1989). Findings of leaching are supported by Robertson (1994) where a tracer experiment was conducted in which 450 mL of a plumbing line cleaner containing dichlorobenzene and 1 kg sodium bromide were injected into a septic system. Dichlorobenzene concentrations of up to 3460 µg/L were observed in the septic tank effluent, up to 650 µg/L in the unsaturated zone 0.45 m below the tile bed, and up to 13 µg/L at the water table at 2 m depth.

However, Wang and Jones (1994) suggest that while dichlorobenzenes may have some potential to leach in soils due to the convective mobility, because of the high volatility of these chemicals, dissipation to the atmosphere will be very rapid, except in those cases where continuous downward movement or a soil cover prevents escape through the soil surface. They further claim that chlorobenzenes in soil have a low potential to leach downward to groundwater. Results from one experiment (details not provided) showed *p*-DCB did not leach through large Dutch soil columns with a water table at a metre depth (Wang and Jones, 1994).

Volatilisation from soil surfaces may be an important transport mechanism; however, this may be mitigated by absorption or leaching. It is possible that the chemical can be slowly biodegraded in soil under aerobic conditions. Chemical transformation processes such as hydrolysis, oxidation, or direct photolysis on soil surfaces are not expected to occur (Howard, 1989).

Biodegradation

Results within the IUCLID data sheet have been assessed, but none of the original reports have been provided. The discussion in the SIAR (1999) with respect to biodegradation is brief, and does not contain many of the results provided in IUCLID. These are limited only to a couple of results from standard test systems (see Tests 1 and 3 in Table 5), where mineralisation is determined.

There appear to be no results from standard tests for soil and sediment.

Aerobic Degradation

Table 5 summarises results from the IUCLID data sheet and the BUA (1994) report. There are some discrepancies between results listed in the IUCLID data sheet and the discussion from the BUA report. Where these discrepancies occur, the results from the BUA report have been used as these have been validated.

Table 5 - Aerobic degradation test results

Test	Inoculum	Method	Result
1	Activated sludge	OECD 301D	67% after 28 days
2	Activated sludge	OECD Confirmatory Test	30% after 21 days
3	Activated sludge.	MITI Test	100% after 28 days (8 ppm)
4	Activated sludge	Respirometric (EU)	20% after 21 days (unpublished)
5	Sewage plant effluent	Biofilm column test	61-79% (time not reported)
6	Primary sewage sludge	Biofilm column test	98% degraded (2 years)
7	Primary sewage sludge	Biofilm column test	40-52% (1.5 hours)
8	Microorganisms from upper layer of slow sand filter	Batch test	100% after 5 days
9	Microorganisms from upper layer of slow sand filter	Biofilm column test	0-49% depending on flow rate (7 days).
10	Aquifer material from the interface of a river/ground water infiltration site	Soil column	90->99.9% (6 d flooding; 16 d drying; 3 cycles)

Test 1 was based on oxygen consumption and showed 1.4% of the original concentration (1.9 ppm) was degraded after 8 days, 49.5% after 15 days and 67% after 28 days. Complete degradation of the transformation products was confirmed

through HPLC analysis. In addition to this test, the author further investigated the degradability of the chemical in a continuous OECD confirmatory test after a 15 day adaptation period (Test 2). While an elimination rate of 97% was determined, 31% of this was attributed to biodegradation with the remainder attributed to volatilisation and adsorption to sewage sludge.

Test 3 determined degradation of *p*-DCB at 8 and 40 ppm in primary sludge with the test medium supplemented by nutrient substances. After 14 days, no degradation was observed at any concentration. In both replicates for the 8 ppm concentration, 100% degradation was observed after 28 days, while 0 and 38% were observed in the 40 ppm test. The mineralisation of *p*-DCB was determined by oxygen consumption, which reached about 80% of the theoretical oxygen demand. The authors assumed the initial concentration of 40 ppm had a toxic effect on the sewage sludge.

Test 4 is unpublished and no details are available.

Biofilm experiments are carried out as a model for trickling filter sewage plants, and several are reported. Test 5 used inoculum from the outlet of a sewage plant nitrification tank, and investigated the degradation of chlorinated benzenes and phenols. At initial concentrations of 1 and 5 ppb degradation of 69 to 79% and 61 to 72% respectively is reported. The duration of the experiment is unclear. The system was closed so losses through other means are excluded.

Test 6 was a two year experiment investigating degradation of approximately 10 ppb *p*-DCB in a mixture of several other chlorinated compounds in a closed system comprising a column filled with glass beads and charged from below, and a biofilm of primary sludge of unknown origin. Sodium acetate (1.39 ppm) was used as the primary substrate and added to the mineral medium. The reaction time in the column was approximately 20 minutes, and organisms adapted to the *p*-DCB within about 10 days. An average *p*-DCB biodegradation of 98% was observed. The authors found no intermediate metabolites in the efflux of the control column.

A 4.5 year biofilm degradation test in a 1 m long column filled with glass beads is described (Test 7). The column was inoculated with municipal primary sludge and charged with an oxygen-saturated solution by a continuous flow through method. The addition of electron acceptors facilitated the formation of aerobic, denitrifying and sulfate-reducing zones with correspondingly different redox potentials. The degradation appeared to be between 40 and 52% for the duration of the experiment. A very limited aerobic zone and large supply of acetate (60 ppm) which is more readily metabolised, led the authors to predict a lower utilisation of *p*-DCB which provides less energy.

Test 8 determined the biodegradation of *p*-DCB through a mixed population of microorganisms originating directly from the upper layer of former slow sand filters in a water processing plant. Bottle tests were performed in glass flasks on shaking apparatuses containing a bacterial suspension and *p*-DCB at concentrations of 3 and 15 ppb. Concentration reductions of 30% and 100% were reported after 1.5 and 5 days respectively. Experiments under sterile conditions showed the decrease of *p*-DCB to be due to biological effects.

The authors of the above study also conducted a biofilm investigation with four glass columns filled with sintered glass material (Test 9). Three columns operated on a flow through system, with one operating by recirculation. Flow rates were either 1 or 5 litres per hour, and degradation rates obtained at 7 days after commencement. The results where the flow rate was 1 litre per hour showed 25 to 40% degradation in the flow through columns and 49% degradation in the recirculation column. With a flow rate of 5 litres per hour, the flow through columns showed degradation of 0 to 32% with 41% degradation reported in the recirculation column. These results indicate that the flow through conditions lead to lower degradation, and degradation is reduced with faster rates of flow.

A flooding/drying schedule was used to investigate the degradation of organic trace substances in sandy soil from a rapid infiltration area for waste water and crude waste water (Test 10). Three cycles, consisting of 6 days of flow through (infiltration rate of 34 cm per day) and 16 days of drying, were conducted both aerobically and then anaerobically. Concentrations tested were 5, 10, 70 and 500 ppb. The aerobic column flowoff showed between 0.03 and 10% of the initial concentration in eluate. No real inferences on biodegradation can be drawn from this as no comment is made on the adsorption processes, and it appears this was not tested.

Experiments not reported in IUCLID that have been summarised in the BUA report.

In one test, biodegradation was determined by means of the biological oxygen demand (BOD) and total oxygen concentration (TOC) content of the test batches in a static enrichment degradation test with microorganisms from settled municipal waste water. Three parallel experiments were mixed after 7 days, separated again, and spiked with new chemical. The initial and additional concentrations of *p*-DCB were 5 and 10 ppm respectively. Degradation was higher in the 5 ppm concentration, ranging from 16 to 55% after 7 days, while for the 10 ppm concentration, it ranged from 0 to 54%. The authors attributed the reduced degradation rate to poisoning effects. It was also highlighted that the concentrations were excessive for environmental conditions, and the static conditions were unrealistic.

A column test detected *p*-DCB degradation of greater than 90%. The columns were charged with material from a river/groundwater infiltration area. Evaluation of the tests showed nearly the same results for the uninoculated columns as for a column inoculated with a xylene enrichment culture. In the flow through method, the columns were protected against volatilisation and charged with synthetic groundwater with *p*-DCB at a concentration of 29.4 ppb. After an initial breakthrough, the concentration in the outflow after 10 days was only 50% of the starting concentration and after approximately 3 weeks, less than 10% was detected. Approximately 80% of the starting concentration was already removed within the first 4.7 cm of the column, although it is not apparent how much of this was through degradation and how much through adsorption.

In a follow up study, 58.8 ppb *p*-DCB in synthetic river water was also degraded to a remainder of about 10%. After the oxygen had been removed from this column, the

chemical was only degraded for a few more hours. During the following 90 days under anoxic conditions, no further degradation was observed.

Conclusion

Tests reported with respect to aerobic degradation generally appeared to follow non-standard conditions. The two results following standard guidelines for ready biodegradability show the chemical to be readily biodegradable although it is not certain if the 10 day window criterion was met in one of these tests.

While other tests seem to be non-standard and hard to interpret due to a lack of details, they generally show *p*-DCB may be expected to degrade relatively quickly under aerobic conditions.

Anaerobic/Anoxic Degradation

Table 6 summarises results from the IUCLID data sheet, with the discussion below outlining further information summarised from the BUA (1994) report.

Test 1 considered primary degradation of 710 ppb under anaerobic conditions in a bottle test with settled, digested sewage sludge. Sodium acetate and sodium propionate were added as nutrients. To determine the proportion of elimination not due to biodegradation, one experiment contained 1% sodium azide. In the digested sewage sludge without sodium azide, elimination steadily increased from 12% at 2 days to 80% at 32 days. This compared with 5% at 2 days with sodium azide to 8% at 32 days, although the 4 day measurement showed 18% elimination. Statistical evaluation of the elimination results of the experiments with and without sodium azide show that, in this investigation, *p*-DCB was degraded anaerobically.

Table 6 - Anaerobic degradation test results

Test	Inoculum	Method	Result
Anaerobic 1	Co-settled digested sludge	Batch test	80% after 32 days
2	Methanogenic mixed culture	Not given	0% after 84 days
3	Primary effluent sludge	Batch test	No significant degradation after 11 weeks.
4	Soil microorganisms from a rapid infiltration field	Soil column	80-96% (6 d flooding; 16 d drying; 3 cycles)
Anoxic	Aquifer material from the interface of a river/groundwater infiltration site	Soil column	0% after 90 days.

This result contrasts to two other anaerobic bottle experiments. The first used methanogenic sludge from an experimental sewage plant (Test 2). The tests were conducted in the dark at 35^0C with a mineral medium. Three concentrations of *p*-DCB were tested, 74, 29 and 7.4 ppb. No degradation was detected at any concentration after 84 days.

The second contrasting result found no significant degradation after 11 weeks (Test 3). To initial primary (aerobic) sewage sludge, a mixture of various halogenated, aliphatic and aromatic hydrocarbons, at concentrations of 40 and 114 ppb per substance, and a mineral medium were added and tested under denitrifying conditions. From the absence of biological degradation under the denitrifying conditions and the previous result using methanogenic sludge, it was concluded that molecular oxygen is necessary for the degradation of *p*-DCB.

Following the aerobic column tests described above which employed a flooding-drying schedule to investigate sandy soil from a rapid infiltration area for waste water and crude waste water (aerobic Test 10), an investigation was carried out on the anaerobic degradation of *p*-DCB and other compounds, under other similar conditions (Test 4). At the three concentrations of 5, 50 and 700 ppb, between 4 and 20% of the initial concentration was found in eluate. As with the aerobic test, no comment has been made with respect to the adsorption of the chemical to the column. However, the concentrations found in the eluate in the anaerobic test are significantly higher than in the aerobic test suggesting that degradation is not as significant under the anaerobic conditions.

As described in Section 7.1.2.5, the anoxic test followed on from an aerobic soil column study where 58.8 ppb *p*-DCB in synthetic river water was degraded. After the oxygen had been removed from this column, the chemical was only degraded for a few more hours. During the following 90 days under anoxic conditions, no further degradation was observed.

Experiments not reported in IUCLID that have been summarised in the BUA report.

Two anaerobic tests are reported in the BUA report which do not appear in the IUCLID data sheet. Column tests with anaerobic Rhine sediment indicate that microorganisms capable of degradation prefer certain chlorinated hydrocarbons. When environmentally relevant concentrations of several chlorinated hydrocarbons were mixed, only dichlorobenzenes were still detectable after 2-6 months in the efflux of a column of only 20 cm in length.

In a follow up experiment, it was demonstrated that under anaerobic conditions, *p*-DCB was formed from 1,2,4-trichlorobenzene (TCB). It was further shown that after 450 days operation of the experimental plant, under various conditions TCB was already dechlorinated to *p*-DCB in the first 0.5 cm of the column, while in the lower 10 cm, a slight decline in the *p*-DCB concentration was accompanied by the formation of monochlorobenzene. 1,3-dichlorobenzene and *p*-DCB were only dechlorinated after the complete removal of 1,2-dichlorobenzene. It was concluded that, under reducing conditions, TCB can be degraded via dichlorobenzene to chlorobenzene and chloride.

Conclusion

As with the aerobic tests, the experiments were largely of a non-standard nature. While two experiments indicate a significant degree of degradation, the rate appears

slower than rates for aerobic degradation. Other results show *p*-DCB to be relatively resistant to degradation under anaerobic conditions.

Bioaccumulation

Several bioaccumulation studies have been reported in the SIAR (1999) (Table 7).

As stated in the SIAR (1999) report, test conditions were not available in detail for all the tests. However, with the exception of the 7 day rainbow trout larvae test and the 60 day flow through test on rainbow trout, the rest appear to have been derived from fairly standard guidelines.

Table 7 - Bioaccumulation results (modified from SIAR, 1999)

Species	Test	Fat (%)	Conc. (μg/L)	Elimination Half-life (d)	BCF[a]
O. mykiss (eggs)	2 d flow through		13.4	See below[b]	45-220[c]
L. macrochirus	14 d flow through	-	10.1	<1	60
O. mykiss (larvae)	7 d -	-	3	<1	112
O. mykiss (larvae)	7 d-	-	15	<1	40
O. mykiss (larvae)	7 d -	-	73	<1	85
P. promelas	28 d flow through	3.2-4.1	570-1000	-	110
J. floridae	28 d flow through	8.5	5	0.7	296
P. reticulata	20 d flow through	6.5	-	-	98
O. mykiss (eggs)	60 d flow through	-	3	-	100-1400[d]

a) BCF = bioconcentration factor; b) Elimination half lives of > 1 day are reported for egg, eyed egg and hatchlings, while < 1 day is reported for adsorbed half yolk, partially adsorbed yolk and alevin; c) The lowest BCF was found in the alevin stage, while the highest was reported for adsorbed half yolk; d) The highest BCF of 1400 was only observed at hatching. It fell to approximately 100 at the end of the test.

These results are indicative of low to medium accumulation potential at all lifestages of the fish, although it generally appears the chemical was readily eliminated from the animals, mostly in the order of less than 24 hours.

Mortimer and Connell (1995) undertook work on the effect of exposure to chlorobenzenes on growth rates of the crab *Portunus pelagicus* (L). In this work, critical body residue concentrations in lipid associated with a growth rate reduction of 50% was determined, and Quantitative Structure-Activity Relationships (QSARs) relating to this concentration were developed including bioconcentration factors (BCF). A BCF of 1445 was reported which is approximately the same as the highest value determined in the above studies.

When considering the general characteristics of organic chemicals which exhibit bioaccumulation, the molecular weight, log Pow and solubility are all suggestive of bioaccumulative compounds. However, the chemical structure and limited expected persistence of *p*-DCB do not indicate bioaccumulation (Connell, 1990).

Summary of Environmental Fate

p-DCB is expected to predominantly partition to the atmospheric compartment of the environment. While it is not expected to undergo direct photolysis to a significant degree, reaction with photochemically produced hydroxyl radicals in the atmosphere will occur. Estimated half-lives in the atmosphere range from a few days to a little under three months.

Experimentally determined Henry's Law Constants for *p*-DCB suggest it is readily volatile in aqueous solution. Aqueous half-lives ranging from <1 to 31 days have been reported in the literature for surface waters. These are similar to those expected for marine waters where mesocosmic experimentation showed half-lives of 10 to18 days for three seasons. Microbial degradation was shown to not be a factor in the elimination of *p*-DCB from this system.

Where *p*-DCB becomes associated with soils or sediment, it can be expected to exhibit medium to low mobility. However, monitoring data from North America, Europe and Japan indicate that adsorption to sediment is a major environmental fate process with significantly higher concentrations found in sediments than surface waters.

Tests reported with respect to degradation generally appeared to follow non-standard conditions. The two aerobic results following standard guidelines for ready biodegradability show the chemical to be readily biodegradable although it is not certain if the 10 day window criterion was met in one of these tests. While other tests seem to be non-standard and difficult to interpret due to a lack of details, they generally show *p*-DCB may be expected to degrade relatively quickly under aerobic conditions.

As with the aerobic tests, the anaerobic experiments were largely of a non-standard nature. While two experiments indicate a significant degree of degradation, the rate appears slower than rates for aerobic degradation. Other results show *p*-DCB to be relatively resistant to degradation under anaerobic conditions.

Based on 7 tests with a maximum measured BCF of approximately 300 (except for 1440 in eggs and a literature calculation of 1445), the chemical is not expected to be highly bioaccumulative.

7.2 Predicted environmental concentrations

7.2.1 Local predicted environmental concentration in air

The local predicted environmental concentration (PEC) in air at 100 m from a point source can be estimated as follows:

C_{air} = Emission x $Cstd_{air}$

where:

C_{air} = concentration in air at 100 m from a point source (kg/m^3)

Emission = emission rate to air (kg/s)

$Cstd_{air}$ = standard concentration in air at source strength of 1 kg/s

= 24×10^{-6} kg/m^3.

Assuming as worst case that all reformulation occurs at the one plant, 50 kg can be expected to be released to the atmosphere per day (see Section 7.1.1). This results in emission of 5.8×10^{-4} kg/s to the atmosphere giving a concentration of *p*-DCB at 100 m from the point source of 13.9 µg/m^3 (2.3 ppb based on the conversion factor provided in Section 4.1).

A crude calculation can be used to determine concentrations in air from end use. Using figures from Connell and Hawker, 1986, the atmospheric component in Canberra can be estimated as 2.21×10^{11} m^3. Canberra has less than 2% of Australia's population. Assuming that 2% of annual release to air occurs in Canberra, then 39 kg per day is expected to be released through use as an air freshener or in toilet blocks. This equates to an air concentration of 0.176 µg/m^3, or 0.029 ppb per day. With no degradation, this suggests an annual concentration in air of 10.7 ppb.

This is clearly an overestimation as removal processes such as degradation through reaction with hydroxyl radicals will reduce the expected concentration. The atmospheric half-life of *p*-DCB is expected to be between 33 and 50 days, so the equilibrium concentration in the atmosphere is expected to be significantly less than that calculated above.

7.2.2 Local PEC in water

To determine a local PEC in water it will be assumed that the use of *p*-DCB will be more concentrated in urban centres where facilities such as those outlined in Section 6.2 are likely to be in greater supply. As a worst case scenario, it will be assumed that 20% of the predicted release to water occurs in an urban area of 1.5 million people serviced by one sewage treatment plant (STP) with a daily output of 250 ML. Table 3 estimates up to 770 kg of *p*-DCB released to water per day. Therefore, this calculation will assume 154 kg (20%) daily to the STP.

p-DCB can be considered biodegradable under aerobic conditions. This, and its high level of volatility, indicate a significant degree of removal through the sewage treatment plant (STP). The SIAR (1999) provides results from both pilot sewage plants and full scale STPs regarding elimination of *p*-DCB. Pilot plants showed removal of 95% and 90%, with the latter being due to 68% removal from stripping, 22% from degradation and 0.8% from adsorption. An OECD confirmatory test (described in Section 7.1.2.4) provided elimination of 97% which was attributed to 67% volatilisation and 30% degradation. Elimination in full scale STPs was significantly less, varying from 60 to 74%.

The SIAR (1999) hypothesises that the difference in removal from the pilot plants and full scale STPs may be explained by the very low influent concentrations in the full-scale STPs (2.2 to 4.2 ppb) compared to the spiked influents in the pilot plants and confirmatory test of 17.7 to 1000 ppb.

The fate of *p*-DCB has been modelled by Clark *et al.*, 1995. This model predicted removal of 72% being through 19% volatilisation, 46% degradation and 7% bound to sludge. Within this paper, the modelling results were compared to removal efficiencies reported in a second paper. In this report (not obtained), removal was measured at 57 to 80% with a medium of 70%, being by 40% volatilisation, 10% biodegradation and 20% bound to sludge.

No test reports have been validated within this assessment. The assumptions for elimination provided in the SIAR (1999) were removal of 70% attributed to 50% to air, 19% through degradation and 1% through adsorption. Total removal seems in general agreement with the literature and modelled results. The separation between volatilisation, degradation and adsorption to sludge appear quite variable but are not essential for this part of the assessment, and 30% of the chemical going through the STP will be assumed to be released to receiving waters. Therefore, the local PEC can be calculated as follows:

Inflow of *p*-DCB per day	154 kg
Quantity after 70% removal	46.2 kg
Concentration in STP	185 μg/L (ppb)
Concentration in receiving waters (10% dilution)	18.5 ppb

7.2.3 Continental PEC in water

The continental PEC can be determined using the same assumptions above with respect to removal from STPs. Based on an Australia population of 18 million people, sewer discharge of 2.7 x 10^3 ML per day can be expected (150 L per person). A daily release of 770 kg per day to sewer will leave 231 kg in effluent after removal processes. This gives a concentration in an STP of 86 ppb, and leads to a prediction of a concentration of 8.6 ppb in receiving waters.

7.2.4 Local PEC in soil

The full extent of application of sewage sludge to land is not known in Australia. However, it is understood that Sydney Water sends in excess of 90% of their sludge (equating to up to 200,000 tonnes per annum) to beneficial use. Some goes to agricultural use and is soil incorporated while some goes to compost and horticulture where surface application may occur. No local measurements of *p*-DCB in sewage sludge, or its subsequent soil concentration after application to land are available, so data obtained from the literature will be relied upon. Wang and Jones (1994b) measured mean concentrations of 1.12 mg/kg in UK sewage sludge, with mean soil concentrations after application of 1.38 μg/L. In another paper, *p*-DCB concentrations in soil of sludge amended plots ranged from 0.02 to 16.6 μg/kg (Wang *et al.*, 1995). In the absence of other information, the highest value of 16.6 μg/kg will be used as a PEC in soil in Australia in the event of sewage sludge application to land.

7.2.5 Comparison with measured environmental concentrations

Effluent

Within Australia, in 1994/95, 320 effluent samples taken from 16 sewage treatment plants discharging effluent to the Hawkesbury-Nepean River system in NSW found no *p*-DCB in excess of the detection limit of 5 ppb (Sydney Water, 1996).

The SIAR (1999) has given measured levels of *p*-DCB within sewage treatment plant effluents in France, Sweden, USA and Canada. The highest reading was 756 µg/L. However, the majority of samples did not detect the chemical. Three STPs in Canada showed levels of 0.9 to 1.4 ppb with effluents from three STPs in the USA showing levels below detection to 53 µg/L.

Surface water

Within Australia, no *p*-DCB was detected in the receiving waters (detection limit 0.5 ppb) when effluent was discharged from 16 sewage treatment plants to the Hawkesbury-Nepean River system in NSW (Sydney Water, 1996).

Several surface water measurements in Germany from 1985-1994 showed *p*-DCB concentrations to be less than 0.1 ppb with only a few exceptions where the concentrations ranged from 0.12 to 2.9 ppb (BUA, 1994). The SIAR (1999) provides levels from several hundred sample sites in Europe, mainly Germany and France. Readings for the majority tend to be less than 0.5 ppb, although several levels are in excess of this with measured levels of up to 4.05 ppb in the Rhine River in France. One set of results indicates levels less than 50 ppb, although it appears this was the detection limit so actual levels are not known.

The IUCLID data sheet provides several measurements within Japanese surface waters. Between 1986 and 1994, 166 samples were taken with *p*-DCB found in 82 of these. Maximum concentrations ranged from 2.5 µg/L in 1989 to 0.18 µg/L in 1991.

While no *p*-DCB was detected in Australian receiving waters, the continental PEC of 8.6 ppb above is higher than the worst case range based on international readings, with the highest measured value of 4.05 ppb in the Rhine River.

Sediment

Sediment studies around the diffuser of a relatively untreated major marine municipal sewage discharge in Canada showed *p*-DCB levels at 1710 µg/kg dw at the point of outfall, and 277 µg/kg dw at 100 metres from the outfall (Chapman *et al.*, 1996).

The SIAR (1999) provides sediment concentrations from Japanese rivers from 1986 to 1994 with maximum concentrations found ranging from 27 µg/kg dw in 1986 to 150 µg/kg dw in 1991 and 1993.

Also, concentrations in sediment and suspended matter are provided for European samples where suspended matter levels of up to 58 µg/kg dw were found in Germany in 1994 and up to 77 µg/kg dw in 1992. In 128 samples in France between 1993/96, no *p*-DCB was detected in suspended matter with the detection limit being

either 50 or 200 μg/kg dw. Sediment readings from rivers in Germany, France, the Netherlands, and Denmark showed concentrations sediments ranging from less than 0.01 to greater than 10^4 μg/kg dw. The highest level of 10540 μg/kg dw appears to be an outlier and was measured in the Elbe in Germany. The next highest reading was 2980 μg/kg dw also from a German river.

Air

The IUCLID data sheet and SIAR (1999) have given background concentrations in air from municipal areas of Europe. In Hamburg, 300 measurements obtained during 1986/87 provided a mean concentration of 0.14 μg/m^3. In the area of Essen, 50 measurements during 1987/88 gave a range in levels of 0.147 to 0.269 μg/m^3. At three unnamed garbage incineration plants (2 samples each), *p*-DCB was found in concentrations of 0.03 to 0.15 μg/m^3. Gaseous effluents from a garbage dump (stated as an anaerobic dump) had *p*-DCB measured at 0.44 μg/m^3. Measurements taken at the border of the Hoechst factory at Frankfurt between 1987/88 gave a maximum value of 4 μg/m^3, although out of 224 measurements around 97% were below the detection limit of 1 μg/m^3.

Further monitoring data are included in the SIAR (1999). Rain samples in 1984 in Portland, Oregon showed a mean concentration of 0.120 μg/m^3 in air and 0.0048 μg/L in rain. As noted in Section 7.1.2.1, *p*-DCB has been detected in rainwater in Canada, although levels are not provided.

The PEC for air calculated above resulted in a daily concentration of 0.176 μg/m^3 which appears within the range of the measured data.

Soil

As noted above, soil concentrations of *p*-DCB after application of sewage sludge are given by Wang and Jones, 1994. Within the sludge, the chemical was present at 1120 μg/kg. After application of wet solid at 69 g/kg soil (following centrifuging and discarding of the supernatant), the soil concentration of *p*-DCB was measured at 1.38 μg/kg.

8. Occupational Exposure

8.1 Routes of exposure

Occupational exposure is likely to occur due to vapour emitted from the volatile solid or from molten material during processing and formulation of imported material into blocks or tablets. Exposure to vapour and dust can occur during handling of the solid material, particularly during the opening, filling or sealing of bags used to transport *p*-DCB, and during milling of the solid. After initial processing of imported *p*-DCB, the product is wrapped in cellophane, which minimises subsequent exposure.

The major routes for occupational exposure to *p*-DCB are by inhalation and dermal contact. Absorption by the oral route is unlikely to be a significant source of exposure under normal occupational use conditions. As *p*-DCB is not manufactured within Australia there is no occupational exposure related to its production in this country.

8.2 Methodology for estimating exposure

The assessment of occupational exposure is ideally based on workplace monitoring data. Where such data is inadequate or unavailable, reliance must be placed on knowledge-based mathematical models that can estimate exposure when the various patterns of use and the physical properties of the substance under investigation are known. Due to a lack of monitoring data for *p*-DCB exposure estimates were made using the UK EASE (Estimation and Assessment of Substance Exposure) model, developed by the UK Health and Safety Executive. The estimates presented are considered to be feasible worst-case scenarios in that they determine exposure at the high-end or maximum concentration of the substance likely to be encountered in the workplace. They do not take into consideration exposures due to accidents, spills or other unusual situations. The information input into the EASE model was derived from industry through survey results and site visits. Monitoring data from overseas situations has also been included, where appropriate, to supplement the modelled data.

8.3 Processing and formulation

In Australia, between 500 and 1000 tonnes of *p*-DCB were processed, formulated and/or handled by at least 19 companies, for either industrial or domestic use, in 1998. Due to the failure of some companies involved in the packaging and distribution of *p*-DCB products to supply adequate information, the number of workers involved in the industry and the numbers hours worked could not be determined accurately. However, the processes are generally semi-automated and typically involve 1 or 2 workers per company.

The manufacture of air freshener and toilet blocks composed of *p*-DCB is a simple process, involving either the milling or melting of the imported material at 60°C followed by blending with small quantities of dye and perfume and then pressing into moulds. Variations of the process are as follows:

- The *p*-DCB is removed from 25 kg bags and milled. The milled material is placed in a mixer where dye and perfume are added. This mixture is then placed into a hopper, which feeds into a press to produce blocks or tablets of the desired weight, typically 25, 50 or 100 g. The blocks are wrapped in cellophane, labelled and boxed.
- The *p*-DCB is removed from 25 kg bags and melted down after which a small quantity of dye and perfume are added. The molten material is poured into moulds and allowed to solidify. The solid material is removed from the mould, wrapped in cellophane, labelled and boxed.
- The contents of 600 kg bags of *p*-DCB flakes (purchased from the major importer/processor and containing pre-mixed *p*-DCB, dye and perfume) are loaded into a hopper, which feeds into a press producing blocks of the required size. The blocks are then wrapped in cellophane, labelled and boxed.

Duration of exposure

From a survey, 7 companies were identified that formulate and or tabulate products containing *p*-DCB. Generally, production is intermittent with consumer demand regulating production activities. Consequently, few workers are involved in the production process, typically 1 to 2 operators per company. Exposure duration for workers in Australia (obtained by survey) are shown in Table 8.

Table 8 - Handling of *p*-DCB in Australia

Activity	Number of Companies	Number of Workers[1] (range)	Hours/day [Days/year] (range)
Formulation/ pressing/wrapping	4	1 - 5	2 [30] - 8 [100]
Pressing/wrapping only	3	1 - 2	6 [12] - 7 [48]
Re-packing only	3	1 - 15	0.5 [1] - 3 [150]
Distribution only	9	NA	NA

[1]Workers handling *p*-DCB; NA, data not available.

Re-packing typically involves the removal of wrapped *p*-DCB from drums or pails and packaging into plastic bags or boxes for distribution. Companies only involved in the distribution of *p*-DCB products generally purchase sealed containers of the material and re-sell it without opening the container.

Levels of exposure

Only one company provided monitoring data. This company is the major importer and formulator of *p*-DCB products in Australia. The company recorded an airborne concentration of 19 ppm of *p*-DCB in the general work area, 34 ppm near a feed hopper and the maximum concentration reached was 59 ppm of *p*-DCB in the bagging area; the data were for April, 1981. More recent data were provided by the company for August, 1998 which indicated an upper concentration range during processing of 10 to 15 ppm near a discharge chute and a position near an exhaust chute in the bagging area gave a level of 6 to 8 ppm. The data were obtained with an air temperature of 22°C and using Dräger tubes (Chlorobenzene 5/a) which provides an approximate measurement only. These exposure levels are in general agreement with data predicted by the UK EASE model which estimated airborne concentrations of 10 to 50 ppm (8 hour TWA) under conditions of 25°C with a non-dispersive pattern of use and with dilution ventilation. The EASE model predicted the same concentration range under conditions where *p*-DCB is handled in the molten state (60°C) with a non-dispersive pattern of use and local exhaust ventilation.

8.4 Hygiene sector

As the greatest use of *p*-DCB in Australia occurs in the hygiene sector where the compound is used extensively as an air freshener and deodorant for toilet facilities, occupational exposure is likely to be greatest amongst cleaners. Cleaners of toilet facilities are likely to be exposed for periods during their work routine by inhalation. Dermal and oral exposure is expected to be negligible under these conditions, particularly if gloves are worn. As much of the cleaning routine is performed after normal daytime working hours when the facilities are not in use, and hence less air flow would be expected, the concentration of *p*-DCB in the air within toilet facilities is likely to be higher than during daytime working hours. No monitoring data were available for Australian conditions. The following basic studies have been conducted in Germany to determine the airborne concentration of *p*-DCB in two public toilet facilities (Globol Werke GmbH, 1986):

- Two toilet facilities were used, toilet 1 contained two urinals and toilet 2 contained 1 urinal. Three *p*-DCB blocks (41.3 g/block) were placed in each urinal. The volumes of toilets 1 and 2 were 39.56 m^3 and 15.42 m^3 respectively. Ventilation was not controlled during the experiment and was dependent on the number of users. The temperature varied from 16 to 22°C. A total of 67 and 57 days were required for the blocks to fully dissipate for toilets 1 and 2 respectively. Measurements taken from toilets 1 and 2 prior to the experiment indicated a concentration of 0.2 ppm (1 mg/m^3) and 0.1 ppm (0.6 mg/m^3) of *p*-DCB respectively (no information about prior recent usage of *p*-DCB in the toilets was provided). Readings taken in the morning and at midday for up to 47

days showed maximal readings of 1.7 ppm (10.1 mg/m^3) for toilet 1 and 2.2 ppm (13.3 mg/m^3) for toilet 2.

- In a further experiment, an air freshener tablet (77.44 g) was placed in a toilet facility 1.6 m above a urinal. The volume of the room was 15.42 m^3. Ventilation was not controlled and was dependent on users. The temperature range was from 16 to 22^{o}C. The duration of the experiment was 30 days although the *p*-DCB block required 67 days to dissipate completely. A background measurement taken prior to the study gave a reading of 0.1 ppm (0.7 mg/m^3) of *p*-DCB. After placement of the *p*-DCB tablet, the morning air concentration of *p*-DCB ranged from 0.5 to 3.8 ppm (3.0 to 23.0 mg/m^3) for the first week and gave a mean of 0.6 ppm (3.6 mg/m^3) for days 12 to 30. The midday concentration of *p*-DCB ranged from 1.1 to 3.7 ppm (6.4 to 22.4 mg/m^3) for the first week with a mean of 0.7 ppm (4.2 mg/m^3) for days 12 to 30. Concentrations in the evening ranged from 0.2 to 4.0 ppm (1.5 to 23.8 mg/m^3) during the first week with a mean of 1.2 ppm (7.5 mg/m^3) for days 12 to 30.

8.5 Automotive/Marine Sector

The car detailing/cleaning and transport sectors were identified as users of *p*-DCB although no monitoring data were available. Use of *p*-DCB was also identified in the marine industry, generally confined to small pleasure craft. It may be expected that the airborne concentrations of *p*-DCB are likely to be higher under these conditions due to the limited and confined air space, limited ventilation and higher than average temperatures found within vehicles and boats. However, the duration of exposure is likely to be short and intermittent.

9. Toxicokinetics and Metabolism

9.1 Absorption

9.1.1 Animals

There have been few studies conducted that address the issue of absorption of *p*-DCB by the oral and inhalation routes.

The absorption of radiolabel after oral administration of *p*-[^{14}C]-DCB (250 mg/day) for 5 days was examined in female rats (strain CFY). Urinalysis revealed that 91 to 97% of the radiolabel was excreted in the urine with approximately 2 to 5% appearing in the faeces and less then 1% in expired breath (Hawkins *et al.*, 1980). As the amount appearing in bile after a single dose amounted to 46 to 63% of radiolabel recovered, indicating considerable enterohepatic circulation of radiolabel, the data suggest that absorption by the oral route is substantially complete at this dose level.

An investigation of the distribution of *p*-[^{14}C]-DCB after oral administration to male and female rats (strain F344; 149 or 305 mg/kg bw) and mice (strain B6C3F$_1$; 310 or 638 mg/kg bw) showed peak blood levels to occur at 1 hour after dosing while peak tissue levels occurred at 6 hours (Wilson, 1990; cited in SIAR 1999).

The kinetics of *p*-DCB absorption was examined by oral administration of *p*-[^{14}C]-DCB (10, 50, or 250 mg/kg bw) dissolved in corn oil to male Wistar rats. At the lowest dose the maximal concentration of the parent compound in the blood (C_{max}[DCB]; determined by gas chromatography) was reached at 4 hours (6.75 µmol/l) while the maximal concentration of radioactivity (C_{max}[Ra]) in the blood occurred at 2 hours. For the mid-dose, C_{max}[DCB] was reached at 6 hours (21.3 µmol/l) and C_{max}[Ra] at 3 hours. At 250 mg/kg bw, C_{max}[DCB] and C_{max}[Ra] both occurred at 6 hours (104 µmol/l). Clearance values for *p*-DCB at 10, 50, or 250 mg/kg bw were 24.1, 23.7 and 22.7 mL/min/kg respectively while the half-life values for the same doses were 8.1, 7.1 and 7.6 hours respectively (Hissink *et al.*, 1997a). The data indicate that rate-limiting conditions, with respect to absorption, had not been reached and that absorption appears to be rapid.

The kinetics of *p*-[^{14}C]-DCB absorption were investigated in male and female rats (strain F344) and mice (strain B6C3F1) following exposure by inhalation. Exposures were conducted in male rats at 160 and 502 ppm and in female rats at 161 and 496 ppm; in mice, exposures at 158 and 501 ppm were used. Absorption via the respiratory system was rapid but not complete. Absorption after inhalation exposure was poor compared to oral exposure. Mice demonstrated increased absorption relative to F344 rats after inhalation (59% in mice versus 25-33% in rats) (Wilson, 1990; cited in SIAR 1999).

No studies have been carried out to specifically address dermal absorption in animals.

9.1.2 Humans

There are no reliable data for the absorption of *p*-DCB by humans.

9.2 Distribution

9.2.1 Animals

The concentration of *p*-DCB in male rats, after a single dose (200 mg/kg bw) by gavage, was found to be highest in adipose tissue with lesser amounts in blood, liver, kidneys, heart, lung and brain tissue (Kimura *et al.*, 1979).

Charbonneau *et al.* (1989) studied the distribution of radiolabel 24 hours after administering a single oral dose of *p*-[^{14}C]-DCB (500 mg/kg bw) to male rats (strain F344). The greatest deposition of radiolabel occurred in the adipose tissue (13,617 nmol/g) followed by the kidneys (535 nmol/g) and liver (458 nmol/g). The plasma accounted for 201 nmol/mL of radiolabel. Within the kidney 266 nmol/g was bound to cytosolic components with 3.3 nmol of *p*-DCB equivalents bound to $\alpha_{2\mu}$-globulin-containing fractions. The radiolabel bound to the fractions was not removed by dialysis unless sodium dodecyl sulfate (0.1%) was present, demonstrating the non-covalent nature of the binding.

Klos and Dekant (1994) investigated the distribution of *p*-[^{14}C]-DCB (900 mg/kg bw) and its metabolites in male and female rats (strain F344) after administration by gavage. The highest content of radiolabel, 72 hours after dosing, occurred in the adipose tissue followed by the liver, kidney, lung, adrenal gland and spleen. The distribution of radiolabel between the tissues of the two sexes was not substantially different.

Administration of *p*-[^{14}C]-DCB (1000 ppm) by inhalation (3 hours/day for up to 10 days; whole-body) to female rats (strain CFY) revealed that 24 hours after the final dose the greatest concentration of radiolabel was found in the adipose tissue with lower concentrations in the kidneys and liver. Levels of radiolabel in lungs and muscle were comparable to plasma levels. Tissue concentrations of radiolabel increased with exposure duration within the first 6 days but reached a plateau or decreased slightly at 8 to 10 days, possibly due to the induction of P450 enzymes. When *p*-[^{14}C]-DCB (250 mg/kg bw per day) was administered for up to 10 days by gavage or by subcutaneous injection a similar distribution of radiolabel occurred (Hawkins *et al.*, 1980).

The distribution of *p*-DCB in rats (strain F344/DuCrj) after inhalation (whole-body) of the vapour (500 ppm) for 24 hours was assessed by determining the level of *p*-DCB in serum, liver, kidneys and adipose tissue at intervals up to 24 hours during and after exposure. Peak serum levels of *p*-DCB for both male and female rats occurred 3 hours after exposure ceased. The level of *p*-DCB in the livers of females increased markedly at 24 hours peaking at 3 hours post exposure and reached significantly higher levels than male livers. Conversely, male rat kidneys took up more of the compound than their female counterparts though the difference was not as marked as with the livers. No significant differences in plasma or adipose tissue

levels were evident between the two genders. For all tissues examined, 24 hours after exposure ceased the level of *p*-DCB declined to below the level detected after the initial 6-hour exposure (Umemura, 1990).

When *p*-DCB was administered as a single oral dose (100 or 1000 mg/kg bw dissolved in polyethylene glycol) to male rats (strain Wistar TNO W47) peak plasma concentrations of *p*-DCB and its metabolite, 2,5-dichlorophenol, occurred at 24 hours for each dose. Similarly, maximal amounts of *p*-DCB and 2,5-dichlorophenol occurred in adipose, hepatic and renal tissue at 24 hours after treatment with 1000 mg/kg bw *p*-DCB. At the 100 mg/kg bw dose, only trace amounts of *p*-DCB or 2,5-dichlorophenol could be detected in hepatic and renal tissue (Bomhard *et al.*, 1998).

In a further series of experiments by Bomhard *et al.*, (1998) rats (strain Wistar TNO W47) were fed a diet containing 100 or 1000 ppm (equivalent to approximately 10 and 100 mg/kg bw respectively) *p*-DCB daily for 4 weeks. At 100 ppm, neither *p*-DCB nor 2,5-dichlorophenol were detectable in the plasma at any time point. After feeding 1000 ppm and measured at day 3, *p*-DCB and 2,5-dichlorophenol levels were at their highest and subsequently declined to give steady-state concentrations from day 6 to day 14. Thereafter, plasma concentrations of both compounds declined to undetectable levels for *p*-DCB and less than 0.5 μg/mL for 2,5-dichlorophenol. Adipose, hepatic and renal tissue levels of *p*-DCB and 2,5-dichlorophenol were also determined. At 100 ppm, adipose tissue levels were constant at low levels (< 5 μg/mL) over the experimental period. Both compounds in the 100 ppm group were undetectable in hepatic and renal tissue over the corresponding period. Within the 1000 ppm group, maximal levels of *p*-DCB occurred at day 3 in hepatic and renal tissue with both tissues exhibiting a rapid decline by day 6. Low steady-state levels of *p*-DCB existed from day 6 to day 28 in hepatic (< 0.5 μg/g) and renal (< 1.0 μg/g) tissue. Hepatic levels of 2,5-dichlorophenol remained constant at low levels (< 0.25 μg/g tissue) during the experimental period while renal levels displayed a biphasic response with maxima occurring at days 3 and 21.

9.2.2 Humans

There are no detailed studies of the distribution of *p*-DCB in humans. Analyses of human samples of adipose tissue, blood and milk have all shown the presence of *p*-DCB.

In a study of Tokyo residents, 34 samples of adipose tissues obtained from local hospitals all had detectable levels of *p*-DCB with an average level of 2.3 μg/g tissue (range 0.2 to 11.7 μg/g). Analysis of six blood samples showed an average level of *p*-DCB of 9.5 ng/mL (range 4 to 16 ng/mL) (Morita and Ohi 1975).

In another report, analysis by mass fragmentography for chlorinated benzenes in a mixture of 15 samples of human adipose tissues obtained from the Tokyo area showed that the main component present was *p*-DCB. The detection level was 0.010 μg/g tissue (Morita *et al.*, 1975).

Mes *et al.*, (1986) reported traces of *p*-DCB in all of 210 human milk samples tested from 5 different regions across Canada.

A survey of 1000 adults in the United States revealed 98% had detectable levels of 2,5-dichlorophenol in their urine (up to 8.7 mg/l) and detectable levels of *p*-DCB in their blood (up to 49 μg/l) (Hill *et al.*, 1995).

9.3 Metabolism

9.3.1 Animals

The oral administration of *p*-DCB (0.5 g/kg) to rabbits resulted in the formation of urinary metabolites consisting of 2,5-dichlorophenol (35%), present as the glucuronic and sulfate conjugates, and 2,5-dichloroquinol (6%) (Azouz *et al.*, 1955).

Administration of *p*-[^{14}C]-DCB (1000 ppm) by inhalation (whole-body) or by oral administration (250 mg/day) to female rats (strain CFY) revealed that the major urinary metabolites were sulfate and glucuronide conjugates of 2,5-dichlorophenol comprising 46 to 54% and 31 to 34% of total radiolabel respectively. Two minor metabolites were dihydroxydichlorobenzene and a mercapturic acid of *p*-DCB (Hawkins *et al.*, 1980).

Administration of *p*-[^{14}C]-DCB (0.9 mmol/kg) to male rats (strain F344) by intraperitoneal (i.p.) injection revealed that the highest concentration of radiolabel in the liver occurred within the first 4 hours with less than 35% of the radiolabel remaining by 12 hours. Analysis of aqueous soluble metabolites indicated that soluble products of *p*-DCB did not exceed 34% of total radiolabel at any time point. The presence of aqueous soluble metabolites correlated with the covalent binding of radiolabel to hepatic proteins which remained within the range 31 to 52 pmol/mg protein bound over a 24 hour period. The role of hepatic glutathione was determined by estimating the total non-protein sulfhydryl content over a period of 5 hours after i.p. injection of *p*-DCB (1.8 mmol/kg), however, no significant difference in glutathione content compared with controls was noted. Depletion of hepatic glutathione by pre-treatment of the animals with phorone resulted in a significant increase in plasma alanine aminotransferase (ALT) levels 24 hours after the administration of *p*-DCB demonstrating a role for glutathione in modulating the hepatotoxicity of *p*-DCB (Stine *et al.*, 1991).

The *in vivo* metabolic fate of *p*-DCB was examined in male rats (strain Wistar) by the administration of *p*-[^{14}C]-DCB (10, 50 or 250 mg/kg bw) by gavage. Metabolites detected in the urine were 2,5-dichlorophenol (8.4 to 9.3%), 2,5-dichlorophenol glucuronide (19.3 to 25.4%), 2,5-dichlorophenol sulfate (56.7 to 62.2%) and mercapturic acids (8.6 to 10.2%). Minor metabolites accounting for the remaining radiolabel (10%) were *N*-acetyl-cysteine-*S*-dihydro-hydroxy-1,4-dichlorobenzene and *N*-acetyl-cysteine-*S*-1,4-dichlorobenzene. No hydroquinone metabolites were detected. The main effect of increasing the dose was a decrease in the amount of sulfate conjugates and an increase in the glucuronide conjugates. Induction of CYP2E1 by prior treatment with isoniazid resulted in an elevated urinary excretion of *p*-DCB metabolites providing evidence for the role of this cytochrome in the *in vivo* metabolism of *p*-DCB (Hissink *et al.*, 1997a).

In a study by Lake *et al*. (1997) male rats (strain F344) were treated daily with *p*-DCB at 0, 25, 75, 150 or 300 mg/kg bw and male mice (strain $B6C3F_1$) at 0, 300 or 600 mg/kg bw by gavage, 5 days/week. The cytochrome P450 content of rat livers increased at all time periods with doses of 75 mg/kg bw or greater. Mouse hepatic cytochrome P450 content increased during all time points only for the 600 mg/kg bw treatment. The identity of the *p*-DCB-induced cytochromes was investigated in the rat and mouse using an immunoblotting technique with antibodies to CYP2B1/2 and CYP3A. A marked induction of CYP2B1/2 in both rat and mouse was detected and a lesser induction of CYP3A occurred in the rat. The authors concluded that *p*-DCB is an inducer of CYP2B in the rat and mouse.

Analysis of urinary metabolites collected from male and female rats (strain F344) 24 to 36 hours after administration of *p*-DCB (900 mg/kg bw) as a single oral dose revealed the presence of conjugates of 2,5-dichlorophenol as the major metabolites. 2-(*N*-acetyl-cysteine-*S*-yl)-1,4-dichlorobenzene was also present, as were conjugates of 2,5-dichlorohydroquinone. In the organs tested, protein-bound radiolabel was below the limit of detection (Klos and Dekant 1994).

The time course of induction of hepatic and renal enzymes was studied in male and female rats (strain F344) by the administration of *p*-DCB (0, 150 or 600 mg/kg bw; 7 days/week for 4 weeks) by gavage. On days 3, 9, 15 and 28 the activities of the phase I enzymes, 7-ethoxycoumarin *O*-deethylase (ECOD; corresponding to cytochrome isoforms CYP1A1, CYP2B1 and CYP2D1), 7-ethoxyresorufin *O*-deethylase (EROD; CYP1A1) and aldrin epoxidase (ADE) and the phase II enzymes, epoxide hydrolase (EH), glutathione S-transferase (GST) and glucuronyl transferase (GLT) were studied. Maximal induction of ECOD in the liver occurred at the highest dose on day 9 at which time a 7 and 10-fold increase in enzyme activity occurred for male and females respectively compared to control animals. At this dose level induction of ECOD was characterised by a biphasic response with a second peak in activity occurring on day 28. At 150 mg/kg bw, *p*-DCB induced ECOD activity was maximal at day 9 for males, although not biphasic, and absent in the female liver. The maximal response to EROD induction by *p*-DCB (600 mg/kg bw) was less than ECOD and occurred in the liver at days 15 and 28 for males and females respectively. Males did not show an increase in hepatic ADE activity at either dose while females demonstrated a 5 and 10-fold increase on day 9 at 150 and 600 mg/kg bw respectively but which subsequently declined towards the end of the study period. The hepatic phase II enzymes, EH, GST and GLT, showed similar patterns of induction in response to *p*-DCB (600 mg/kg bw). Maximal activity occurred on day 9 with a subsequent decrease in activity on succeeding days. Compared to control animals, EH activities increased 6 and 4-fold for males and females, GS-T activities increased 4-fold and 2-fold for males and females and GLT activities increased 8-fold and 4-fold for males and females respectively. At 150 mg/kg bw, only marginal increases in the phase II enzyme activities were recorded. The renal response to *p*-DCB (600 mg/kg bw) was a maximal induction of ECOD activity for both sexes on day 15, 7-fold for females and 3.5-fold for males compared to control animals. Of the other enzymes studied, only EH exhibited a slight induction of less than 2-fold on day 15. No renal induction was seen for any enzyme at 150 mg/kg bw (Bomhard 1998).

9.3.2 Humans

The metabolism of *p*-DCB has not been extensively investigated in humans.

In an early study, Pagnotto and Walkley (1965) reported the presence of dichlorophenol in the urine of workers exposed to *p*-DCB vapour in the range 10 to 233 ppm.

9.3.3 *In vitro* studies

In a study of the metabolism of *p*-DCB by rat liver microsomes (derived from male Wistar rats) the main metabolites produced were 2,5-dichlorophenol with lesser amount of 2,4-dichlorophenol detected. 2,5-Dichlorophenol was further metabolised to 2,5-dichlorohydroquinone and 2,4-dichlorophenol was metabolised to 3,5-dichlorocatechol and, to a lesser extent, 1-chlorobenzoquinone. Subsequent oxidation of the dichlorohydroquinone/dichlorocatechol species produced reactive dichlorobenzoquinones. These reactive species were capable of protein binding although they demonstrated less affinity for DNA. Significantly less protein binding was observed when the reductant, ascorbic acid, was added to the incubation medium (Den Besten *et al.*, 1992).

The metabolism of *p*-DCB (1 mM) by rat liver slices (strains Sprague-Dawley and F344) was examined. After 2 and 6 hours of incubation, the amount of metabolites derived from *p*-DCB were comparable between the two strains giving 2.1 to 2.7 nmol/mg protein. The metabolites formed were also comparable in terms of chemical species produced with glutathione/cysteine conjugates being the major metabolites. Covalent binding of reactive *p*-DCB metabolites to liver slices was assessed and revealed comparable levels of binding for F344 and Sprague-Dawley tissues (0.471 and 0.359 nmol bound/mg protein respectively) after 6 hours incubation (Fisher *et al.*, 1995).

To further delineate the species and strain differences in hepatic microsomal cytochrome P450-mediated metabolism of *p*-DCB, Hissink *et al*. (1997b) undertook a comparative study of male rats (strains Wistar, Sprague-Dawley, and F344) and male mice (strain B6C3F$_1$). Conversion of *p*-[^{14}C]-DCB by microsomes ranged from 16% for mice to 1.3% for Wistar and F344 rats while Sprague-Dawley rats gave 0.6% conversion. Prior treatment of Wistar rats with phenobarbital increased the conversion of *p*-DCB by 4-fold. The main metabolites identified are listed in Table 9. In all cases, 2,5-dichlorophenol accounted for more than 50% of converted material. In the absence of ascorbic acid, hydroquinone metabolites accounted for 10.2 to 27.1% of the total conversion. Addition of ascorbic acid significantly increased the recovery of hydroquinone metabolites while decreasing covalent binding. Covalent binding of *p*-DCB metabolites to microsomal protein was highest for mouse microsomes (20.9% bound) with rat microsomes ranging between 6.8% to 10.1% bound. Prior treatment of Wistar rats with phenobarbital significantly increased the response from 8.1% to 13.4% bound. In all cases, the addition of ascorbic acid or glutathione reduced microsomal covalent binding. In the absence of exogenous glutathione, mouse microsomes produced no detectable glutathione-epoxide conjugates whereas rat microsomes produced detectable levels (range 5.0 to 15.0% of

total conversion) for each strain. Addition of glutathione to microsomal preparations resulted in undetectable levels of glutathione-epoxide conjugates from mouse preparations and a significant increase (range 39.7 to 52.2%) for all rat strains.

Further studies of the metabolism of *p*-DCB were undertaken using hepatic microsomes derived from male and female rats (strain Wistar) and mice (strain $B6C3F_1$) with or without induction of CYP3A or CYP2E1. The formation of soluble metabolites after the addition of *p*-[^{14}C]-DCB (0.1 mM) to microsomal preparations was 3-fold greater for male than for female mice. Treatment of animals with pregnenolone 16α-carbonitrile to induce CYP3A resulted in a 7-fold increase in covalently bound metabolites in male rats and a 5-fold decrease in the formation of soluble metabolites by male and female mice compared to controls. Induction of CYP2E1 (by benzene inhalation) did not result in any significant change in soluble or covalently bound metabolite formation by rats or mice. Inhibition of CYP2E1 by diethyldithiocarbamate resulted in significantly less *p*-DCB oxidation by rat and mouse microsomes (Nedelcheva *et al.*, 1998).

Table 9 - Metabolites formed by hepatic microsomal metabolism of *p*-DCB

Species (strain)	Conversion (% of total radioactivity)	Metabolite (% of total conversion)			
		GS-epoxide[1]	Hydroquinone	2,5-DCP	GS-quinone
Mouse	15	ND	16.1	50.5	3
Rat (F344)	1.1	5.0	27.1	56.8	6.0
Rat (S-D)	0.6	7.3	10.2	70.7	ND
Rat (Wistar)	1.3	15.0	10.5	50.0	ND
Human	0.3	ND	16.8	66.2	ND

[1] derived from endogenous GSH; ND, not detected in the absence of exogenous GSH; S-D, Sprague-Dawley; GS, glutathione conjugate; DCP, dichlorophenol (Hissink *et al.*, 1997b).

Incubation of human liver slices (from 10 individuals; 5 male, 5 female; 7 from head injury victims and 3 from resections with metastatic colon cancer) with *p*-DCB (1 or 2 mM) for up to 6 hours resulted in a decrease in protein synthesis and an increase in leakage of the cytosolic enzyme, lactate dehydrogenase (LDH), only at the higher concentration. The effect was time-dependent with statistically significant ($p<0.05$) results occurring at 4 hours (Fisher *et al.*, 1991).

In another study by Fisher *et al.* (1995), the metabolism of *p*-DCB (1 mM) by human liver slices (from 7 individuals) was examined after 2 and 6 hours of incubation. *p*-DCB metabolites amounted to 2.6 and 3.3 nmol/mg protein respectively. Covalent binding of reactive metabolites to liver slices was assessed and amounted to 0.482 nmol bound/mg protein after 6 hours.

Comparative studies undertaken with microsomes derived from human cell lines transfected with cDNA expressing specific cytochrome P450 isoforms have demonstrated that *p*-DCB is metabolised predominantly by CYP2E1 to 2,5-dichlorophenol. CYP1A2 demonstrated the next highest activity, which amounted to 6.0% of the CYP2E1 activity. CYP1A1, CYP3A4 and CYP2D6 possessed little activity towards *p*-DCB (Bogaards *et al.*, 1995).

The role of hepatic microsomal cytochrome P450-mediated metabolism of *p*-DCB (150 μM) was further investigated by Hissink *et al.* (1997b). Human cell lines expressing specific cytochrome P450 isoforms (from 5 individuals) were utilised. Total conversion of *p*-[^{14}C]-DCB by microsomes was 0.3%. 2,5-Dichlorophenol accounted for 66.2% of the metabolites produced with hydroquinone species accounting for a further 16.8%. Addition of ascorbic acid increased the recovery of hydroquinone metabolites to 27.9%. Covalent binding comprised 5.8% of the total conversion.

Nedelcheva *et al.* (1998) found that inhibition of CYP2E1 by diethyldithiocarbamate and CYP3A by triacetyloleandomycine resulted in substantial inhibition of the metabolism of *p*-[^{14}C]-DCB by human microsomes derived from the livers of male brain injury victims. No correlation could be found, using immunoblotting techniques, for an association between cytochrome type and rate of metabolism.

The major metabolic routes for the metabolism of *p*-DCB by hepatic microsomes derived from humans, rats and mice and the proposed bio-reactive metabolites are shown in Figure 2. The metabolism of *p*-DCB by humans is limited and proceeds by aromatic hydroxylation by CYP2E1 to give the 2,3-epoxide (Bogaards *et al.*, 1995). The epoxide, in a non-enzymatic process, converts to 2,5-dichlorophenol which is excreted or conjugated with sulfate or glucuronic acid prior to excretion. Lesser amounts are converted by γ-glutamyl transferase which results in excretion of mercapturic acid derivatives. In the rat and mouse aromatic hydroxylation involves other P450 enzymes in addition to CYP2E1 so that 1,2-epoxide and 2,3-epoxide derivatives are formed, both of which form 2,4- and 2,5-dichlorophenols respectively. By secondary oxidation hydroquinone derivatives are produced which may autoxidise to their corresponding benzoquinone forms (Den Besten *et al.*, 1992). The 1,2-epoxide can conjugate with glutathione and be excreted as a mercapturic acid. Alternatively, both epoxides can form glutathionyl, sulfate or glucuronide derivatives prior to excretion (Hissink *et al.*, 1997a,b).

9.4 Elimination and excretion

9.4.1 Animals

In rabbits, the excretion of *p*-DCB metabolites occurred predominantly in the urine when *p*-DCB was administered by gavage (1.5 g/animal). Only glucuronides and sulfates of 2,5-dichlorophenol were detected, which peaked two days after administration of the parent compound. Detectable levels of metabolites continued to be found in the urine six days after dosing. Analysis over six days failed to detect *p*-DCB or its metabolites in the faeces (Azouz *et al.*, 1955).

Hawkins *et al.* (1980) found that *p*-[^{14}C]-DCB (1000 ppm) administered by inhalation (3 hours/day for up to 10 days; whole-body) to female rats (strain CFY) was rapidly

eliminated after termination of exposure. Analysis of excreta collected over 4 days post-exposure showed 97.4% of the radiolabel to be in the urine, 2.5% in the faeces and 0.2% in expired air. Similarly, when administered by gavage, *p*-DCB (250 mg/kg bw per day) was excreted predominately in the urine with less than 10% in the faeces.

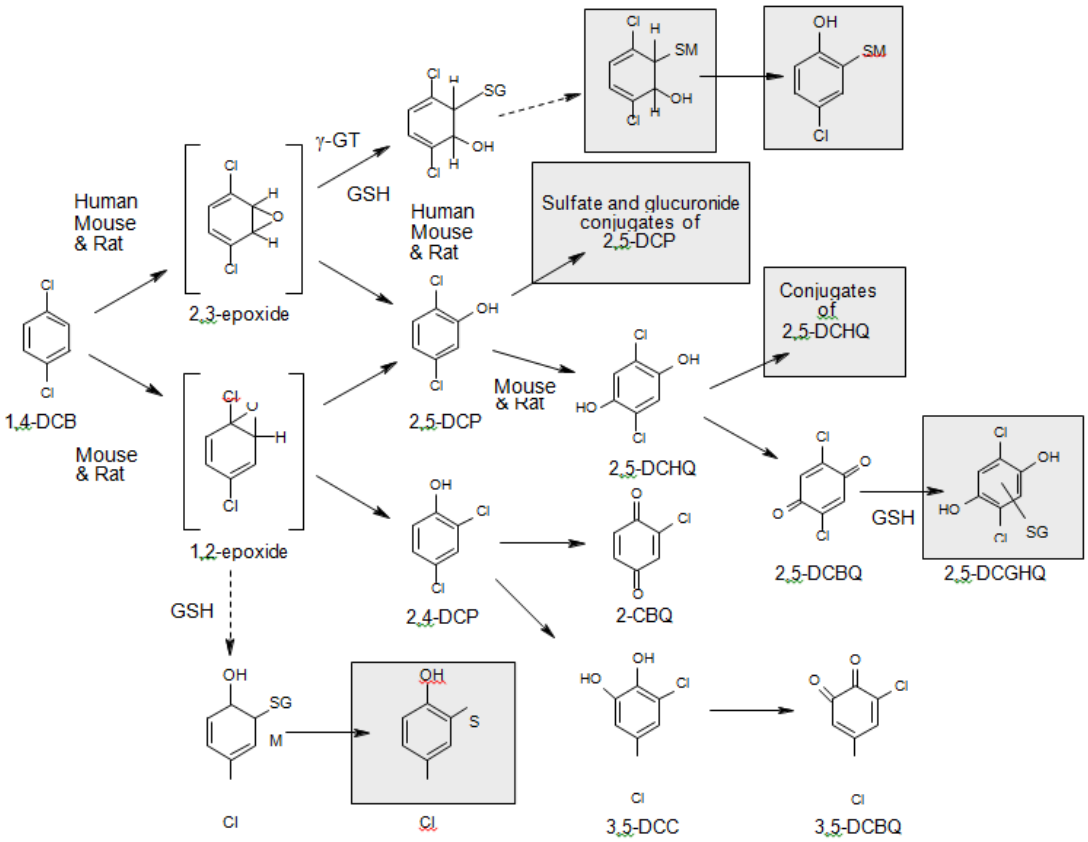


Figure 2 - Metabolic pathways for the metabolism of *p*-DCB in humans, rats and mice. The major urinary products are depicted in boxes. CBQ, chlorobenzoquinone; DCB, dichlorobenzene; DCBQ, dichlorobenzoquinone; DCC, dichlorocatechol; DCGHQ, dichlorogluthionyl-hydroquinone; DCHQ, dichlorohydroquinone; DCP, dichlorophenol; DCBQ, dichloro-1,2-benzoquinone; γ-GT, gamma glutamyl transferase; GSH, reduced glutathione; SG, glutathione-*S*-yl-metabolite, SM, *N*-acetyl-cysteine-*S*-yl-metabolite. Dashed arrows represent multi-step pathways (After Den Besten *et al*., 1992, Klos and Dekant 1994 and Hissink *et al*., 1997).

Umemura *et al*. (1990) observed that tissue levels of *p*-DCB in male and female rats (strain F344; 3 animals/group) exposed to *p*-DCB vapour (500 ppm) for 6, 12, and 24 hours fell approximately 90% within the following 24-hour period.

The excretion of *p*-[^{14}C]-DCB (900 mg/kg bw) by male and female rats (strain F344) was determined over 72 hours after a single oral dose. Urine was collected over 12-hour intervals. The results indicated that 72 hours after dosing 41.3% and 37.8% of the radiolabel was excreted in the urine by males and females respectively. Faeces accounted for 3.6% of the radiolabel from males and 2.5% from females. Peak excretion for both sexes occurred during the 24 to 36 hour interval. No treatment-related changes in urine volume were observed. At the end of the study only 0.05% and 0.04% of the total radiolabel remained in the pooled tissues from males and females respectively. Similar results were obtained with unlabelled *p*-DCB. The major excretion products were conjugates of 2,5-dichlorophenol. These were higher in the male although females excreted higher amounts of 2-(*N*-acetyl-cysteine-*S*-yl)-1,4-dichloro-benzene than males. Males also excreted more conjugates of 2,5-dichlorohydroquinone than females (Klos and Dekant 1994).

The elimination of *p*-DCB by male rats (strain Wistar) was studied following the administration of a single dose of *p*-[^{14}C]-DCB (10, 50 or 250 mg/kg bw) by gavage. Less than 1% of all dose levels was eliminated by the lungs. Examination of all organs for radiolabel after 168 hours accounted for less than 0.05% of the dose. Elimination in urine accounted for 80% of the dose while the faeces contained 4% of radiolabel with changes in dose levels having no significant effect on these values. Most of the radiolabel was excreted between 8 and 24 hours after dosing. Metabolites detected in the urine were 2,5-dichlorophenol (5-10%), the 2,5-dichlorophenol glucuronide (20-30%) and 2,5-dichlorophenol sulfate (50-60%). Minor metabolites including mercapturic acids (10%) accounted for the remaining radiolabel. Excretion in bile was dose-dependent with 4% of total radiolabel excreted in the first 12 hours after administering 10 mg/kg bw; faeces contained less than 2.5% of the dose. At 250 mg/kg bw, 10 to 30% of total radiolabel appeared in the bile with less than 5% in the faeces suggesting that enterohepatic circulation of *p*-DCB and its metabolites is significant with reabsorption by the intestinal route high (Hissink *et al.*, 1997a).

9.4.2 Humans

A study of workers exposed to dichlorobenzenes (predominantly *p*-DCB) by inhalation found that urinary excretion of the major metabolite, 2,5-dichlorophenol, commenced soon after initial exposure with maximal excretion occurring at the end of the exposure period. Air sampling indicated a range of concentrations from 8 to 49 ppm of dichlorobenzene and urinary dichlorophenol formed from 10 to 233 mg/l. The excretion of metabolites was found to be biphasic with an initial rapid decrease in urinary metabolites followed by a prolonged reduction over several days (Pagnotto and Walkley, 1965).

In a controlled experiment using human volunteers it was determined that the biological residence time of *p*-DCB in humans, based on exhaled breath measurements and using a least-squares fit to a one compartment pharmacokinetic model, to be 20 to 30 hours (Wallace *et al.*, 1989).

Hill *et al.*, (1995), in a study of 1000 adults in the United States, demonstrated a significant correlation ($p<0.0001$) between the concentration of *p*-DCB in the blood and urinary *p*-dichlorophenol concentration.

9.5 Other studies

In vivo studies

Covalent binding of radiolabel to DNA, RNA and protein 22 hours after i.p. administration of *p*-[^{14}C]-DCB to male rats (strain Wistar) and mice (strain BALB/c) was examined. Covalent binding of radiolabel to DNA extracted from rat liver, kidney, lung and stomach was not detected whereas binding for the corresponding mouse DNA samples were 0.14, 0.09, 0.60 and 0.08 pmol/mg respectively. Covalent binding of radiolabel to RNA from rat and mouse was: liver (0.60 and 1.83 pmol/mg), kidney (2.28 and 1.60 pmol/mg), lung (0.95 and 4.28 pmol/mg) and stomach (0.08 and the mouse was not determined). Covalent binding of radiolabel to protein from rat and mouse tissue was: liver (0.12 and 0.75 pmol/mg), kidney (0.63 and 0.74 pmol/mg), lung (0.60 and 0.73 pmol/mg) and stomach (0.54 and 0.38 pmol/mg) (Lattanzi *et al.*, 1989).

Renal cell proliferation in response to the oral administration of *p*-DCB (0, 118 or 294 mg/kg bw) to male rats (strain F344) for 7 days was examined. A significant increase ($p<0.05$) was observed in treated animals (Charbonneau *et al.*, 1989).

Eldridge *et al.* (1992) investigated hepatocellular proliferation in female rats (strain F344) and mice (strain B6C3F$_1$) of both sexes after administration of a single oral dose of *p*-DCB (600 mg/kg bw). Cell proliferation increased (as determined by incorporation of 5-bromo-2'-deoxyuridine (BrdU)) reaching a maximum for female mice and rats at 24 hours and declined thereafter. For male mice, no increase was observed during the first 24 hours but a maximal response was observed at 48 hours. Cell proliferation returned to control levels 4 days after treatment. Further experiments over 13 weeks with doses of 0, 300 or 600 mg/kg bw per day for mice and 0 or 600 mg/kg bw per day for rats were performed. Hepatocellular proliferation was observed during the first week at 600 mg/kg bw per day in both sexes of mice and in female rats. Cell proliferation was not observed at the lower 300 mg/kg bw dose in mice.

Cell proliferation in response to *p*-DCB exposure was investigated in male and female rats (strain F344) and mice (strain B6C3F$_1$) using the BrdU technique. Male rats were administered *p*-DCB by gavage for 4 days at 0, 150 or 300 mg/kg bw. Another group, composed of male and female rats and female mice were administered doses of 0, 300 or 600 mg/kg bw. Increased cell proliferation was observed in the proximal convoluted tubules and to a lesser extent the proximal straight tubules but not distal tubules of treated male rats. Female rats or mice of either sex did not exhibit increased cell proliferation of the renal tubules. Increased cell proliferation was noted in the livers of both sexes of rats and mice at 300 mg/kg bw (Umemura *et al.*, 1992).

The role of endogenous glutathione in protecting against *p*-DCB-induced hepatotoxicity was demonstrated in male mice (strain ddY). The mice were pre-treated by i.p. injection with buthionine sulfoximine (BSO; 2 mmol/kg) to deplete glutathione prior to the oral administration of *p*-DCB (100 to 400 mg/kg). Hepatotoxicity was demonstrated by an increase in serum ALT and hepatic calcium levels which both peaked 24 hours after the administration of *p*-DCB (300 mg/kg); histopathology showed microvacuolar lipid accumulation of the periportal and midzonal areas within 4 hours of exposure. By 24 to 30 hours necrosis of the centrilobular and midzonal regions was evident. Control animals not treated with BSO and receiving *p*-DCB (1200 mg/kg) had serum ALT levels of 37.5 units/mL 30 hours after treatment while BSO treated animals exposed to *p*-DCB (100 to 400 mg/kg) had ALT levels of 342 to 3820 units/mL (Mizutani *et al.*, 1994).

The role of *p*-DCB as an inducer of DNA synthesis was assessed using hepatocytes derived from male $B6C3F_1$ mice. Mice where administered *p*-DCB (0, 750 or 1500 mg/kg bw) by the oral route and hepatocytes prepared 24, 39 or 48 hours later. Replicative DNA synthesis was assessed after the addition of [methyl-^{3}H]thymidine followed by autoradiography. A positive result was obtained with the lower dose at 48 hours while the higher dose gave positive results at 39 and 48 hours (Miyagawa *et al.*, 1995).

In a study by Lake *et al*. (1997) male rats (strain F344) were treated daily with *p*-DCB at 0, 25, 75, 150 or 300 mg/kg bw and male mice (strain $B6C3F_1$) at 0, 300 or 600 mg/kg bw by gavage 5 days/week. Replicative DNA synthesis in hepatocytes and renal proximal tube cells was assessed *in vivo* at weeks 0 to 1, 3 to 4 and 12 to13 (i.e., for six-day periods). Histological examination showed that a dose of 150 mg/kg bw or less did not increase the hepatocyte labelling index for rats whereas 300 mg/kg bw resulted in an increase of 255% of the control value at week one. No increases were observed for the same parameter for weeks 4 and 13. In the mouse, the hepatocyte-labelling index increased for all doses for weeks 1 and 4 with no significant differences observed at week 13. Replicative DNA synthesis in renal proximal tubule cells labelling increased at weeks 1, 4 and 13 in rats while the mouse kidney labelling index increased only during the 4 week period.

The relationship between the expression of the immediate-early genes, c-*fos*, c-*jun* and c-*myc* to hepatocyte proliferation after exposure to *p*-DCB has been investigated. Male rats (strain F344) were given a single oral dose of *p*-DCB (300 mg/kg bw). Examination of liver sections 48 hours after dosing revealed an increase of 4- to 5-fold in the hepatic labelling index (S-phase; as determined by BrdU labelling) compared to controls. The increase in labelling index was proceeded by an increase in the expression, in some cells, of c-*fos*, c-*jun* and c-*myc* at one hour post-dosing. Considerable inter-animal variation was observed in the expression of the immediate-early genes. A direct correlation between the expression of c-*myc*, but not c-*fos* or c-*jun*, with the hepatocyte labelling index was observed. *In situ* hybridization analysis indicated that cells expressing c-*jun* and c-*fos* were randomly distributed across the liver lobules while cells expressing c-*myc* were mainly midzonal and, to a lesser extent, periportal (Hasmall *et al.*, 1997a).

To further delineate the relationship between *p*-DCB-induced cell proliferation and hepatocarcinogenesis, Umemura *et al*. (1998) treated male rats (strain F344) and male mice (strain B6C3F_1) with *p*-DCB (0, 75, 150 or 300 mg/kg bw or 0, 150, 300 or 600 mg/kg bw respectively) by gavage for 1 and 4 weeks. Relative liver weights increased at both time points in mice at 600 mg/kg bw and in rats at 150 and 300 mg/kg bw. At week 4 a significant decrease ($p<0.01$) was noted in glutamine synthetase-expressing hepatocytes, a marker of hepatocyte injury, in mice at 150 mg/kg bw but not at week 1 or either time point for rats. The hepatic cumulative replicating fraction, determined by BrdU labelling, of mice increased at 300 and 600 mg/kg bw at week 1 with the response declining at week 4 to reach significance ($p<0.05$) only at 600 mg/kg bw. The cumulative replicating fraction in rat livers increased at 150 and 300 mg/kg bw at week 1 but returned to control levels at week 4. The absence of any hepatotoxic effect in the rat at 75 mg/kg bw and the mouse at 150 mg/kg bw suggests that a threshold level is required for hepatocarcinogenesis.

The relationship between cell proliferation and apoptosis in the maintenance of hepatic homeostasis after the oral administration of *p*-DCB to male rats (strain F344) and mice (strain B6C3F_1) at 300 and 600 mg/kg bw respectively, was investigated. After 2 days of treatment, DNA synthesis was significantly elevated ($p<0.05$) in both species while the percentage of apoptotic hepatocytes, both spontaneous and transforming growth factor β1-induced, was significantly ($p<0.05$) decreased. In all mice and 4 out of 5 rats apoptosis decreased to undetectable levels. Using Western blot analysis, an increase in the expression of mouse hepatic CYP2B1/2 was detected in response to treatment with *p*-DCB (James *et al.*, 1998).

In vitro studies

DNA synthesis by isolated rat (strain F344) and mouse (strain B6C3F_1) hepatocytes exposed to *p*-DCB (500 μM) for 40 hours was significantly elevated ($p<0.05$) in cells of both species while the percentage of apoptotic cells, both spontaneous and transforming growth factor β1-induced, was significantly ($p<0.05$) decreased (James *et al*., 1998).

10. Effects on Laboratory Mammals and Other Test Systems

Toxicological studies made available for assessment by applicants and notifiers and relevant studies identified after extensive literature searches have been evaluated and are summarised in this section. Use was also made of international assessment reports for *p*-DCB (BUA 1994, ATSDR 1998 (draft) and SIAR 1999 (draft)). In certain cases, studies cited in such reports, but not accessible for evaluation for this assessment, were utilised and have been acknowledged as such in the appropriate place.

10.1 Acute toxicity

10.1.1 Lethality

Lethality studies have demonstrated that *p*-DCB presents a low level of toxicity by the oral, dermal and inhalation routes. The LD_{50} and LC_{50} values from several published studies are presented in Table 10.

Table 10 - Summary of *p*-DCB acute lethality studies

Route	Species	Result	Reference
Oral	Rat	$LD_{100} \leq 4000$ mg/kg bw	Hollingsworth *et al*., 1956
	Rat	$LD_{50} = 2512$ mg/kg bw	Varshavskaja, 1967
	Rat	$LD_{50} = 3790$ to 3863 mg/kg bw	Gaines and Linder, 1986
	Guinea pig	$LD_{100} \leq 2800$ mg/kg bw	Hollingsworth *et al*., 1956
Dermal	Rat	$LD_{50} > 6000$ mg/kg bw	Gaines and Linder, 1986
Inhalation	Rat	$LC_{50} > 5.07$ mg/L/4 hours	Hardy, 1987
Intraperitoneal	Rat	$LD_{50} = 2562$ mg/kg bw	Zupko and Edwards, 1949

10.1.2 Systemic effects

Acute toxic effects reported in animals due to *p*-DCB are generally nephrotoxicity in male rats and hepatotoxicity in both sexes of mice. A summary of the acute toxic effects is presented in Table 11.

Table 11 - Summary of acute systemic effects due to *p*-DCB

Species (strain) animals/group	Sex	Exposure[1] (mg/kg bw)	Method	Clinical observations and pathology	Reference
Rat (F344) 6 animals/group	M/F	500	Corn oil gavage	A 2.6 fold increase in renal protein droplet formation in males.	Charbonneau *et al.*, 1989
Mice ($B6C3F_1$) 5 animals/group	M/F	600	Corn oil gavage	Significant increase* in liver weight at 48 hrs. Increased hepatic cell proliferation at 24 hrs for females and 48 hours for males. No change in plasma ALT, AST or LDH.	Eldridge *et al.*, 1992
Rats (F344) 5 animals/group	F	600	Corn oil gavage	Liver weight significantly increased* at 24 hrs. Increased hepatic cell proliferation occurred at 24hrs. No increase in plasma ALT, AST or LDH.	Eldridge *et al.*, 1992
Mice ($B6C3F_1$) 5 animals/group	M/F	0 600 900 1200	Corn oil gavage	Vacuolation of male periportal hepatocytes and centrilobular hepatocytes developed a granulated cytoplasm; females developed these features to a lesser extent. No increase in plasma ALT, AST or LDH at 24 or 48 hrs..	Eldridge *et al.*, 1992
Rats (F344) 5 animals/group	F	0 600 900 1200	Corn oil gavage	Slight vacuolation of the centrilobular hepatocytes. No evidence of necrosis. No increase in plasma ALT, AST and LDH at 24 or 48 hrs.	Eldridge *et al.*, 1992
Rats (F344) 1 animal/group	M	0 to ~2800	Corn oil gavage	Hepatic cytochrome P450 levels increased ~ 30% at ≥ 380 mg/kg bw. Centrilobular vacuolar degeneration observed at ≥ 475 mg/kg bw but no necrosis. No increase in plasma ALT or AST.	Allis *et al.*, 1992
Rat (F344) 8 animals/group	M	132, 265 397, 529 662, 794	i.p. injection	No change in serum levels of ALT at any dose level 24 hours after injection. No histopathological changes to the liver at any dose level.	Stine *et al.*, 1991
Rat (Wistar)	M	147 294 588	i.p. injection	No change in renal or hepatic tissue GSH or serum ALT. Plasma levels of total thyroxine (T4) decreased at the highest dose; triiodothyronine (T3) levels were not altered. A slight hepatic centrilobular hypertrophy observed at 588 mg/kg bw and renal protein droplets present at all doses.	Den Besten *et al.*, 1991
Mice ($B6C3F_1$) 5 animals/group	M	0 600 1000 1800	Corn oil gavage	Serum ALT significantly increased* at 1800 mg/kg bw at 48 hrs. No significant necrosis present but some hepatocyte swelling. A significant increase* in cell proliferation at ≥1000 mg/kg bw. Cell proliferation present at days 2, 3 and 4 but absent at day 7.	Umemura *et al.*, 1996
Rat (F344)	M	300	Corn oil gavage	Increase in hepatocellular proliferation and expression of c-*fos*, c-*jun* & c-*myc*.	Hasmall *et al.*, 1997a.[1]

[1] single dose only; * $p<0.05$

Charbonneau *et al*. (1989) reported the formation of protein droplets in the renal tissue of male, but not female rats (strain F344), 24 hours after a single oral dose of *p*-DCB (500 mg/kg bw).

Eldridge *et al.* (1992) found no increase in plasma enzyme levels, indicative of cellular injury, (alanine aminotransferase (ALT), aspartate amino-transferase (AST) or lactate dehydrogenase (LDH)), from male and female mice (strain $B6C3F_1$) and female rats (strain F344) which were administered a single dose of *p*-DCB (600 mg/kg bw). However, an increase in the liver weights of mice and rats was observed and an increase in the hepatocellular labelling index, (as determined by incorporation of 5-bromo-2'-deoxyuridine (BrdU)), a measure of cell proliferation, occurring at 24 and 48 hours after exposure for mice and rats respectively. The labelling index returned to normal 4 days after treatment. Histological examination of the livers revealed vacuolation of periportal hepatocytes and granulated cytoplasm of centrilobular hepatocytes of male mice and to a lesser degree in female mice. Rats developed slight vacuolation of the centrilobular hepatocytes although necrosis was not evident. Further experiments over 13 weeks with doses of 0, 300 or 600 mg/kg bw per day for mice and 0 or 600 mg/kg bw per day for rats were performed. Hepatocellular proliferation was observed during the first week at 600 mg/kg bw per day in both sexes of mice and in female rats. Cell proliferation was not observed at the lower 300 mg/kg bw dose in mice.

Similar effects were observed in another study of rats (strain F344) above 450 mg/kg bw in which hepatocellular degeneration was observed (Allis *et al*., 1992).

10.1.3 Metabolites of *p*-DCB

The administration of 2,5-dichloro-3-(glutathion-*S*-yl)-1,4-benzoquinone (DCGBQ; 0, 50, 100, 150 or 200 μmol/kg), a metabolite of *p*-DCB, to male rats (Sprague-Dawley strain) by i.v. injection resulted in the development of a dose-dependent renal proximal tubular necrosis. The condition was characterised at 19 hours post administration by an increase in blood urea nitrogen (BUN) at the highest dose and elevated excretion of glucose, LDH and γ-glutamyl transferase (γ-GT) at 100 μmol/kg and above. Histopathological examination of the kidneys demonstrated extensive necrosis of the proximal tubules at 19 hours post exposure. The investigators concluded that DCGBQ derived from the hepatic metabolism of *p*-DCB is responsible for the early onset of nephrotoxicity in the male rat (Mertens *et al*., 1991).

10.2 Irritation and corrosivity

There is little published information relating to skin and eye irritation in animals due to acute exposure to *p*-DCB. Hollingsworth *et al*. (1956) reported that solid *p*-DCB has a negligible irritating action on intact uncovered human skin but can produce a burning sensation when in close contact for an excessive period. Eye irritation due to *p*-DCB vapour (798 ppm) was also reported in rabbits, rats and guinea pigs.

In an unpublished study, it was reported that *p*-DCB (500 mg in paraffin oil) was slightly irritating to rabbit skin (erythema but no oedema) after exposure for 4 hours. Similarly, when the same preparation was applied to the eyes of rabbits for 24 hours slight irritation was observed as judged by the presence of isolated damage to the conjunctiva (erythema and oedema) (Maertins 1988, cited in SIAR 1999).

There have been no studies reporting *p*-DCB as being corrosive.

10.3 Sensitisation

A Magnusson and Kligman test was conducted on guinea pigs to examine the sensitisation potential of *p*-DCB. Animals were tested at induction concentrations of 0.1% intradermally, 25% topically and a challenge concentration of 25% in petrolatum. Irritation was slight after 0.1% intradermal application. All animals were negative for sensitisation at 24 hours and 21% of animals were positive at 48 hours (Bornatowicz *et al.*, 1995, cited in SIAR 1999).

Two non-validated sensitisation studies have also been reported in which the results were negative (Leung *et al.*, 1990 and Suzuki *et al.*, 1991).

10.4 Immunotoxicity

Human lymphocytes exposed for 4 hours to *p*-DCB (100 μM to 10 mM) proved to be cytotoxic only at the highest concentration as judged by the trypan blue dye exclusion assay (Perocco *et al.*, 1983).

Total thyroxine (T4) and total triiodothyronine (T3) plasma levels of male rats (strain Wistar) at 24 hours after a single intraperitoneal injection of *p*-DCB (1 or 2 mmol/kg) were examined. *p*-DCB decreased T4 levels at 2 mmol/kg but not at the lower dose and T3 levels were not altered (Den Besten *et al.*, 1991).

10.5 Repeated dose toxicity

10.5.1 Oral administration

Male and female rats (strain F344) were administered *p*-DCB (0, 60, 125, 250, 500 or 1000 mg/kg bw) by gavage for 14 days. All treated animals survived with the exception of one male (125 mg/kg bw), the death being attributed to gavage error. The final average body weights for males were decreased relative to controls; female body weights were not affected (NTP, 1987).

A second 14-day study of male and female rats (strain F344) was conducted with increased doses of *p*-DCB (0, 500, 1000, 2000, 4000 or 8000 mg/kg bw) by gavage. A dose of 1000 mg/kg bw resulted in the death of 4 of 5 females and one male (attributed to gavage error). Doses of 2000 mg/kg bw or greater proved fatal for all animals within 14 days (NTP, 1987). The results of the two 14 day studies in rats are inconsistent and both sets of results were confounded by a high incidence of gavage errors that may have contributed to the deaths of some animals.

Male and female mice (strain B6C3F$_1$) were administered *p*-DCB (0, 250, 500, 1000, 2000 or 4000 mg/kg bw) by gavage for 14 days. All mice dosed at 4000 mg/kg bw died by day 4. Two male and one female control animals died due to gavage errors. The final mean body weight of male mice at 2000 mg/kg bw was 15% lower than controls. No dose-related histopathological results were observed (NTP, 1987).

In a second 14-day study, male and female mice (strain B6C3F$_1$) were administered *p*-DCB (0, 250, 500, 1000, 2000 or 4000 mg/kg bw) by gavage. No *p*-DCB-related deaths were observed. One treated male (125 mg/kg bw) and 2 male and 1 female control animals died due to gavage errors. Final mean body weights were not different from control animals (NTP, 1987).

Renal cell proliferation in response to the oral administration of *p*-DCB (0, 118 or 294 mg/kg bw) to male rats (strain F344) for 7 days was examined. A significant increase ($p<0.05$) was observed in treated animals (Charbonneau *et al.*, 1989).

A 1-year oral study in which *p*-DCB was administered via capsule was conducted with Beagle dogs (5 male and 5 female dogs/group) using doses of 0, 10, 50 or 150 mg/kg/day. Due to severe toxicity at the highest dose, the maximum dose was adjusted to 100 mg/kg/day for males at 3 weeks and subsequently decreased to 75 mg/kg/day for both sexes. Two males and 1 female died within the first 4 weeks of the study and 1 male control died at a later date. The effects at 150 mg/kg (and for the deceased control male) included hypoactivity, dehydration, decreased defecation, blood-like faecal colour, emesis, emaciation and pale oral mucosa. Cumulative body weight gain was decreased at 150 mg/kg/day although, after the reduced dosage regimen was introduced, final body weights were comparable at the conclusion of the study. High-dose animals exhibited a mild anaemia, characterised by decreased erythrocyte and haematocrit levels at 6 months, but returned to normal due to a compensatory hemopoietic response. Doses of 50 mg/kg/day or greater resulted in an increase in hepatic enzymes (ALT, AST and GGT) and were associated with increases in liver weights compared to control animals. Histopathological findings included hepatocellular hypertrophy, hepatocellular pigment deposition, bile duct hyperplasia and hepatic portal inflammation. Renal effects observed included collecting duct epithelial vacuolation in a high-dose male and at all dose levels in females and, at 50 mg/kg/day or greater, was associated with increased renal weights with renal discolouration (Naylor and Stout, 1996). The NOAEL was 10 mg/kg/day and a LOAEL of 50 mg/kg/day based on hepatotoxicity.

The effect of *p*-DCB (300 mg/kg bw) on male rat (strain F344) hepatocyte ploidy and nuclearity were examined after 7 days following daily administration of the compound by gavage. Examination of isolated untreated hepatocytes showed the dominant cell population to be tetraploid cells with lesser populations of diploid and octoploid cells. Following *p*-DCB treatment the number of octoploid cells increased by 18% and a decrease in the tetraploid and diploid cell populations resulted. *p*-DCB also caused an 11% increase in the population of mononucleated octoploid cells but not of binucleated octoploid or mononucleated and binucleated tetraploid cells. It was further shown that *p*-DCB treatment resulted in a 12-fold increase in the hepatic labelling index as determined by BrdU labelling. The distribution of BrdU labelling within each ploidy and nuclearity class showed significant increases in the labelling

index of diploid, mononucleated tetraploid and mono- and binucleated octoploid cells. The labelling index of binucleated tetraploid cells was not increased by *p*-DCB (Hasmall *et al.*, 1997b).

Oral administration of *p*-DCB to male rats (strain not specified) and delivered by gavage, five days/week for four weeks at doses of 10, 100 and 500 mg/kg bw, resulted in the development of liver necrosis and swelling of the renal tubular epithelium at the highest dose. No adverse effects were observed with the lower doses (Hollingsworth *et al.*, 1956).

A long term study was performed with female rats given *p*-DCB (0, 18.8, 188, or 376 mg/kg bw) by gavage for 5 days/week over 192 days (138 doses). The results demonstrated an increase in average liver weight and a slight increase in kidney weight at 188 and 376 mg/kg bw. At the higher dose histopathological findings indicated slight cirrhosis and focal necrosis of the liver. There were no adverse haematological findings. The NOAEL for this study was 18.8 mg/kg bw (Hollingsworth *et al.*, 1956).

A 13-week study investigated the effect of *p*-DCB on male and female rats (strain F344). Rats were administered *p*-DCB (0, 300, 600, 900, 1200 or 1500 mg/kg bw) 5 days/week by gavage. Examination of the animals revealed that *p*-DCB at all doses induced histopathological changes in the kidneys of all male rats; the changes where characterised by renal tubular cell alterations with hyaline droplet formation. At 1,200 and 1,500 mg/kg bw survival of male rats decreased and female survival decreased at 1,500 mg/kg bw. Weight gain decreased for male rats at 300 mg/kg bw or greater and for females at 1,200 and 1,500 mg/kg bw. A statistically significant increase in liver weights was observed at 900 mg/kg bw for both sexes. At doses of 1,200 and 1,500 mg/kg bw pathological conditions were seen in both sexes including degeneration and necrosis of hepatocytes, bone marrow hypoplasia, lymphoid depletion of the thymus and spleen and epithelial necrosis of the nasal turbinates. Haematological changes observed in male rats at doses of 300 to 1,200 mg/kg bw included statistically significant decreases in haematocrit, erythrocyte count and haemoglobin level. No significant changes in female blood parameters were observed (NTP, 1987). The NOAEL for females was 600 mg/kg bw while a NOAEL was not identified for males.

A second 13-week NTP study examined the effect of lower doses of *p*-DCB (0, 37.5, 75, 150, 300 or 600 mg/kg bw) on male and female F344 rats when administered by gavage. The findings indicated that the NOAEL for female rats was 600 mg/kg bw per day and for male rats the NOAEL was 300 mg/kg bw per day where renal cortical degeneration was observed at 600 mg/kg bw (NTP, 1987).

A 13-week study of male and female mice (strain B6C3F_1) dosed with *p*-DCB (0, 600, 900, 1000, 1500 or 1800 mg/kg bw) by gavage 5 days/week resulted in hepatocellular degeneration in all treatment groups. The survival of males and females at 1,500 and 1,800 mg/kg bw was decreased compared to control groups. Administration of *p*-DCB to male mice at 600 mg/kg bw or more resulted in leukopenia and females at 1,000 mg/kg bw or more displayed a similar condition. No renal pathologies were observed in any treatment group (NTP, 1987). A LOAEL of 600 mg/kg bw for both sexes of mice was determined.

A second 13-week gavage study of mice (strain B6C3F$_1$) treated with lower doses of *p*-DCB (0, 85, 338, 675 or 900 mg/kg bw) for 5 days/week. Both sexes developed hepatocellular cytomegaly at 675 mg/kg bw or greater but was not observed at lower doses. No renal pathologies were observed in any treatment group (NTP, 1987). The NOAEL was 338 mg/kg bw.

A study of the nephrotoxic effects of *p*-DCB for male and female rats (strain F344) was conducted. Animals received oral doses of *p*-DCB (0, 75, 150, 300 or 600 mg/kg bw per day), 7 days/week for 4 or 13 weeks. No treatment-related effects were observed for food consumption, growth or haematocrit. Urinalysis showed a dose-dependent shift to acidic values and an increase in the presence of epithelial cells in the males at 4 and 13 weeks. The presence of LDH was elevated in the urine of males at day 9 and was dose-dependent from 75 to 300 mg/kg bw. LDH was similarly elevated at 4 and 13 weeks. Gross pathology of the kidneys at 4 weeks showed an increase in absolute and relative weights for the males at 300 mg/kg bw and females at 600 mg/kg bw. At week 13 the increase occurred at 150 mg/kg bw for males while the female weight increase was significant at 600 mg/kg bw. Histopathological changes were evident in the male kidneys after 4 weeks of exposure to *p*-DCB at doses of 150 and 600 mg/kg bw and consisted of dilated tubules, cellular degeneration and hyaline droplet formation. The kidneys of the 75 mg/kg bw group showed no sign of structural alterations. At 13 weeks male kidneys in the 150 to 600 mg/kg bw groups showed signs of epithelial regeneration and chronic nephropathy with extensive hyaline droplet formation in the proximal tubular epithelial cells. The female kidneys showed no sign of hyaline droplet formation and no structural alterations due to *p*-DCB (Bomhard *et al.*, 1988). The LOAEL for males was 75 mg/kg bw for both the 4 and 13 week studies while the NOAEL for females was 300 mg/kg bw for both time periods.

A 13-week study was undertaken to determine the effects of *p*-DCB on male and female mice (strain B6C3F$_1$) and female rats (strain F344) (5 animals/group) in which the mice received 0, 300 or 600 mg/kg bw per day and the rats 0 or 600 mg/kg bw per day by gavage. One experimental group from each species was given *p*-DCB up to week 5 and for the remainder of the study received the vehicle (corn oil). At the highest dose, liver weights increased in all treated animals. Hepatocellular proliferation was observed during the first week at 600 mg/kg bw per day in both sexes of mice and in female rats but not in mice at 300 mg/kg bw per day. There were no changes in liver-associated plasma enzymes for any treatment group compared to control animals. In the groups in which the treatment with *p*-DCB ceased at 5 weeks the increase in liver weight halted and reverted to normal weight by the end of the study demonstrating the reversible nature of the effect. These results indicate that, under the experimental conditions described, *p*-DCB can induce a mitogenic response in the liver in the absence of a necrotic response (Eldridge *et al.*, 1992). The LOAEL was 600 mg/kg bw for each species due to hepatocellular proliferation.

In a study by Lake *et al*. (1997) male rats (strain F344) were treated daily with *p*-DCB at 0, 25, 75 150 or 300 mg/kg bw and male mice (strain B6C3F$_1$) at 0, 300 or 600 mg/kg bw by gavage, 5 days/week. The animals were sacrificed at weeks 1, 4 and 13. Treatment of rats with *p*-DCB at 300 mg/kg bw resulted in a marked increase in relative liver weights at weeks 1, 4 and 13 and became significant at 150 mg/kg bw

at week 4. A significant increase in kidney weight in rats at weeks 4 and 13 was noted at a dose of 150 and 300 mg/kg bw. Relative liver weights for mice were increased at weeks 1, 4 and 13 at 300 mg/kg bw but kidney weights were not significantly different from controls. Histological examination of liver sections revealed no pathological changes in response to 300 mg/kg bw in the rat for 1 week and slight centrilobular hypertrophy in the mouse. At 300 mg/kg bw rats developed a mild centrilobular hypertrophy after 13 weeks exposure while mice receiving 600 mg/kg bw showed a marked centrilobular hypertrophy. The NOAEL for rats was 75 mg/kg bw and for mice the NOAEL was 300 mg/kg bw.

A 2-year study of the effects of oral administration of *p*-DCB on both sexes of rats (strain F344) and mice (strain $B6C3F_1$) was conducted. Male rats were dosed at 0, 150 or 300 mg/kg bw and female rats and male and female mice at 0, 300 or 600 mg/kg bw for 5 days/week. Survival of high dose male rats was significantly lower than controls. Male rats exhibited nephropathy due to epithelial hyperplasia of the renal pelvis, mineralisation of the collecting tubules of the renal medulla and focal hyperplasia of the renal tubular epithelium at both doses. Female rats showed increased nephropathy at either dose compared to controls. Both sexes of mice exposed to *p*-DCB developed hepatic lesions characterised by cytomegaly, karyomegaly, cellular degeneration and individual cell necrosis. Male mice displayed an increase in nephropathy while females exhibited renal tubular regeneration. The LOAEL for male and female rats were 150 and 300 mg/kg bw respectively. The LOAEL for both sexes of mice was 300 mg/kg bw (NTP, 1987). Male rats developed renal tumours while both sexes of mice developed hepatic tumours which are described in further detail in Section 10.8.

10.5.2 Inhalation

Male and female rats (strain not specified), guinea pigs and rabbits were exposed to *p*-DCB vapour (0, 96, 158, 173, 341 or 798 ppm) 7 hours/day (8 hours/day for 798 ppm), 5 days/week from 1 to 69 exposures. At the highest dose the animals displayed tremors, weakness, an unkempt appearance and unconsciousness. Histological findings included slight to moderate cloudy swelling and central necrosis of the liver of all animals and swelling of the tubular epithelium of female rat kidneys. Rabbits, but not rats or guinea pigs, exhibited reversible eye ground changes and 2 exhibited pulmonary congestion with emphysema. One group of animals were treated at 341 ppm *p*-DCB for 6 months. Male rats developed a slight increase in liver and kidney weights and the growth of male guinea pigs was depressed. Histological findings in male guinea pigs included cloudy swelling of the liver with fatty degeneration, focal necrosis and slight cirrhosis in some animals. Exposure to 173 ppm *p*-DCB for 16 days produced a moderate increase in the average weights of livers and kidneys of rats and degeneration of the central areas of female rat livers. The average weight of the spleen in male guinea pigs was depressed. Histological examination of lung tissue revealed slight interstitial oedema and congestion in male rats and female rabbits and guinea pigs while some animals developed alveolar haemorrhages and oedema. Similarly, treatment with 158 ppm produced an increase in the average weights of male livers and kidneys and female rat and guinea pig livers but no other adverse effects. Exposure to *p*-DCB at 96 ppm for six months produced no adverse effects

(Hollingsworth *et al.*, 1956). A NOAEL of 96 ppm and a LOAEL of 158 ppm were identified for rats.

In a study reviewed by Loeser and Litchfield (1983), exposed rats (strain Wistar-derived) and mice (strain Swiss) to long term exposure of *p*-DCB vapour (0, 75 and 500 ppm), 5 hours/day, 5 days/week for 76 and 57 weeks for rats and mice respectively. They found no treatment-related changes to the rats with respect to mortality, changes in body weight, or food and water intake. At 500 ppm, urinary coproporphyrin was elevated with the authors concluding that this may be related to an observed increase in liver and kidney weights of this group. There was no change in hepatic aminopyrine demethylase. It was concluded that while long-term exposure of rats to *p*-DCB at 500 ppm induced some changes in liver function there was no toxicological effect at 75 ppm. Mice exposed to *p*-DCB under identical conditions as the rats did not demonstrate any signs of toxicity giving a NOAEL of 500 ppm, however, due to recurrent respiratory infections the results reported are of questionable value.

In the 2-year JBRC (1995) study, to determine the carcinogenicity of *p*-DCB, rats (strain F344) were exposed to the vapour at 0, 20, 75 or 300 ppm 6 hours/day, 5 days/week. Treatment of male rats at 300 ppm resulted in an increase in kidney weight with mineralisation of the of the papilla collecting tubule and urothelial hyperplasia. Respiratory effects were observed (respiratory metaplasia in nasal cavity gland and eosinophilic changes in respiratory epithelium) in females at 300 ppm and eosinophilic changes in olfactory epithelium in both sexes of control and treated animals at 300 ppm and females at 75 ppm. The apparent presence of an underlying respiratory pathology in control and test animals makes the interpretation of treatment-related respiratory effects difficult. Based on renal pathology alone a NOAEL of 75 ppm was determined.

A 2-year study was undertaken to determine the carcinogenicity of *p*-DCB for mice (strain BDF1) exposed to the vapour of *p*-DCB at 0, 20, 75 or 300 ppm 6 hours/day, 5 days/week. Liver weights were increased at 300 ppm for both sexes and an increase in hepatic enzymes (AST, ALT, LDH and alkaline phosphatase) was observed. Histological examination revealed slight local necrosis in both sexes and hepatocellular hypertrophy in males. Kidney weights of both sexes was increased at 300 ppm (JBRC, 1995). A NOAEL of 75 ppm was determined.

The carcinogenic effects observed in the above 2-year studies are discussed in Section 10.8

10.5.3 Dermal

A dermal toxicity study was conducted with male and female rats (strain Sprague-Dawley) by application of *p*-DCB (0 to 300 mg/kg bw per day) in mineral oil, 5 days/week for 3 weeks. No evidence of any toxicity or significant dermal effect was observed (Arletta, 1990; cited in SIAR 1999).

10.6 Reproductive and developmental toxicity

10.6.1 Reproductive toxicity

A two-generation reproductive study was conducted with Sprague-Dawley rats. Weanling rats (F_0) were exposed to *p*-DCB vapour (0, 66, 211 or 538 ppm) for 6 hr/d for 10 weeks before mating and during mating, gestation and lactation. F_1 weanlings were then exposed for 11 weeks and mated under the conditions described above. Exposure to *p*-DCB at 538 ppm resulted in a decrease in body weight for both sexes and a reduction in body weight gain along with reduced food consumption. Body weights for F_0 and F_1 females during gestation and F_1 females during lactation were decreased at 538 ppm. No reproductive effects were observed for either generation. The F_1 and F_2 litter body weights were decreased and an increase in perinatal deaths occurred at 538 ppm. All levels of exposure to *p*-DCB resulted in F_0 and F_1 male nephrotoxicity (hyaline droplet nephropathy) with increases in kidney weights. Doses of 211 or 538 ppm resulted in increased male and female liver weights with hepatocellular hypertrophy at 538 ppm in both F_0 and F_1 sexes (Neeper-Bradley *et al.*, 1989). No NOAEL could be determined for males. A NOAEL for females of 66 ppm was determined based on hepatotoxicity observed at 211 ppm and for reproductive effects a NOAEL of 211 ppm was determined.

In a two-generation study of the effects of *p*-DCB on reproduction (OECD guideline 416) rats (strain Sprague-Dawley) were administered *p*-DCB (0, 30, 90 or 270 mg/kg bw per day) by gavage. Treatment was initiated 77 days prior to mating for male (F_0) rats and for F_0 females 14 days before mating and continued during mating, gestation, and lactation and for 21 days postnatal. In parents, in both generations at 270 mg/kg bw, the organ weights of male rat livers and kidneys (associated with nephrotoxicity) were increased while spleen weights decreased. Parameters examined were time between beginning of mating and evidence of copulation, time of gestation, fertility index, gestational index, percentage of dams with dead pups, total number of pups at birth, percentage of pups with positive ear reflex, grasping and orientation reflex, absolute and relative weights of testes, epididymides and ovaries, absolute and relative weights of female livers, kidneys and spleens. Treatment with *p*-DCB at 270 mg/kg bw per day resulted in both generations in a reduction in the number of live pups at birth and mean body weights of pups only at birth and an increased pup mortality between days 1 to 4 and 5 to 21 of lactation. Developmental effects included retardation of the erection of ears and opening of eyes, a statistically significant ($p<0.05$) reduction in the number of pups with a positive draw up reflex and an increase incidence of dry skin. At 90 mg/kg bw per day, reversible reduced mean body weight at birth in the F_0/F_1 generation and a statistically significant increase in the number of deceased pups between days 1 to 4 only in F_1/F_2 generation was noted. The NOAEL for fertility was estimated to be 270 mg/kg bw per day while the NOAEL for parents F_0 and F_1 was 90 mg/kg bw per day. The NOAEL for developmental effects was 30 mg/kg bw per day with reversible reduced mean body weight at birth in F_0/F_1 pups, increased number of deceased pups in 1 time period in 1 generation and slight behavioural anomalies in 1 generation (Bornatowicz *et al.*, 1994, cited in SIAR 1999).

10.6.2 Developmental toxicity

The developmental toxicity of *p*-DCB in New Zealand rabbits has been investigated. Inseminated rabbits were exposed to *p*-DCB (0, 100, 300 or 800 ppm) for 6 hr/day on days 6 to 18 of gestation. Maternal toxicity was observed, described as slight and based on a decrease in body weight gain during the first three day of exposure, at 800 ppm. No significant differences were observed when animals were examined for number of pregnancies, number of litters, corpora lutea/dam, foetuses/litter, litters totally resorbed, resorptions/litters with resorptions, sex ratio and foetal body weight. A significant, but non dose-dependent, increase in the percent implantations resorbed and percent litters with resorptions at 300 ppm was observed. At 800 ppm a significant increase in retro-oesophageal right subclavian artery development was observed (controls, 1/115; 800 ppm, 6/119; historical controls for the laboratory, 5%) but was not considered to be a teratogenic response to treatment. No other significant treatment-related changes in offspring were observed (Hayes, 1985). A NOAEL of 300 ppm for maternal toxicity was determined. The NOAEL for developmental toxicity was 800 ppm.

Pregnant female rats (strain CD) were treated from day 6 to day 15 of gestation with *p*-DCB (0, 250, 500, 750 or 1000 mg/kg bw per day) by gavage. A dose of 500 mg/kg bw or higher resulted in a decrease in maternal weight gain. No significant change was observed in the number of implantations, corpora lutea/dam, number of live foetuses, percent pre-implantation and post-implantation loss or percent resorptions compared to control animals. At the highest dose foetal weights were significantly lower ($p<0.01$) than control or other treated groups. No abnormalities were observed with the exception of the 750 and 1000 mg/kg bw groups where skeletal variations were detected and a dose-related incidence of extra rib formation at 500 to 1000 mg/kg bw (Giavini *et al.*, 1986). The NOAEL for maternal toxicity and developmental toxicity was 250 mg/kg bw per day.

Table 12 – Summary of NOAEL and LOAEL values for *p*-DCB

Species (strain)	Study type and duration	Sex	NOAEL (mg/kg bw per day)	LOAEL and associated pathologies	Reference
Rat (strain not specified)	Oral, 4 weeks	Male	100	500 mg/kg bw; liver necrosis and swelling of the renal tubular epithelium.	Hollingsworth *et al.*, 1956
Rat (strain not specified)	Oral, 6 months	Female	18.8	188 mg/kg bw; increase in liver weight and slight increase in kidney weight	Hollingsworth *et al.*, 1956
Rat (F344)	Oral, 13 week (high dose)	Male	NI	300 mg/kg bw; renal tubular cell alteration and hyaline droplet formation	NTP, 1987
		Female	600	900 mg/kg bw; increase in liver weight	
Rat (F344)	Oral, 13 week (lower dose)	Male	300	600 mg/kg bw; renal cortical degeneration.	NTP, 1987
		Female	300	600 mg/kg bw; renal cortical degeneration	
Mice ($B6C3F_1$)	Oral, 13 week (high dose)	Male	NI	600 mg/kg bw; hepatocellular degeneration, leukopenia.	NTP,1987
		Female	NI	600 mg/kg bw; hepatocellular degeneration	
Mice ($B6C3F_1$)	Oral, 13 week (lower dose)	Male	338	675 mg/kg bw; hepatocytomegaly	NTP, 1987
		Female	338	675 mg/kg bw; hepatocytomegaly	
Rat (F344)	Oral, 4 weeks	Male	NI	75 mg/kg bw; increase in urinary LDH, hyaline droplet formation.	Bomhard, 1998
		Female	300	600 mg/kg bw; increase in relative kidney weight.	
Rat (F344)	Oral, 13 weeks	Male	NI	75 mg/kg bw; increase in urinary LDH, hyaline droplet formation.	Bomhard, 1998
		Female	300	600 mg/kg bw; increase in kidney weight.	
Mice ($B6C3F_1$)	Oral 13 weeks	Male	300	600 mg/kg bw; increased liver weight, increased hepatocyte proliferation	Eldridge *et al.*, 1992
		Female	300	600 mg/kg bw; increased liver weight, increased hepatocyte proliferation	
Rat (F344)	Oral, 13 weeks	Female	NI	600 mg/kg bw; increased liver weight, increased hepatocyte proliferation.	Eldridge *et al.*, 1992
Rat (F344)	Oral, 13 weeks	Male	75	150 mg/kg bw; decrease in body weight, increase in relative liver & kidney weight.	Lake *et al.*, 1997
Mice ($B6C3F_1$)	Oral, 13 weeks	Male	NI	300 mg/kg bw; increase in hepatic labelling index; increase in relative liver weight	Lake *et al.*, 1997
Rat (F344)	Oral, 2 year	Male	NI	150 mg/kg bw; renal hyperplasia, mineralisation,	NTP, 1987
		Female	NI	300 mg/kg bw; nephropathy.	
Mice ($B6C3F_1$)	Oral, 2 year	Male	NI	300 mg/kg bw; hepatocellular degeneration, nephropathy.	NTP, 1987
		Female	NI	300 mg/kg bw; hepatocellular degeneration, renal tubular regeneration.	

Table 12 – Summary of NOAEL and LOAEL values for *p*-DCB (cont.)

Species (strain)	Study type and duration	Sex	NOAEL (mg/kg bw per day)	LOAEL and associated pathologies	Reference
Dog	Oral 1year	Male	10	50 mg/kg bw; increased kidney and liver weights. Increased hepatic enzymes (ALT, AST, GGT), hepatocellular hypertrophy and pigment deposition, bile duct hyperplasia and hepatic portal inflammation. Renal effects included collecting duct epithelial vacuolation and renal discolouration.	Naylor & Stout, 1996
		Female	10	50 mg/kg bw; increased kidney and liver weights. Increased hepatic enzymes (ALT, AST, GGT), hepatocellular hypertrophy and pigment deposition, bile duct hyperplasia and hepatic portal inflammation. Renal effects included collecting duct epithelial vacuolation and renal discolouration.	
Rat (strain not specified)	Inhalation, 13 weeks	Male	96 ppm	158 ppm; increase in average liver & kidney weights.	Hollingsworth *et al.*, 1956
		Female	96 ppm	158 ppm; increase in average liver weight.	
Rat (F344)	Inhalation, 76 weeks	Male	75 ppm	500 ppm; increased liver & kidney weights, increase in urinary protein and coproporphyrin output.	Loesser & Litchfield, 1983
		Female	75 ppm	500 ppm; increased liver & kidney weights, increase in urinary protein and coproporphyrin output.	
Mice (F344)	Inhalation, 57 weeks	Male	500 ppm	No treatment-related pathologies observed.	Loesser & Litchfield, 1983
		Female	500 ppm	No treatment-related pathologies observed.	
Rat (F344)	Inhalation, 2 year	Male	75 ppm	300 ppm; nephropathy & increased liver weight.	JBRC, 1995
		Female	75 ppm	300 ppm; increased liver weight.	
Mice (BDF1)	Inhalation, 2 year	Male	75 ppm	300 ppm; increases in liver weight, AST, ALT, LDH & alkaline phosphatase, hepatocellular hypertrophy & slight necrosis. Increased kidney weight.	JBRC, 1995

NI = not identified (i.e. effects seen at lowest dose).

10.7 Genotoxicity

In vitro

A series of Ames tests were conducted with several strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1538), with or without metabolic activation by the S9 system. In one experiment, *p*-DCB was used in the gas phase (0, 94, 299 or 682 ppm) and in two other experiments the plate incorporation method (4, 20, 100, 500 or 2500 μg *p*-DCB/plate) was used. All strains gave negative results with the exception of the TA1535 strain which was positive at 500 μg/plate in one experiment only (Anderson, 1976, cited by Loeser and Litchfield, 1983).

Other Ames tests by Shimizu *et al.*, (1983) using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 and Haworth *et al.*, (1983) using strains TA98, TA100, TA1535 and TA1537 produced negative results with or without metabolic activation.

Tests with *p*-DCB using other species of bacteria (*Bacillus subtilis* and *Escherichia coli*) have reported negative results (Waters 1982). However, a mutagenic effect due to *p*-DCB was found in the eukaryotic *Aspergillus nidulans* assay (Prasad, 1970).

Human lymphocytes exposed for 4 hours to *p*-DCB (0.01 to 1 mM) failed to induce unscheduled DNA synthesis by a [^{3}H]-thymidine uptake assay (Perocco *et al.*, 1983).

Studies of unscheduled DNA synthesis in the HeLa cell line proved to be negative with or without metabolic activation (Instituto di Recerche Biomediche 1986a) as did gene mutation assays involving Chinese hamster lung cells (Instituto di Recerche Biomediche 1986b; cited in SIAR, 1999).

The mutagenic potential of *p*-DCB was assessed in the L5178Y mouse lymphoma forward mutation assay, however, the results proved to be inconclusive due to one metabolic activation test giving a negative result and two other tests giving positive results, one positive at high doses, the other at an intermediate dose. The test was negative without metabolic activation (McGregor *et al.*, 1988).

Human lymphocytes from 2 donors, when incubated for 48 hours with 0.05, 0.1 or 0.2 μg/mL *p*-DCB and without metabolic activation, resulted in a significant increase in the sister-chromatid exchange rate at the two highest doses. At 0.1 and 0.2 μg/mL, *p*-DCB was cytotoxic as judged by a decrease in third and second metaphases. The lowest tested concentration of *p*-DCB (0.05 μg/mL) was without effect (Carbonell *et al.*, 1991).

Treatment of rat hepatocyte primary cultures with *p*-DCB (0.56 to 3.2 mM) resulted in a biphasic response with a significant increase ($p = 0.05$) in micronuclei at 1.0 to 1.8 mM and a return to control levels at 3.2 mM. The frequency of DNA strand breaks did not alter in response to treatment with *p*-DCB. Human hepatocytes, from 2 donors, under the same conditions did not exhibit clastogenic effects (Canonero *et al.*, 1997).

The binding of radiolabel to calf thymus DNA after the addition of *p*-[^{14}C]-DCB was mediated by rat and mouse microsomes (from liver and lung) and was enhanced by the addition of glutathione suggesting that activation of *p*-DCB by cytochromes P450 and microsomal glutathione transferases are important for DNA binding to occur (Lattanzi *et al.*, 1989).

In vivo

A dominant lethal assay using male CD-1 mice exposed to *p*-DCB (0, 75, 225 or 450 ppm) 6 hr/day for 5 days found *p*-DCB to be non-mutagenic at any maturation stage of the 8-week spermatogenic cycle. No significant increases in post implantation early foetal deaths, early foetal deaths or percentage of early foetal deaths per total implants per pregnancy were detected which could be attributed to *p*-DCB (Anderson and Hodge, 1976, cited in Loeser and Litchfield, 1983).

A micronucleus assay performed on male and female mice (strain B6C3F$_1$) after oral administration of *p*-DCB (0, 600, 900, 1000, 1500 and 1800 mg/kg bw) in corn oil for 13 weeks produced no evidence of peripheral micronucleated erythrocytes at any dose (NTP, 1987).

The clastogenic activity of *p*-DCB was assessed using an *in vivo* bone marrow micronucleus test. The *p*-DCB (0, 355, 710, 1065 or 1420 mg/kg bw) was administered in two doses 24 hours apart by i.p. injection to male NMRI mice and the animals sacrificed 6 hours after the final injection. At 30 hours after first exposure, a dose-dependent increase in micronuclei was observed in the femoral bone marrow of treated mice compared to control animals. Data on cytotoxicity were not presented (Mohtashamipur, 1987).

The covalent binding of radiolabel to DNA has been investigated in male Wistar rats and BALB/c mice after intraperitoneal injection of *p*-[^{14}C]-DCB (0.4 mg/kg bw). The amount of radioactivity covalently bound to DNA in the liver, kidneys, lungs and stomach was determined 22 hours later. The results demonstrated no DNA binding in the rat and low level binding to the DNA of all organs tested in the mouse. Examination of mice 72 hours after injection revealed no detectable binding of radiolabel to DNA (Lattanzi *et al.*, 1989).

In another micronucleus assay, i.p. doses of *p*-DCB at 355 and 710 mg/kg bw produced negative test results. Cytotoxicity was noted at the highest dose based on a change in the polychromatic/normochromatic erythrocyte ratio (Herbold, 1988, cited in SIAR 1999).

No evidence of micronucleus formation was found in male mice (strain CD-1) after double i.p. injections of up to 75% of the LD_{50} or double oral doses of up to 2000 mg/kg bw by gavage (Morita *et al.*, 1997).

Damage to the DNA of male mice (strain CD-1) after treatment with *p*-DCB (2000 mg/kg bw, suspended in 2% Tween-80) by i.p. injection was assessed by the single-cell gel electrophoresis (Comet) assay. At 3 hours post-injection DNA damage was evident in the liver and, to a lesser extent, in the spleen but absent in both organs at

24 hours. Kidney, lung and bone marrow were negative for DNA damage at all time points. The frequency of apoptotic cells was not recorded (Sasaki *et al.*, 1997).

Hepatocytes derived from male and female rats (strain F344) and female mice (strain $B6C3F_1$) were examined for unscheduled DNA synthesis (UDS) and replicative DNA synthesis after administration by gavage of *p*-DCB (0, 300, 600 or 1,000 mg/kg bw) dissolved in corn oil. The mouse liver and rat kidney were negative for UDS. Hepatocytes from both sexes of mice showed a dose-dependent increase in S-phase cells. Renal tissue from male rats also showed an increase in S-phase cells but not females (Sherman *et al.*, 1998).

Exposure of *Drosophila* to *p*-DCB vapour produced no mutagenic effect in a sex-linked recessive lethal test. Significant mortality of flies occurred from 6000 to 15600 ppm. (Bioassay Systems Corp. 1982; cited in SIAR, 1999).

Metabolites

When calf thymus DNA was incubated in the presence of the *p*-DCB metabolites, 2,5-dichlorohydroquinone (DCHQ) or 2,5-dichlorobenzoquinone (DCBQ), an increase in the oxidation product, 8-oxo-7,8-dihydro-2'-deoxyguanosine, was observed. In addition, DCHQ and DCBQ induced dose-dependent increases in single and double strand DNA breaks. DCHQ required the presence of Cu(II) ions while DCBQ required Cu(II) and NADH for the observed effects to occur. Analysis of DNA fragments showed site specificity for thymine residues. Inhibitor studies indicated that formation of the semiquinone radical reduces molecular oxygen to superoxide which, by dismutation, produces hydrogen peroxide. The observed DNA changes resulted from the combined action of copper and hydrogen peroxide (Oikawa and Kawanishi, 1996).

10.8 Carcinogenicity

10.8.1 Oral exposure

A 2-year study by the National Toxicology Program was conducted in which rats (strain F344) and mice (strain $B6C3F_1$) were administered *p*-DCB by gavage (5 days/week). Male rats received 0, 150 or 300 mg/kg bw per day and female rats and both sexes of mice received 0, 300 or 600 mg/kg bw per day. The survival of female rats and both sexes of mice treated with *p*-DCB was comparable to the control groups while high dose male rats had a significantly lower survival rate (40%). Male and high dose female rats showed a dose-dependent increase in nephropathy. The non-carcinogenic effects are summarised in Section 10.5 Treated male rats showed an increased incidence (not significant and within the range of the laboratory historical controls) of mononuclear cell leukaemia and a dose-dependent increase (statistically significant at 300 mg/kg bw per day, $p = 0.011$ by life table test) in the formation of renal tubular cell adenocarcinoma (controls 1/50; low-dose, 3/50; high-dose, 7/50). One case of tubular cell adenoma was also recorded in a high-dose male rat. An increase in hyperplasia of the parathyroid gland was observed in male rats (control, 4/42; low-dose, 13/42; high-dose 20/38) but not females.

In mice, there was no was no significant difference in survival time between the three dosage groups for either gender. Males and females showed an increase, which was statistically significant in the high-dose animals, in the incidence of hepatocellular carcinoma (males: controls, 14/50; low-dose, 11/49; high-dose, 32/50; females: controls, 5/50; low-dose, 5/48; high-dose, 19/50), hepatocellular adenoma (males: controls, 5/50; low-dose, 13/49; high-dose, 16/50; females: controls, 10/50; low-dose, 6/48; high-dose, 21/50) and hepatocellular carcinoma and adenoma (combined) (males: controls, 17/50; low-dose, 22/49; high-dose, 40/50; females: controls, 15/50; low-dose, 10/48; high-dose 36/50). Four high-dose males developed rare hepatoblastomas although statistical significance was not reached. A high incidence of spontaneous hepatic adenomas and carcinomas were observed in control animals and in historical controls. An increase (not significant) in pheochromocytomas in male mice (controls, 0/47; low-dose, 2/48; high-dose, 4/49) and thyroid gland follicular cell hyperplasia was also evident (NTP, 1987).

An investigation of the hepatocarcinogenic activity of *p*-DCB in male rats (strain F344), when administered by gavage at doses up to 58.8 mg/kg bw per day gave negative results in an initiation/promotion assay (Gustafson *et al.*, 1998).

10.8.2 Inhalation exposure

A study involving the inhalation of *p*-DCB vapour (0, 75 and 500 ppm) for 5 hours/day, 5 days/week for 76 weeks by rats (strain Wistar-derived) and 56 weeks by mice (strain Swiss) found no evidence for increased tumour or multiple tumour formation. However, the study in mice was compromised by the presence of respiratory infections and both studies were of limited duration. See Section 10.5.2 for further details (Loesser and Litchfield, 1983).

In a two-year study to determine the carcinogenicity of *p*-DCB, rats (strain F344) were exposed to the vapour at 0, 20, 75 or 300 ppm 6 hours/day, 5 days/week. The non-carcinogenic treatment effects are summarised in section 10.5.2. No treatment-related tumours were observed (JBRC, 1995).

A two-year study was undertaken to determine the carcinogenicity of *p*-DCB for mice (strain BDF1) exposed to the vapour of *p*-DCB at 0, 20, 75 or 300 ppm 6 hours/day, 5 days/week. An increase in the incidence of hepatocellular carcinoma was observed which was statistically significant at 300 ppm ($p<0.01$) (males: control 12/49, low-dose 17/49, mid-dose 16/50, high-dose 38/49; females: control 2/50, low-dose 4/50, mid-dose 2/49, high-dose 41/50). Females showed an increase in hepatocellular adenomas which became significant at 300 ppm (control 2/50, low-dose 10/50, mid-dose 6/49, high-dose 20/50). A statistically significant ($p<0.05$) increase in hepatic histiocytosarcomas was observed at 300 ppm (control 0/49, 3/49, 1/50, 6/49) only in males with hepatocellular carcinomas. Hepatoblastoma-like tumours were observed in animals with hepatocarcinoma (males: control 0/12, low-dose 2/17, mid-dose 1/16, high-dose 8/38; females: high-dose 6/41). As with the oral study (NTP, 1987) a high spontaneous incidence of hepatic adenomas and carcinomas were observed in control animals, despite this, statistical significance was achieved. High-dose females exhibited a statistically significant increase ($p = 0.0430$) in combined bronchiolar-alveolar adenomas and bronchiolar-alveolar carcinomas

(experimental controls, 1/50; high-dose females, 7/50). However, the combined incidence of bronchiolar-alveolar adenomas and bronchiolar-alveolar carcinomas for historical controls for the laboratory was 7.6% (range 0/50 to 9/50). There were no increases in bronchiolar-alveolar adenomas or bronchiolar-alveolar carcinomas in male mice (JBRC, 1995).

11. Human Health Effects

There are few reports concerning the effects of *p*-DCB on humans and no clinical studies on human volunteers. In cases of occupational and accidental exposure or misuse the extent of exposure is not known and the involvement of other chemicals uncertain.

11.1 Acute toxicity

Only one case report describing the acute effects after *p*-DCB ingestion is reported in the literature. Following ingestion of an unknown quantity of moth crystals containing *p*-DCB, a 3-year-old male was hospitalised with acute haemolytic anaemia. He became listless, jaundiced and his haemoglobin fell to 36%. Urinalysis revealed methemoglobinuria and a compound stated to be 2,5-dichloroquinol (Hallowell, 1959).

11.2 Irritation and sensitisation

Occupational exposure to the vapour of *p*-DCB in the range 50 to 80 ppm was associated with irritation to the eyes and nose and severe irritation apparent in the range 80 to 160 ppm. It was reported that due to respiratory irritant effects workers required the use of a respirator at concentrations above 160 ppm although some workers, who had became acclimated to the effects of *p*-DCB, did not require their use (Hollingsworth *et al*., 1956).

Hollingsworth *et al*. (1956) reported that solid *p*-DCB has a negligible irritating action on intact uncovered human skin but can produce a burning sensation if held in close contact for an excessive period.

A 69-year-old male developed allergic purpura attributed to dermal contact with an armchair that had been treated with *p*-DCB crystals and in which he had sat the same day. The symptoms appeared 24 to 48 hours later. An indirect basophil degranulation test was positive for *p*-DCB. The level and duration of exposure to *p*-DCB were unknown (Nalbandian and Pearce, 1965).

11.3 Repeated exposure

11.3.1 Case reports

There are a number of case reports of long-term occupational or domestic exposure to *p*-DCB. In addition, several cases are known of deliberate long-term ingestion or inhalation of *p*-DCB.

In an older report, five cases of poisoning following exposure to preparations containing *p*-DCB used for moth killing were described (Cotter, 1953). However, no clear cause-effect relationship was established. The cases are as follows:

- A 36-year-old female developed symptoms of periorbital swelling, headache and profuse rhinitis after domestic exposure to a *p*-DCB preparation (purity and duration of exposure not given) for killing moths. The symptoms resolved themselves within 24 hours without treatment.
- A 34-year-old female with a history of demonstrating *p*-DCB preparations (while enclosed in a glass cabinet) for a department store reported a long period of general malaise. After returning to work from a break, she subsequently developed acute symptoms of headache, nausea and vomiting and showed signs of jaundice after renewed handling of *p*-DCB. She was diagnosed with subacute yellow atrophy and cirrhosis of the liver.
- A 60-year-old male domestically exposed to mothball vapour for 3 to 4 months developed a persistent headache, weight loss and irregular bowel movements. In addition, he suffered an unsteady gait and paresthesia of the lower extremities. On admission to hospital he was found to have slurred speech and anaemia; the patient subsequently died. A diagnosis of acute yellow atrophy of the liver was made.
- The wife (age not stated) of the above male also died within a year and following "persistent and severe" exposure to *p*-DCB. She was diagnosed with acute yellow atrophy of the liver, Laennec's cirrhosis and splenomegaly.
- A 52-year-old male who had used *p*-DCB for two years began to suffer weakness and nausea and became jaundiced. He was found to be anaemic and neutropenic. A diagnosis of subacute yellow atrophy of the liver was made.

Other cases include:

- A 19-year-old female consumed 4 to 5 mothballs (composed of *p*-DCB, purity unknown) daily for 2.5 years which resulted in a fixed drug eruption characterised by symmetrical, well demarcated areas of increased skin pigmentation ranging from 3 to 7 cm in diameter. The symptoms disappeared within 4 months once consumption of the mothballs ceased (Frank and Cohen, 1961).
- Two cases of acute myeloblastic lymphoid leukaemia and a chronic lymphoid leukaemia, related to the use of a cleaning fluid containing *p*-DCB were described (Girard, 1969). A cause-effect relationship could not be demonstrated due to a lack of information on the duration and level of exposure and to the presence of other substances in the product and lack of information on exposure to other chemicals.
- A 21-year-old pregnant female consumed 1 to 2 toilet air-freshener blocks (predominantly *p*-DCB) per week throughout her pregnancy. The patient developed hypochromic, microcytic anaemia with polychromasia and marginal nuclear hypersegmentation of the neutrophils. Liver function and urinalysis

findings were normal. A complete recovery was made upon removal of the chemical from her diet. The neonate was reported to display no abnormalities (Cambell and Davidson, 1970).

- A 68-year-old female was admitted to hospital following an epistaxis episode and the presence of ecchymoses and petechiae. On examination, she complained of recent tiredness, asthenia, dyspnea and palpitations on exertion, oedema of the lower limbs and a nasal discharge. Haematological examination revealed aplastic anaemia with a haematocrit of 22%. Prior to her admission, the woman had been working at a clothing shop and had spent the previous three weeks in a poorly ventilated room packing clothing. She had handled 5.5 kg of *p*-DCB and 7 kg naphthalene during that time; the average outdoor temperature was 27°C (Harden and Baetjer, 1978). The role of *p*-DCB in this case is not clear due to the confounding presence of naphthalene.
- A 25-year-old female presented with symptoms of severe cerebral ataxia, dysarthria, weakness of the limbs and hyporeflexia. A comprehensive clinical examination produced negative results with the exception of delayed brainstem auditory evoked potentials. The woman had been exposed to *p*-DCB for approximately 6 years due to a phobia relating to ticks, which resulted in excessive household use of mothballs including grinding the mothballs to powder and spreading it amongst her bed clothes and clothing. The symptoms disappeared within 6 months after her exposure to *p*-DCB ended (Miyai *et al.*, 1988).
- A 16-year-old female who regularly inhaled *p*-DCB vapour from a toilet bowl for several months developed encephalopathy with bilateral reduction of visual acuity, ataxia, behavioural disturbances, asthenia, impaired memory, apathy and sleepiness. Haematological symptoms included anaemia. After discontinuing the practice of inhaling *p*-DCB vapour the symptoms disappeared within 6 months (Reygagne *et al.*, 1992).

11.4 Epidemiological studies

No well-controlled epidemiological studies have been conducted. Confounding factors in epidemiological studies of chemical exposure are concomitant exposure to other agents and inadequate details of exposure conditions, previous or existing medical conditions, medication or substance abuse.

Hollingsworth *et al.*, (1956) conducted a study of 58 men employed in a plant producing *p*-DCB continuously or intermittently for 8 hours/day, 5 days/week. The average work time at the plant was 4.75 years (range 0.7 to 25 years). During an initial survey, the average airborne *p*-DCB concentration was 85 ppm (range of 10 to 550 ppm). A second, later survey of the same plant found two sets of working conditions: those uncomfortable to employees (due to irritation of the eyes and nose) with an average concentration of 380 ppm (range 100 to 725 ppm) and tolerable conditions with an average of 90 ppm (range 5 to 275 ppm). A third survey was conducted after extensive plant modifications. Areas where there were continued

complaints from employees gave average *p*-DCB concentrations of 105 ppm (range 50 to 170 ppm) while other areas of the plant which received no complaints recorded an average of 45 ppm (range 15 to 85 ppm). Medical examination of the employees, including a comprehensive haematological examination, found no evidence that could be attributed to a *p*-DCB-related effect on health other than eye and nose irritation.

Nine males with a mean age of 54.1 years (range 32 to 66 years) and a mean working time of 24.1 years (range 13 to 35 years) in a factory using chlorobenzenes (*mono*-, *para*- and *ortho*-dichlorobenzene) were identified as having chloracne based on the presence of polymorphic dermatosis, predominately comedones and cysts. All patients had conjunctivitis and reported gastrointestinal complaints including nausea with occasional vomiting and as having paresthesia of the lower extremities. Five of the workers had developed a diffuse melanotic discolouration and four developed hyperpigmentation of the face. Liver function tests were abnormal and radiology indicated enlargement of the liver. The symptoms described were present for at least two years after leaving the company. Analysis of water from the work site gave 15 ppm of chloracne inducing substances. Analysis of air samples were recorded as being inconclusive (Vazquez *et al*., 1996). Due to the inadequacy of the data on exposure levels and the absence of data relating to other work related compounds or other substances including medication, a causal relationship cannot be demonstrated.

12. Health Hazard Assessment and Classification

The purpose of this section is to evaluate the physicochemical data, kinetic and metabolism studies, human and animal experimental studies (including *in vivo* and *in vitro* data) in order to determine the potential hazard to human health that exposure to *p*-DCB might entail.

Workplace substances are classified as 'hazardous' to health if they meet the NOHSC *Approved Criteria for Classifying Hazardous Substances* (the Approved Criteria) [NOHSC:1008(1999a)], and 'dangerous' in terms of physicochemical hazards, if they satisfy the criteria of the *Australian Code for the Transport of Dangerous Goods by Road and Rail* (ADG Code) (Federal Office of Road Safety, 1998).

The classifications for *p*-DCB are incorporated in the following assessment of physicochemical and health hazards.

12.1 Physicochemical hazards

p-DCB is a white crystalline solid which undergoes sublimation (vapour pressure 1.35 hPa at 25^{o}C) resulting in dissipation of the solid over time. The melting point is 53.5^{o}C and the flash point (closed crucible) is 65^{o}C. The flammability limit is 2.5%.

Classification status:

p-DCB is not classified under the ADG Code.

12.2 Kinetics, metabolism and mechanisms

Absorption of *p*-DCB is rapid by inhalation and the oral route in animals. Dermal absorption is considered to be low based on acute dermal and repeated dose toxicity studies. In animals, *p*-DCB is distributed to all tissues although principally in adipose tissue, the kidneys and liver. Due to the lipophilic nature of *p*-DCB it is likely that the major mechanism for the transport of this compound across cellular membranes is by passive diffusion. The biological residence time is short with almost complete excretion of the metabolised compound occurring in the urine by 24 to 48 hours. Minor amounts are lost by the faeces and breath. There appears to be considerable enterohepatic circulation of *p*-DCB and its metabolites in the rat.

In vitro studies indicate that *p*-DCB is metabolised by hepatic cytochrome P450 enzymes in animals and humans. A substantial number of metabolic studies, including comparative studies with mice, rats and humans, have been undertaken. In all mammal species thus far examined, the metabolism of *p*-DCB proceeds by hydroxylation of the aromatic ring resulting in the formation of an intermediate epoxide. The subsequent metabolic fate of the epoxide is species-dependent. The

epoxide may form a conjugate with glutathione, undergo catalysis by epoxide hydrolase to yield a dichlorophenol or dihydrodiol, or undergo secondary metabolism to form a hydroquinone derivative. In the rat and mouse the major metabolites are dichlorophenols, dichlorohydroquinones and glutathione-epoxide and glutathione-quinone conjugates. Covalent binding to the tissues of the liver and kidneys in the rat and mouse has been reported and is linked to the formation of reactive epoxides and benzoquinones, the latter being derived from oxidation of hydroquinones.

In contrast to the rat and mouse, human metabolism of *p*-DCB, *in vitro*, results in a non-reactive epoxide which is converted to one major metabolite, 2,5-dichlorophenol. In humans, excreted metabolites consist predominately of 2,5-dichlorophenol and its conjugated sulfate and glucuronide derivatives.

The metabolism of *p*-DCB by rat, mouse and human microsomes and proposed toxic effects are summarised in Figure 3.

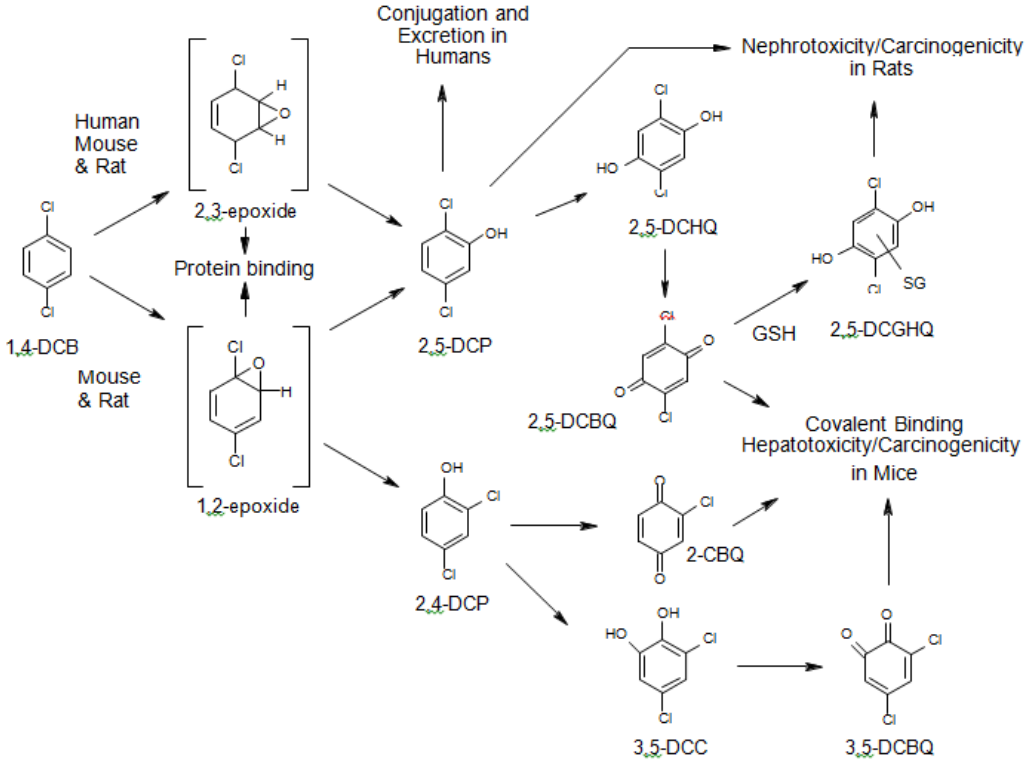


Figure 3 - Metabolism of 1,4-dichlorobenzene (*p*-DCB) by rat, mouse and human hepatic microsomes. Pathways for the formation of bioactive metabolites in rats, mice and humans are shown. CBQ, chlorobenzoquinone; DCB, dichlorobenzene; DCBQ, dichlorobenzoquinone; DCC, dichlorocatechol; DCGHQ, dichlorogluthionyl-hydroquinone; DCHQ, dichlorohydroquinone; DCP, dichlorophenol; DCBQ, dichloro-1,2-benzoquinone; GSH, reduced glutathione; SG, glutathione-*S*-yl-metabolite (After Den Besten *et al.*, 1992; Klos and Dekant, 1994 and Hissink *et al.*, 1997).

12.3 Health hazards

12.3.1 Acute effects

Animal studies with rats and mice have shown *p*-DCB to induce reversible, acute nephrotoxic and hepatotoxic effects. In acute studies the oral LD_{50} for the rat was in the range 3790 to 3863 mg/kg bw. The LD_{50} for the rat due to dermal exposure was >6000 mg/kg bw. The LC_{50} in rats by inhalation is given as >5.07 mg/L/4 hours (845 ppm).

Only one case report of acute toxicity has been described involving a 3-year old male who ingested an unknown quantity of *p*-DCB, which resulted in haemolytic anaemia with methemoglobinuria and jaundice.

Classification status:

p-DCB does *not* meet the Approved Criteria (NOHSC, 1999a) for *acute lethal effects* by oral, inhalation or dermal exposure.

p-DCB does *not* meet the Approved Criteria (NOHSC, 1999a) for *non-lethal (irreversible) effects after a single exposure*.

12.3.2 Irritant effects

Skin irritation

Few data are available from animal studies addressing the irritant potential of *p*-DCB. Hollingsworth *et al*. (1956) reported that solid *p*-DCB has a negligible irritating action on intact uncovered human skin but can produce a burning sensation if held in close contact for an excessive period. Maertins (1988, unpublished) indicated that *p*-DCB was slightly irritating to rabbit skin producing erythema but no oedema. A case study of a woman exposed to *p*-DCB crystals and powder for approximately 6 years as a result of a neurosis showed no sign of skin irritation. Despite the widespread use of *p*-DCB over many years there are no convincing reports of skin irritation effects in the literature.

Classification status:

p-DCB does *not* meet the Approved Criteria (NOHSC, 1999a) for *skin irritation*.

Although *p*-DCB does not meet the criteria for classification as a skin irritant, due to concerns that prolonged exposure may produce irritation, the notation 'Avoid contact with skin (S24)' should be retained.

Eye irritation

Hollingsworth (1956) reported that occupational exposure to *p*-DCB vapour (above 50 ppm; 300 mg/m^3) led to increased complaints of eye irritation which was described as painful at concentrations greater than 80 ppm ($481mg/m^3$). It was further reported that rabbits, rats and guinea pigs experienced eye irritation when exposed to

p-DCB vapour at 798 ppm. In a case study, a woman was reported to develop periorbital oedema after domestic use of *p*-DCB (Cotter, 1953).

Maertins (1988, unpublished) reported that rabbits (3 only) appeared to experience only slight ocular irritation, characterised by slight erythema and oedema of the conjunctivae which was reversible by the third day.

The Hollingsworth (1956) study is insufficiently detailed with respect to the nature of the eye irritation for purposes of classification. Similarly, the only animal study is inadequate for the purpose of classification.

Classification status:

Insufficient data exists to classify *p*-DCB for *eye irritation* (R36). However, based on limited human data, sufficient evidence exists for precaution to be exercised in the use of *p*-DCB under industrial conditions and for which the following safety phrases are recommended: *Avoid contact with eyes* (S25) and *Use only in well ventilated areas* (S51).

Respiratory irritation

Hollingsworth (1956) reported that occupational exposure to *p*-DCB vapour (above 50 ppm; 300 mg/m^3) has been reported to cause irritation to the nose and upper respiratory tract. Irritation to the nose was reported as painful at concentrations of 80 to 160 ppm (481 to 962 mg/m^3) while higher concentrations were irrespirable. The development of profuse rhinitis after *p*-DCB exposure was reported in one case study although the level of exposure was not known (Cotter, 1953). Another case study reported a woman experiencing an epistaxis episode and dyspnea due to persistent exposure to *p*-DCB, however, the presence of naphthalene was a confounding factor (Harden and Baetjer, 1978).

The effects of *p*-DCB exposure on animals during a 2-year inhalation study included histopathological changes to the olfactory epithelium (eosinophilic changes) in male rats at 300 ppm (1803 mg/m^3) and female rats at 75 ppm (451 mg/m^3) and 300 ppm (1803 mg/m^3). Female rats were also observed to develop eosinophilic changes to the respiratory epithelium and metaplasia of the nasal cavity gland at 300 ppm (1803 mg/m^3). Under identical conditions, mice did not develop any upper respiratory tract lesions (JBRC, 1995). In a study of 16 days duration at an exposure level of 173 ppm (1040mg/m^3) with rats, guinea pigs and rabbits, slight pulmonary interstitial oedema and congestion were observed in all male rats and female guinea pigs and rabbits with alveolar haemorrhage and oedema observed in some animals (Hollingsworth *et al.*, 1956).

The case reports (Cotter, 1953) are insufficiently detailed for purposes of classification as the vapour concentrations were not available and potential confounding factors, such as allergy or co-existing infections, were not discussed. Other cases of *p*-DCB exposure reported by Cotter (1953) and others do not include adverse respiratory symptoms. Similarly, the Hollingsworth (1956) study is considered inadequate as the use of other chemicals was not described nor were co-existing medical conditions of the workers. The animal studies do not provide

sufficient evidence for upper respiratory tract irritation. While female rats developed marked changes to the olfactory epithelium at 75 ppm (451 mg/m^3) the control animals displayed a high incidence (98%) of slight to moderate olfactory lesions. Such lesions were not observed in mice.

Classification status:

Insufficient data exists to classify *p*-DCB for *irritation to the respiratory system* (R37). However, based on limited human data, sufficient evidence exists for precaution to be exercised in the use of *p*-DCB under industrial conditions and for which the following safety phrases are recommended: *Do not breath vapour* (S23) and *Use only in well ventilated areas* (S51).

12.3.3 Sensitisation

Few studies have been conducted addressing the issue of sensitisation as a result of exposure to *p*-DCB. A case of acute petechial purpura attributed to *p*-DCB has been reported although insufficient details were provided by the study to establish a causal relationship (Nalbandian and Pearce, 1965). Extensive industrial experience with *p*-DCB has not resulted in reports of sensitisation.

A study of guinea pigs indicated that exposure by dermal contact causes weak sensitisation (Bornatowicz *et al*., 1995, cited in SIAR 1999).

Classification status:

p-DCB does *not* meet the Approved Criteria (NOHSC, 1999a) for *sensitising effects (skin or inhalation)*.

12.3.4 Severe effects (non-carcinogenic) after repeated or prolonged exposure

Cases involving prolonged human exposure to *p*-DCB have indicated the development of neurological symptoms all of which appear to be reversible following cessation of exposure. Haematological disorders have also been noted, particularly anaemia. There have been two deaths (related individuals) attributed to *p*-DCB exposure, however, other factors which may have contributed to mortality were not discussed in the case report. Adverse liver effects have been noted in some case studies. In all case reports of repeated exposure the cause-effect relationship with respect to *p*-DCB has not been clearly established. Repeated ingestion of *p*-DCB or inhalation of its vapour, due to intentional misuse, may result in drowsiness, incoordination and anaemia.

Several repeated dose studies, of up to 2 years duration, via inhalation and oral routes have been conducted in mice and rats. A 1-year oral study in dogs has also been conducted. Only 1 dermal study, of 3 weeks duration, has been reported.

Prolonged exposure of animals to *p*-DCB results in neurological disturbances including tremor, unsteady gait and an unkempt appearance. Severe effects include increases in liver and kidney weights and the development of hepatic lesions, an increase in hepatocellular proliferation in rats and mice and death at high doses. The

most relevant studies are the 2-year inhalation and oral studies in mice and rats and the NOAELs and critical effects are described below.

A NOAEL in male rats by the oral route was not identified and for females was 300 mg/kg. A LOAEL was 75 mg/kg bw for male rats for nephropathy and 600 mg/kg bw for females for nephropathy and increased liver weights (NTP, 1987; Bomhard, 1998; Eldridge *et al.*, 1992).

A NOAEL by inhalation for the rat (both sexes) was 75 ppm (451 mg/m^3 or 0.451 mg/L) and a LOAEL of 300 ppm (1803 mg/m^3 or 1.803 mg/L) identified for nephropathy in males and hepatotoxicity in both sexes.

In the mouse, by the oral route, a NOAEL was not identified and a LOAEL of 300 mg/kg bw for both sexes was determined based on hepatocellular degeneration and increased kidney weight. By inhalation, a NOAEL of 75 ppm (451 mg/m^3 or 0.451 mg/L) was determined for both sexes of mice and a LOAEL of 300 ppm for hepatotoxicity and increased kidney weights.

For dogs exposed to *p*-DCB by the oral route a NOAEL of 10 mg/kg bw was determined and a LOAEL of 50 mg/kg bw for hepatic toxicity and increased kidney weights with some epithelial cell degeneration.

Classification status:

p-DCB does *not* meet the Approved Criteria (NOHSC, 1999a) for *severe effects after repeated/prolonged exposure*.

12.3.5 Reproductive effects

There have been no reports of reproductive effects in humans described in the literature. A neonate delivered to a woman who regularly ate toilet air-freshener blocks composed of *p*-DCB throughout her pregnancy showed no developmental problems or other adverse effects.

The effects of *p*-DCB on reproductive and developmental toxicity have been studied in rats and rabbits. In oral and inhalation 2-generation reproductive studies no fertility effects were observed at or below exposure levels which did not induce maternal toxicity. Slight developmental effects (reversible reduced mean body weight at birth in F_0/F_1 pups, increased number of deceased pups in 1 time period in 1 generation and slight behavioural anomalies in 1 generation) were observed in rats in a 2-generation oral reproductive study. The effects were observed at 90 mg/kg bw per day. These slight developmental effects occurred at doses where no parental toxicity was evident (NOAEL maternal toxicity was 90 mg/kg bw per day). However, similar effects were not observed in a 2-generation inhalation study or in two well-conducted developmental studies (oral and inhalation).

Classification status:

p-DCB does *not* meet the Approved Criteria (NOHSC, 1999a) for *reproductive effects*.

12.3.6 Genotoxicity

Several test systems including the Ames test (with or without metabolic activation), gene mutation, chromosomal aberration and DNA effect assays have given negative results for *p*-DCB.

In vitro, unscheduled DNA synthesis tests with human lymphocytes have given negative results while a mouse lymphoma forward mutation assay proved to be inconclusive. Positive results have been obtained for human lymphocytes showing an increase in the sister-chromatid exchange rate.

Under *in vivo* conditions using several test methods, including dominant lethal, micronucleus and unscheduled DNA synthesis assays, *p*-DCB was found to give negative genotoxic responses. Using the alkaline Comet assay, damage to DNA occurred in the liver and spleen, but not other tissues tested, of male mice exposed to *p*-DCB while a sex-linked recessive lethal test with *Drosophila* produced negative results.

Classification status:

From the results of *in vitro* and *in vivo* studies, *p*-DCB does *not* meet the Approved Criteria (NOHSC, 1999a) for *mutagenic effects*.

12.3.7 Carcinogenicity

Investigations of the carcinogenic potential of *p*-DCB are limited to two well-conducted 2-year studies in rats and mice, the US NTP (1987) oral study and the JBRC (1995) inhalation study. An older inhalation study with a duration of 76 weeks for rats and 56 weeks for mice was also conducted (Loeser and Litchfield, 1983), however, this study is of limited value due to its short duration.

The effects of oral exposure to *p*-DCB over 2 years were characterised by the development of renal tumours in male rats (LOAEL 150 mg/kg bw per day) and hepatic tumours in both sexes of mice (LOAEL 600 mg/kg bw per day; NOAEL 300 mg/kg bw per day). The inhalation studies showed no evidence of treatment-related neoplasia in rats of either gender while both sexes of mice developed hepatic tumours (LOAEL 300 ppm) in the 2-year study.

Available evidence from *in vitro* and *in vivo* genotoxicity testing indicate that *p*-DCB itself does not appear to induce DNA alterations and, consequently, separate epigenetic mechanisms have been proposed to account for the observed sex- and species-dependent renal and hepatic tumours.

Renal carcinogenesis

Exposure of mature male rats to oral doses of *p*-DCB results in the development of nephrotoxicity and ultimately renal tumour formation (adenocarcinoma). Extensive investigations of the mechanisms involved in renal carcinogenesis have revealed two apparently separate pathologies; a proximal tubular necrosis due to hyaline droplet formation and a second nephropathy associated with reactive metabolites of *p*-DCB.

Role of $\alpha_{2\mu}$-Globulin in renal carcinogenesis

Several studies in which *p*-DCB was administered orally to rats resulted in a male-specific pathology characterised by the formation of hyaline droplets in proximal tubule epithelial cells (NTP, 1987; Bomhard, 1989). Early studies of the effects of *p*-DCB on male rat kidneys considered the development of hyaline droplet nephropathy to be the prelude to the formation of kidney tumours. It has been proposed that *p*-DCB forms a reversible complex with the male rat-specific protein, $\alpha_{2\mu}$-globulin. In untreated rats, $\alpha_{2\mu}$-globulin is catabolised by lysosomal proteases thus preventing its accumulation. In *p*-DCB-treated rats the modified protein appears to be resistant to degradation and accumulates as a precipitate within phagolysosomes of the proximal convoluted tubule epithelial cells thus producing the hyaline droplet pathology (Charbonneau et al., 1989). Subsequent studies, *in vitro*, have demonstrated that the *p*-DCB metabolite, 2,5-dichlorophenol, is responsible for inhibiting the degradation of $\alpha_{2\mu}$-globulin and not the parent compound (Lehman-McKeeman et al., 1990). The role of modified $\alpha_{2\mu}$-globulin in hyaline droplet nephropathy is supported by the observation that immature male rats, which do not produce significant amounts of $\alpha_{2\mu}$-globulin, do not develop renal lesions when exposed to chemicals which induce hyaline droplet formation. Similarly, old male rats which are deficient in the protein failed to develop renal lesions in response to chemical induction (reviewed in Melnick, 1992; Hard et al., 1993).

The presence of *p*-DCB-induced intracellular hyaline droplets ultimately induce cell death and a proliferative response in adjacent cells which subsequently leads to the development of a low incidence of renal tubular cell tumours in male rats (Charbonneau et al., 1989). Further studies of cell proliferation in response to *p*-DCB exposure have been undertaken in male and female rats and mice using immunohistochemical techniques. Increased cell proliferation was observed in the proximal convoluted tubules and to a lesser extent the proximal straight tubules but not distal tubules of treated male rats. Female rats or mice of either sex did not exhibit increased cell proliferation of the renal tubules (Umemura et al., 1992). However, in a 2-year oral study by Dominick et al. (1991), it was demonstrated that a direct cause-and-effect relationship between chemically induced $\alpha_{2\mu}$-globulin-dependent hyaline droplet formation and renal carcinogenesis could not be established. In support of this, exposure of male rats to *p*-DCB by inhalation for 76 weeks did not result in renal carcinogenesis (Loeser and Litchfield, 1983) despite several studies demonstrating the presence of hyaline droplets after short term *p*-DCB exposure (Bomhard et al., 1988; Charbonneau et al., 1989; Den Besten et al., 1991). It has been suggested that the failure of male rats to develop renal tumours during the inhalation studies was due to differences in tissue concentrations of *p*-DCB as they were not comparable to concentrations achieved by oral administration of the compound (Barter et al., 1999). Thus a threshold effect may be required for the development of *p*-DCB-induced renal carcinogenesis in male rats.

The relevance of $\alpha_{2\mu}$-globulin-dependent hyaline droplet formation as a potential cause of human carcinogenesis has been extensively examined. Synthesis of $\alpha_{2\mu}$-globulin occurs only in the liver of the mature male rat under hormonal, particularly androgenic, control. The protein is filtered from the blood by the kidneys with

approximately 60% being resorbed and the remainder excreted in the urine. The physiological function of $\alpha_{2\mu}$-globulin in the rat remains speculative although it has been postulated that the protein may function as a carrier for lipophilic ligands, possibly pheromones. The $\alpha_{2\mu}$-globulin protein has been sequenced and belongs to a superfamily of low molecular weight proteins, the lipocalin family. With the exception of the mouse major urinary proteins, the sequence homology of $\alpha_{2\mu}$-globulin with other members of the superfamily is low and *in vitro* studies have shown that their affinities for various ligands appear to be unrelated (Cavaggioni et al., 1990). It has been proposed that $\alpha_{2\mu}$-globulin can transport *p*-DCB (or its metabolites) to the kidneys where reactive metabolites form due to renal metabolism with the consequent development of nephrotoxicity and carcinogenesis in addition to hyaline droplet formation (Melnick, 1992). Support for this hypothesis has been provided by Charbonneau et al. (1989) who have shown that, after adipose tissue, the male rat kidney accumulated the highest amount of radiolabel after oral dosing with *p*-DCB. It was further shown that male rat kidneys acquire more radiolabel that female kidneys (Umemura, 1990).

The mechanism of $\alpha_{2\mu}$-globulin-mediated nephropathy and carcinogenesis has been generally adopted by the scientific community as not predictive of carcinogenic risk in humans and in particular for *p*-DCB (Rice et al., 1999).

Metabolite-induced nephropathy

A second renal pathology is associated with reactive metabolites of *p*-DCB. In the rat liver, *p*-DCB is metabolised to a number of compounds including 2,5-dichloro-1,4-benzoquinone, that can react with glutathione to give 2,5-dichloro-3-(glutathion-*S*-yl)-1,4-benzoquinone (DCGBQ). The administration of DCGBQ to male rats by i.v. injection resulted in the development of a dose-dependent renal proximal tubular necrosis. Histopathological examination of the kidneys demonstrated extensive single cell necrosis (with pyknotic nuclei) of the proximal tubules, however, there was no evidence of hyaline droplet formation. Co-administration of ascorbic acid to reduce the DCGBQ to the corresponding hydroquinone enhanced the nephrotoxic effect. These findings suggest that DCGBQ and its parent hydroquinone derived from the hepatic metabolism of *p*-DCB can contribute to the early onset of nephrotoxicity in the rat (Mertens *et al.*, 1991). In *p*-DCB-treated rats this condition is likely to be independent of, although concurrent with, the above-described hyaline droplet nephropathy and supports the hypothesis of Melnick (1992). At present, it is not known if DCGBQ binds to $\alpha_{2\mu}$-globulin and, if so, the extent of renal accumulation of the compound. Similarly, the relationship between DCGBQ-induced nephropathy and renal carcinogenesis is uncertain. However, this mechanism is considered not to be relevant to humans as the metabolites involved are not produced to the same extent during human metabolism of *p*-DCB.

Hepatocarcinogenesis

Exposure of rats and mice to *p*-DCB resulted in the development of hepatotoxicity in both species and hepatocarcinogenicity in mice. Rats exhibited a reversible increase in liver weight but no increase in serum levels of hepatic enzymes while histological findings revealed mild centrilobular hypertrophy. Mice, when exposed to *p*-DCB by

the oral route, exhibited non-neoplastic hepatic lesions characterised by cytomegaly, karyomegaly, hepatocellular degeneration and individual cell necrosis (NTP 1987) while exposure via the inhalation route resulted in centrilobular hepatocellular hypertrophy in high-dose males (JBRC 1995). Neoplastic lesions consisted of hepatocellular adenoma and carcinoma and a limited number of rare hepatoblastomas. Of significance was the high spontaneous incidence of hepatocellular adenoma and carcinoma in control mice and in historical control animals for the two testing laboratories suggesting the presence of pre-neoplastic cell populations in the animals involved. The experimental data for the NTP testing laboratory (NTP, 1987) were: male controls (adenoma, 15.0%; carcinoma, 37.5%; combined adenoma and carcinoma, 43.4%) and female controls (adenoma, 27.4%; carcinoma, 13.2%; combined adenoma and carcinoma, 39.0%). The experimental data for the JBRC testing laboratory (JBRC, 1995) were: male controls (adenoma 28.9%; carcinoma, 25.6%; combined adenoma and carcinoma, 43.6%) and female controls (adenoma, 4.8%; carcinoma, 7.1%; combined adenoma and carcinoma,10.7%). The $B6C3F_1$ mouse is considered to be a sensitive strain based on its tumour response to chemical testing (Tennant *et al.*, 1986; Gold *et al.*, 1989). Less data are available for the Crj:BDF_1 mouse, however, as this strain is genetically similar to the $B6C3F_1$ mouse, in that they share the same maternal lineage, it is expected that a similar sensitivity towards chemicals would be observed.

A number of studies have failed to demonstrate, either *in vitro* or *in vivo*, a genotoxic mechanism by which *p*-DCB could induce a carcinogenic response, consequently, alternative mechanisms must be evaluated. It has been shown, by several *in vivo* studies, that *p*-DCB is an inducer of hepatocellular proliferation and that the response is mitogenic in nature. Eldridge *et al.*, (1992) observed that *p*-DCB produced a mitogenic response in rats and mice characterised by an increase in hepatocellular proliferation and which occurred in the absence of hepatocellular necrosis. Other studies have concluded that *p*-DCB-induced cell proliferation is not sufficient to induce hepatic carcinogenesis as rats showed increased hepatocellular proliferation without developing tumours in long-term studies and that the cumulative replicative fraction of rat hepatocytes is significantly greater than that of mouse hepatocytes (Umemura *et al.*, 1992). These data suggest that, in order to maintain hepatic homeostasis, a higher rate of hepatocyte apoptosis would be required in the rat. James *et al.* (1998) have confirmed that the basal apoptotic rate of rat hepatocytes is substantially higher than that of their murine counterparts. It was further demonstrated that *p*-DCB suppresses the apoptotic response in both species, *in vitro* and *in vivo*. The proliferative response in the rat liver was shown to be proceeded by the expression of the immediate-early genes c-*fos*, c-*jun* and c-*myc* and the localisation of c-Myc protein with areas of proliferation was demonstrated (Hasmall *et al.*, 1997). The products of these proto-oncogenes (c-Fos and c-Jun) form a heterodimer, activator protein-1 (AP-1), which recognises a DNA specific sequence motif, the AP-1 binding site. AP-1-induced gene transcription is associated with increased cell proliferation (Chiu *et al.*, 1988). Umemura *et al.* (1998) established a threshold effect for *p*-DCB-induced cell proliferation (75 mg/kg bw for rats and 150 mg/kg bw for mice) below which a proliferative response was not observed. It was further demonstrated that, above the threshold dose, the proliferative response in the rat liver was transient whereas the response in the mouse liver, while attenuated, was

prolonged. A prolonged response is considered to be predictive of carcinogenesis (Melnick and Huff 1993). However, the lack of concordance in the responses between species and between doses of *p*-DCB which induce cell proliferation and carcinogenesis suggest that additional factors are involved in the development of hepatic tumours in mice.

Several lines of evidence indicate that the differences in species-related hepatotoxicity of *p*-DCB would appear to be due to differences in the metabolism of the compound by hepatic cytochromes P450. Lake *et al*. (1997) observed, *in vivo*, a marked induction of CYP2B1/2 in both the rat and the mouse in response to *p*-DCB exposure. CYP2E1 is also involved in the metabolism of *p*-DCB and results in the formation of the 2,3-epoxide whereas metabolism by CYP2B1/2 produces the 1,2-epoxide. Subsequent metabolism of the 1,2-epoxide results in the formation of mono- and dichlorohydroquinones (Klos and Dekant 1994). Studies with human cell lines transfected with cDNA expressing specific cytochrome P450 isoforms revealed that only CYP2E1 participated in the metabolism of *p*-DCB (Hissink *et al*., 1997b).

Hissink *et al*. (1997b) demonstrated the existence of substantial interspecies differences between mice, rats and humans with respect to the hepatic microsomal metabolism of *p*-DCB. The rank order for hepatic microsomal metabolism was determined to be (as a percentage of total conversion): mice (16%) >> rats (0.6% to 1.3%) > humans (0.3%). Covalent binding of *p*-DCB metabolites to microsomal protein was demonstrated to have the following rank order (as a percentage of total conversion): mice (21%) > rats (10%) > humans (6%). In all cases, the addition of ascorbic acid reduced microsomal covalent binding indicating that benzoquinone species, derived from hydroquinones, rather than epoxides are primarily involved. For mice, the ascorbate-dependent reduction was 92% compared to 25% for humans from which it may be concluded that quinone/protein binding is not extensive for human microsomes. Addition of glutathione to microsomal preparations and analysis for glutathione-epoxide conjugates resulted in undetectable levels from mouse preparations, a 6% increase from human preparations and a significant increase (range 40 to 52%) for all rat strains.

It has been established that the metabolism of *p*-DCB in mice proceeds by the action of CYP2E1 and CYP2B1/2 with substantial hydroquinone formation whereas with humans, metabolism proceeds by the action of CYP2E1 only with relatively minor amounts of hydroquinones produced. As humans do not express CYP2B1/2, the 1,2-epoxide and subsequent hydroquinones are not produced in the human liver. Total hydroquinone formation (as a percentage of total hepatic microsomal metabolites and in the presence of ascorbate as reductant) was 8.86% for mice while rat and human microsomal metabolism resulted in a maximum conversion of 0.4% and 0.08% respectively.

A number of studies have provided evidence implicating redox cycling of hydroquinone species, derived from *p*-DCB, in murine hepatocarcinogenesis. Lattanzi *et al*. (1989) demonstrated, *in vivo*, the binding of radiolabeled metabolites of *p*-DCB to the DNA of mouse liver but found no evidence for binding to rat liver DNA. Sasaki *et al*. (1997) also showed, *in vivo*, lesions to DNA derived from the liver and spleen of mice treated with *p*-DCB but no damage to the DNA of other

organs. Under *in vitro* conditions, 2,5-dichlorohydroquinone (2,5-DCHQ) induced single and double strand breaks in DNA and DNA base alterations including the formation 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) when incubated with native DNA. The effect was enhanced when 2,5-DCHQ was able to redox cycle in the presence of the intracellular reductant, nicotinamide adenine dinucleotide (NADH) (Oikawa and Kawanishi, 1996). Inhibitor studies revealed a direct role for reactive oxygen species (superoxide and hydrogen peroxide) in 2,5-DCHQ-mediated DNA damage. The formation of DNA lesions were completely prevented when catalase, a scavenger of hydrogen peroxide, was present. The hydrogen peroxide arises by dismutation of superoxide which is produced by the autoxidation of hydroquinone species. Oxidative modification of DNA bases, particularly the 8-oxodG base alteration, has been shown to result in DNA misreplication (Shibutani, *et al.*, 1991) and has been implicated in mutations leading to cancer formation (Floyd 1990). It is recognised that hydroquinone species and hydrogen peroxide can act as tumour promoters. Tumour promotion can arise from the ability of these substances to alter intracellular redox status, mediate immediate-early gene expression, disrupt intercellular gap junction communication and promote cell proliferation (Gopalakrishna *et al.*, 1994; Joseph *et al.*, 1998; Huang *et al.*, 1999).

While the modulation of cellular function by hydroquinone species provides a plausible mechanism for the increased numbers of hepatic tumours observed in long term carcinogenicity studies of mice after exposure to *p*-DCB, the mechanism, at present, is not generally accepted by the scientific community.

Classification

As the rat renal tumours are not considered relevant to humans the only evidence for carcinogenicity is the occurrence of liver tumours in mice.

The Approved Criteria (NOHSC, 1999a) for classifying carcinogenic effects are provided in full in Appendix 2.

It is clear from the available data that *p*-DCB does not fall into either Category 1 or 2 of the criteria. In considering whether *p*-DCB should be classified in Category 3 or 'no classification' based on increased liver tumour formation in mice, the following were taken into consideration:

- there was no evidence of liver tumours of any type from the rat studies;
- increases in hepatic adenoma and carcinoma formation in male and female mice occurred only at doses above or close to the maximum tolerated dose;
- there was no evidence for a genotoxic mechanism;
- the strains of mice used ($B6C3F_1$ and $Crj:BDF_1$) exhibited a high spontaneous incidence of hepatocellular adenoma and carcinoma;
- the $B6C3F_1$ and $Crj:BDF_1$ mice are considered to be a sensitive strains based on their tumour responses to chemical testing; and
- there are substantial differences in the hepatic metabolism of *p*-DCB by mice in comparison to rats and humans.

Classification status:

p-DCB does *not* meet the Approved Criteria (NOHSC, 1999a) for *carcinogenicity*.

Review of carcinogenicity by other countries/agencies

The renal tumours induced by *p*-DCB in male rats are considered by IARC not to be predictive of a human carcinogenic hazard due to the role of $\alpha_{2\mu}$-globulin in the formation of these tumour. However, on the bases of the mouse liver tumours, for which a satisfactory mechanism of tumour formation is considered lacking, IARC has classified *p*-DCB as Group 2B: possibly carcinogenic to humans on the basis of IARC criteria (Rice *et al.*, 1999).

The SIAR (1999) concluded that the available data do not justify the classification of *p*-DCB as a carcinogen (Category 3) on the assumption that the liver tumours were probably not relevant to humans and that they were likely to occur as a consequence of chronic liver damage, despite the hypothesis not being clearly demonstrated. Based on the conclusions of the SIAR, France recommended to the European Commission Working Group on the Classification and Labelling of Dangerous Substances (October, 1998) that classification of *p*-DCB as a carcinogen was not warranted. This was on the basis that the rat kidney and mouse liver tumours induced by *p*-DCB were species-specific and the mechanisms not relevant for humans. Agreement was reached by the Working Group in 1998 that the *p*-DCB-related carcinogenic events in animals were not relevant to humans. The Australian classification criteria are the same as those used by the European Union.

13. Environmental Effects

No ecotoxicity tests were provided by applicants. In the following tables, results have been taken from the SIAR (1999). Additional studies not included in that report have been taken from the IUCLID datasheet although these results can not be assumed to be valid.

No QSAR results are reported here due to the large amount of experimental results available. Comments relating to tests are provided from the SIAR report (1999) where available.

13.1 Aquatic toxicity

13.1.1 Toxicity to fish

Based on the scale of Mensink (1995) *p*-DCB can be considered slightly to moderately toxic to fish when tested acutely, and when subject to chronic exposure, the chemical can be classed as slightly toxic to fish. The acute and chronic data are summarised in Table 13.

No observations regarding sublethal effects are made about the acute tests in either the SIAR (1999) or BUA (1994) reports. They followed OECD, American Standard Test Method (ASTM) or US EPA guidelines. Early life stage (ELS) testing on *P. promelas* followed a flow-through methodology and was conducted during development from egg to larvae. Each test was initiated by placing 30 embryos, 4 to 12 hours old, into the aquarium with the percentage of normal larvae hatching and surviving (abnormal developing fish included) used as endpoints.

ELS testing on *J. floridae* was similar to that for fathead minnow. Water samples were analysed 5 days per week during 28 day exposure. Two age groups were used simultaneously: eggs/embryo/larval fish with data collected on hatching success 4 to 6 days after exposure and 10 day larval survival; and one week old fry with data generated on survival and growth over 28 days. No influence was demonstrated on hatching rate while significant effects with respect to survival of larvae up to 10 days of age in two parallel tests were observed at 0.31 and 0.32 mg/L.

ELS testing on rainbow trout appeared to be conducted with 1000 eggs and 25 embryos per test concentration. No morphological and histological effects at any tested concentration were observed. A cumulative mortality of 30% for all the treatments as well as the controls is reported.

Table 13 - Acute and chronic toxicity of *p*-DCB to Fish

Species	Test Duration	Result (mg/L)	Reference
Acute			
Brachydanio rerio (zebra fish)	96 hours	LC_{50} = 2.1	SIAR, 1999
Pimephales promelas (fathead minnow)	96 hours	LC_{50} = 3.6 (larvae)	SIAR, 1999
		LC_{50} = 14.2 (juveniles)	
		LC_{50} = 11.7 (subadults)	
Pimephales promelas	96 hours	LC_{50} = 4.2	SIAR, 1999
Jordanella floridae (American flagfish)	96 hours	LC_{50} = 4.5 (semi static)	SIAR, 1999
		LC_{50} = 2.1 (flow through)	
Oncorhynchus mykiss (Rainbow trout)	96 hours	LC_{50} = 1.12	SIAR, 1999
Cyprinodon variegatus (Sheepshead minnow)	96 hours	LC_{50} = 7.4	SIAR, 1999
		NOEC = 5.6	
Lepomis macrochirus	96 hours	LC_{50} = 4.28	IUCLID
Chronic			
Brachydanio rerio	14 days	NOEC = 0.44	SIAR, 1999
		LOEC = 0.7	
Poecilia reticulata	14 days	LC_{50} = 4	IUCLID
Pimephales promelas (early life stage)	28 days	NOEC = 0.57	SIAR, 1999
		LOEC = 1	
Jordanella floridae (early life stage)	14 days	NOEC = 0.2	SIAR, 1999
	28 days	NOEC = 0.35	
Oncorhynchyus mykiss (early life stage)	60 days	NOEC = 0.12	SIAR, 1999

13.1.2 Toxicity to aquatic invertebrates

Using the scale described in Mensink (1995), *p*-DCB can be said to be moderately to highly toxic to aquatic invertebrates under acute exposure, and slightly toxic under chronic exposure. Toxicity data for representative aquatic invertebrates are given in Table 14.

Table 14 - Toxicity of *p*-DCB to aquatic invertebrates

Species	Test Duration	Result (mg/L)	Reference
Acute			
Daphnia magna	24 hours	EC_{50} = 1.6	SIAR, 1999
		EC_{50} = 3.2	
	48 hours	LC_{50} = 2.2	SIAR, 1999
		EC_{50} = 0.7	
Mysidopsis bahia	96 hours	EC_{50} = 1.99	IUCLID
Tanytarsis dissimilis (midge)	48 hours	EC_{50} = 13	IUCLID
Chronic			
Daphnia magna	21 days	NOEC = 0.4	SIAR, 1999
	28 days	NOEC = 0.22	SIAR, 1999

No observations are made regarding sublethal effects for the acute tests in either the SIAR (1999) or BUA (1994) reports. The 21 day chronic *Daphnia* test used the most sensitive parameter stated as the time of the first birth. Animals were transferred every second day to new, closed test bottles and the test concentration was observed to decrease from 0.5 to 0.3 mg/L before renewal. No observed effects were noticed, and the mean nominal concentration of 0.4 mg/L was used as a NOEC. The 28 day fertility tests used 25 new born animals exposed to different concentrations for 28 days. Toxic and alimentation solutions were changed daily and at the same time the number of dead and new-born animals were observed. Concentration loss never exceeded 15% of the initial concentration.

In addition to these tests, Mortimer and Connell (1995) investigated the effects of *p*-DCB on the crab *Portunus pelagicus* (L). Juvenile crabs were exposed to aqueous concentrations, and the end point was growth rate. Concentrations tested were 31, 62, 125 and 250 ppb. The result is expressed as a 96 hour LC_{50} of 1.37 μmol/L which has been equated to 201 μg/L making this species the most sensitive aquatic invertebrate tested both acutely and chronically.

13.1.3 Toxicity to aquatic plants

Using the scale described in Mensink (1995), *p*-DCB can be said to be toxic to slightly toxic to algae and aquatic plants. No observations from the tests are available. The data for aquatic plant toxicity tests are presented in Table 15.

Table 15 - Toxicity of *p*-DCB to aquatic plants

Species	Test Duration	Result (mg/L)	Reference
Ankistrodesmus falcatus (algae)	4 hours	EC_{50} = 20 (photosynthesis)	Wong *et al.*, 1984
Scenedesmus pannonicus	72 hours	$E_{\mu}C_{50}$ = 31	SIAR, 1999
Scenedesmus subspicatus	48 hours	$E_{\beta}C_{50}$ = 28 $E_{\mu}C_{50}$ = 38	SIAR, 1999
Selenastrum capricornutum	96 hours	$E_{\mu}C_{50}$ = 1.6	SIAR, 1999
Cyclotella meneghiniana	48 hours	$E_{\mu}C_{50}$ = 34.3	SIAR, 1999
Selenastrum capricornutum	3 hours	EC_{50} = 5.2 (photosynthesis)	IUCLID

$E_{\beta}C_{50}$ is based on inhibition of reproduction. $E_{\mu}C_{50}$ is based on inhibition of growth rate.

13.1.4 Toxicity to micro-organisms

p-DCB can be described as slightly to practically non-toxic to micro-organisms (Table 16). Specifically, sewage sludge and methanogenic sewage sludge only showed slight to practically no toxic effects when exposed to the chemical. No observations from the tests are available.

Table 16 - Toxicity of *p*-DCB to micro-organisms

Species	Test Duration	Result (mg/L)	Reference
Nitrosomonas sp (Bacteria)	12 hours	IC_{50} = 86	SIAR, 1999
Photobacterium phosphorem (Bacteria)	5 minutes	IC_{50} = 4.3	IUCLID
Active sludge	12 hours	IC_{50} = 330	SIAR, 1999
Methanogenic sewage sludge	48 hours	IC_{50} = 86	SIAR, 1999

13.1.5 Predicted no effect concentration for the aquatic environment

There are a significant number of experimental test results for this chemical to the aquatic compartment, covering all trophic levels. A number of chronic results are available for both fish and aquatic invertebrates.

The predicted no effect concentration (PNEC) can therefore be determined by taking the lowest chronic value (14-day NOEC for *J. floridae* = 0.2 ppm), and using an assessment factor of 10 to reflect the large amount of data. The outcome would be the same if the most sensitive acute effect (96h LC_{50} = 200 µg/L for *P. pelagicus* (L)) is used.

The resulting PNEC is 20 µg/L (ppb) in water.

13.2 Terrestrial toxicity

The SIAR (1999) has provided toxicity results for two worm species, *Eisenia andrei* and *Lumbricus rubellus*. In both tests, at least five concentrations and a control were used with 20 adults per test concentration. The tests were carried out in an open static system in two soils, one a natural sand soil (KOBG) and the other an artificial soil (OECD). The characteristics of the soils are presented in Table 17.

Table 17 - Characteristics of soil types

Soil type	pH	% OM[1]	% sand	% silt	% clay
KOBG	4.8	3.7	86.5	1.4	7.5
OECD	5.9	8.1	72.1	8.1	7.4

[1]OM = organic matter

The *p*-DCB was first combined with a small amount of dry soil, and this mixture was combined with the remaining soil. With mortality as an endpoint the LC_{50} for *E. andrei* and *L. rubellus* was 128 (KOBG soil) to 229 (OECD soil) and 184 (KOBG soil) to 615 (OECD soil) mg/kg soil dw respectively. When results were based on calculated concentrations in soil porewater, the LC_{50} was lower, ranging from 17.8 to 51 and 26.2 to 229 mg/L respectively. Tests in the KOBG soil appeared to result in higher toxicity. This may be due to higher removal of the chemical through

adsorption in the OECD soil as there is a significantly higher concentration of organic matter.

The SIAR (1999) has normalised the results to an organic matter content of 3.4% in line with the EU TGD which resulted in recalculated LC_{50} of 118 (KOBG soil) to 96 (OECD soil) and 169 (KOBG soil) to 258 (OECD soil) mg/kg soil dw for *E. andrei* and *L. rubellus* respectively.

Based on these results, *p*-DCB can be considered only slightly toxic to earthworms.

In a published report, the lethal body burden (LBB) of *p*-DCB was determined for the earthworm (*E. andrei*) with the contact paper toxicity test method (Belfroid *et al.*, 1993). Exposure to 735 and 220 μg/cm^2 of *p*-DCB resulted in death within 8 hours. The LBB was measured at 0.33 μmol/g (approximately 48.5 mg/kg). This is not comparable with an LC_{50}. This paper showed that the LBB of *p*-DCB was lower than four other chlorobenzenes (by up to an order of magnitude). It is stated that this observation has been made in other research, citing another paper where LBBs of *p*-DCB for midge larvae were 0.14 μmol/g and for other chlorobenzenes ranged from 0.29 to 1.24 μmol/g. An hypothesis for this apparent higher toxicity of *p*-DCB is given in that it may be expected there is a higher possibility of transformation for a lower chlorinated benzene than higher chlorinated benzenes such as those tested (eg penta-chlorobenzene and hexa-chlorobenzene). The appearance of toxic metabolites would result in death at lower internal concentrations, and therefore in lower LBBs.

13.2.1 Terrestrial plants

The SIAR (1999) summarises a 7 to 14 day growth test on the dicotyledon *Lactuca sativa*, and a second 16 to 21 day growth test on the same plant is described in the IUCLID data sheet.

The 7 to 14 day test followed OECD Test Guideline 208. Soil from two collections in an orchard were used, with pH 7.5; percent organic matter (%OM) 1.4 to 1.8; clay 12 to 24% with moisture at 80% of water holding capacity. Concentrations were spaced by a factor of 3.2 with at least 3 concentrations and a control. Ten seeds per tray were used with two replicates. Only the 5 first germinated seedlings were kept. The 7 day EC_{50} was 213 mg/kg soil dw (although it is stated that this result may be underestimated). The 14 day EC_{50} was 248 mg/kg soil dw (based on nominal concentrations).

The second test followed older (1968) methodology. The test was conducted under semi-static conditions with renewal three times a week. The structure used 5 seedlings (1 week old with minimum root length of 3 cm) per pot with two replicates. Concentrations were spaced by a factor of 3.2. The test reports an EC_{50} was 5.1 mg/kg soil dw.

The phytotoxicity of *p*-DCB was tested using soybean, carrot and tomato cell cultures during their periods of fast growth (Wang *et al.*, 1996). The cells were grown under standard conditions until day 4, 5 and 6 for soybean, tomato and carrot respectively when the cultures were transferred into sealed flasks and incubated for 1 day with 6 different concentrations of *p*-DCB. Preliminary experiments showed that the standard

cultivation method is not suitable due to the volatility of the compound. Sealing the culture flasks during the whole growth period affected the growth of cells more than the chemical itself. However, when cell cultures were incubated during the period of their fastest growth, an incubation time of 1 day proved sufficient to determine the differences in the growth rates in relation to the concentration of the chemical. The results showed that *p*-DCB is toxic to plant cells. A 50% inhibition in growth was produced by 0.05 mM for tomato and 0.5 mM for soybean and carrot cultures. This does not appear to be a standard phytotoxicity test.

13.2.2 PNEC for soil

Only short term toxicity tests for soil are available for *p*-DCB. The most sensitive result was LC_{50} for *E. andrei* of 128 mg/kg soil dw. Due to the lack of data available, an assessment factor of 1000 will be used, giving a PNEC in soil of 0.128 mg/kg soil dw (ppm).

14. Occupational Risk Characterisation

In this section, the results of the health hazard and occupational exposure assessments have been integrated to characterise the risk of adverse effects to workers potentially exposed to *p*-DCB.

Results from the risk characterisation process provide the basis for health risk management strategies (i.e., methods to reduce exposure and/or increase worker awareness of potential hazards and safe handling of *p*-DCB).

14.1 Methodology

The risk to human health from exposure to *p*-DCB has been characterised using margin of exposure methodology commonly adopted in international assessments (EC, 1994; OECD 1994).

For health effects caused by repeated or prolonged exposure, risk(s) have been characterised as follows:

1. Identification of the critical effect(s).

2. Identification of the most appropriate/reliable NOAEL (if available) for the critical effect(s).

3. Where appropriate, comparison of the NOAEL with the estimated human dose or exposure (EHD), to provide a margin of exposure (MOE), that is:

$$MOE = \frac{NOAEL}{EHD}$$

   Where actual exposure monitoring data are unavailable or insufficient, the EHD may be determined using exposure assessment models, such as the UK EASE model.

4. Characterisation of risk, by evaluating whether the MOE indicates a concern for the human population under consideration.

The MOE provides a measure of the likelihood that a particular adverse health effect will occur under the conditions of exposure. As the MOE increases, the risk of potential adverse effects decreases. In deciding whether the MOE is of sufficient magnitude, expert judgement is required. Such judgements are usually made on a

case-by-case basis, and should take into account uncertainties arising in the risk assessment process, such as the completeness and quality of the database, the nature and severity of effect(s) and intra/inter species variability.

14.2 Critical health effects and exposures

14.2.1 Acute effects

The critical effects from acute exposure to *p*-DCB vapour to humans are eye and respiratory irritation. Eye and nose irritation has been reported at atmospheric levels as low as 50 to 80 ppm with severe discomfort experienced in the range 80 to 160 ppm. Severe respiratory irritation has been observed at 160 ppm and above.

Acute oral toxicity may present as haemolytic anaemia with methaemoglobinuria following ingestion of *p*-DCB. No deaths directly attributable to acute *p*-DCB toxicity have been reported.

14.2.2 Chronic effects

Effects from long-term, repeated (chronic) exposures are not well characterised in human populations. Therefore, the risk characterisation is based upon the critical health effect in animals. A number of chronic studies have been carried out in a variety of animal species (by different routes of exposure). *p*-DCB elicits kidney tumours in male rats and liver tumours in both sexes of mice. The results of genotoxicity testing, both *in vitro* and *in vivo*, have yielded negative results and therefore threshold based risk assessment has been conducted.

Consistent findings associated with repeated human exposure to *p*-DCB include reversible neurological and haematological disorders.

Oral exposure

Chronic oral studies have been conducted with rats, mice and dogs. In rats the critical non-neoplastic endpoints were renal and hepatic toxicity and the critical neoplastic endpoint was renal tumour formation. In mice the critical non-neoplastic and neoplastic endpoints were hepatic effects. For dogs, the critical endpoint was hepatotoxicity.

For the most sensitive species, the dog, the $NOAEL_{(oral)}$ was 10 mg/kg bw per day and a $LOAEL_{(oral)}$ of 50 mg/kg bw per day was observed which produced hepatotoxicity.

Inhalation

Inhalation effects were observed in chronic (2-year) inhalation studies with rats and mice. Both species exhibited hepatotoxicity and increased kidney weights. The $NOAEL_{(inhalation)}$ was 75 ppm and the $LOAEL_{(inhalation)}$ was 300 ppm for rats and mice.

Dermal exposure

No treatment-related effects were seen in a 3-week dermal study with rats.

14.3 Occupational health and safety risks

Occupational health risks may result from acute and/or chronic exposure to *p*-DCB via inhalation exposure (the most relevant route of exposure). Other occupational safety risks may arise from physicochemical hazards.

14.3.1 Risk from physicochemical hazards

Risks of fire and/or explosion during handling and use of *p*-DCB are low.

14.3.2 Acute health risks

While *p*-DCB has a high vapour pressure (0.84 hPa at 20°C) which results in sublimation of the compound, it is considered unlikely that, for uses identified in Australia, vapour levels would reach those required to elicit acute systemic effects (from inhalation). Based on old monitoring data from one Australian company and the UK EASE model, levels could reach above 50 ppm. Thus, irritation to the eyes and upper respiratory tract may occur where ventilation is inadequate, such as during bagging operations.

14.3.3 Chronic health risks

Despite the fact that exposures are not well characterised for occupational scenarios with potential exposure to *p*-DCB either in Australia or overseas, information on known use profiles and data obtained from the UK EASE model have enabled broad estimates of risk to be made. Dermal exposure is unlikely to contribute significantly to body burden due to the solid nature of *p*-DCB and the use of gloves in the workplace. Consequently, only exposure via inhalation has been assessed in the following occupational scenarios. The critical effects are liver and kidney effects in rats and mice via inhalation. Margins of exposure (MOE) were calculated using the $NOAEL_{(inhalation)}$ of 75 ppm (450 mg/m^3) reported in the JBRC (1995) study.

Formulation *p*-DCB products

The formulation of *p*-DCB products is basically an open process involving either the milling or melting at 60°C of *p*-DCB followed by blending with dyes and perfume.

Monitoring data from 1981 indicated exposures of 10 to 60 ppm, with the highest exposures in the bagging area. Similar levels were predicted by EASE. However, more recent but not very accurate monitoring data suggest that levels are more likely to be 5 to 15 ppm giving a MOE of 15 to 5. Taking into account the intermittent exposure (approximately 100 days per year) and the LOAEL of 300 ppm, the risk of chronic effects are likely to be low. However, the poor basis of the exposure data must be acknowledged.

Pressing and wrapping *p*-DCB products

The pressing and wrapping of air freshener and deodorant products composed of *p*-DCB occurs at ambient temperatures and, based on survey results, is generally an intermittent process occupying approximately 50 days per year. Monitoring data for these activities are not available. However, as the exposure levels are likely to be lower than during formulation and exposure frequency much less, it is likely that the risk of chronic effects will be low.

Hygiene sector

Due to the extensive use of *p*-DCB as an air freshener/deodorant in public and workplace toilet facilities, cleaners are regularly exposed to the vapour of *p*-DCB by inhalation. No Australian workplace monitoring data are available for these conditions. Two experimental studies conducted in Germany found the highest airborne concentrations of *p*-DCB to be 1.8 ppm in one facility and 3.8 ppm in another giving MOE of 41.7 and 19.7 respectively. However, the values can only be considered as rough approximations of toilet facilities in general as the concentration of *p*-DCB vapour will depend on several variables including the number of *p*-DCB blocks used, the internal volume of the facility, the type of ventilation, the temperature and whether the blocks are wrapped or unwrapped. Due to the intermittent nature of exposure and the low levels of *p*-DCB likely to be encountered, the risk of chronic effects to workers in the hygiene sector from exposure to *p*-DCB is likely to be low.

14.3.4 Uncertainties in the calculation of margins of exposure

A consideration of uncertainties in the risk characterisation process is necessary when discussing the acceptability and implications of estimated MOE. Examples of uncertainties inherent in the assessment of risk for *p*-DCB are as follows:

Inadequate data

- lack of exposure monitoring data;
- lack of representative worker exposure profiles (i.e., degree of worker exposure may vary from factory to factory); and
- inadequate data on human health effects following chronic exposure.

Assumptions in the assessment process

- that occupational dermal absorption (of vapour) is minimal;
- that absorption and bioavailability of *p*-DCB via inhalation is similar in humans and rats;
- that dose-response relationships are likely to be similar (on a ppm in air basis) in rats and humans.

15. Environmental Risk Characterisation

p-DCB is a volatile and water soluble chemical with its major use in Australia being as an air freshener, and toilet blocks. This leads to widespread release to the atmosphere and aquatic compartment through direct release to the sewer. Monitoring data from around the world confirms the widespread transport of this chemical with substantial detection obtained in air, surface water and sediments overseas. While monitoring in Australia has tended to not detect *p*-DCB, the level of monitoring has not been substantial, and not all environmental compartments have been tested.

15.1 Atmospheric risk

No experimental data on environmental organisms exposed through the gas phase are available, so it is not possible to conduct a hazard assessment for those residing in the atmosphere. However, abiotic effects can be assessed. While direct photolysis is not considered likely, the atmospheric half-life is relatively short (expected to be <50 days) due to reaction with photochemically produced hydroxyl radicals. The chemical contains chlorine substituents which suggests it may have a potential effect on stratospheric ozone. However, with half-lives for migration to the stratosphere of 3 to 10 years (Bunce, 1994), this chemical would not be expected to persist long enough in the troposphere to be of concern.

Nonetheless, Webster *et al.* (1998) state that transport times to the Arctic can be measured in weeks. Therefore, with a half-life of 5 to 7 weeks for *p*-DCB, it can be expected that the chemical could undergo significant transport in the atmosphere and may migrate to the poles. No measurements appear to be available from these regions.

For chemicals to be considered persistent organic pollutants (POPs), they need to meet certain criteria with respect to persistence, bioaccumulation and the potential for long range transport. *p*-DCB meets the draft UNEP criteria for persistence in air (half life > 2 days), and therefore, possibly the draft criterion for long range transport. The draft criteria indicate half-lives in soil and sediments need to be greater than six months, but there are no measurements in this area so no conclusions can be drawn. However, *p*-DCB fails the draft persistence in water criterion of either two or six months, and also fails the draft bioaccumulation criterion of BCF>5000. Therefore, *p*-DCB is unlikely to be considered a POP.

15.2 Aquatic risk

PEC/PNEC ratios for the aquatic compartment can be calculated using the worst-case local scenario, in this instance, the PEC_{local} of 18.5 µg/L and $PEC_{continental}$ of 8.6

µg/L. The ratio of PEC/PNEC has been calculated for local and continental compartments as follows:

$PEC/PNEC_{local}$ = 0.925
$PEC/PNEC_{continental}$ = 0.43

In order to predict a low potential for an environmental hazard, the PEC/PNEC ratio must be less than 1. The PNEC has been conservatively determined by taking the lowest effect from a large data source, and applying a further safety factor of 10.

In determining the PEC/PNEC ratio, the PEC is clearly an overestimate using worst-case assumptions. Where surface waters were monitored in Australia, *p*-DCB failed to be detected at 0.5 µg/L. While the estimated continental PEC is above the highest monitored surface water level in the Northern Hemisphere (4.05 µg/L), the majority of monitoring samples in surface waters from around the world were significantly less than this, and often involved no chemical being detected.

Within sediments, evidence suggests *p*-DCB will be present at higher concentrations than receiving waters where exposed. However, no benthic tests are available in which to conduct a meaningful risk assessment for sediments. It is reasonable to assume that *p*-DCB associated with the sediments is in fact adsorbed and so not bioavailable. If this were not the case, the chemical would be expected to volatilise. Based on this, the hazard to benthic organisms is anticipated to be low. However, anaerobic degradation studies indicate that *p*-DCB is relatively resistant to biodegradation under the conditions expected in sediments. Additionally, the much higher levels found in sediments (see Section 7.2.5) than surface waters indicate possible accumulation in this compartment, which may be an area of concern.

p-DCB is classified under the International Maritime Dangerous Goods code as a UN3077 Class 9 Environmentally Hazardous Substance. However, based on current usage patterns, the evidence supports a conclusion of a low expected risk to the aquatic environment in Australia.

15.3 Terrestrial risk through agricultural use

In the event of application of sewage sludge to land, a PEC of 16.6 µg/kg has been determined with a PNEC of 128 µg/kg. These values give a PEC/PNEC ratio of 0.13. Data for terrestrial organisms were only limited to earthworms and one plant species, and there is a possibility other terrestrial organisms may be more sensitive. However, an assessment factor of 1000 was used to reflect this scenario.

In summary, the use of *p*-DCB is expected to present a low terrestrial environmental risk.

16. Public Health Assessment

Public exposure will arise from the use of *p*-DCB in toilet deodorant blocks and air fresheners. When used as air freshener/deodoriser the cellophane wrapping is punctured and the *p*-DCB block or disk undergoes sublimation and the vapour disperses. Blocks or disks are unwrapped and placed into urinals. Consequently, public exposure will occur principally by inhalation, with the potential for dermal exposure reduced by the containment of *p*-DCB in cellophane wrapping during handling. There have been no reports of skin irritation or sensitisation in widespread human use, except for one case of acute petechial purpura in a 69-year old man. Consequently the hazard of dermal irritation or sensitisation is considered to be low.

Several products are available in disk form for domestic use as air deodorisers with insect repellent activity and are used to protect clothes in cupboards and wardrobes from silverfish and moths. The sublimation of such *p*-DCB products may lead to a concentration of *p*-DCB vapour in an enclosed space.

A study undertaken to establish a baseline for the trace compound composition of expired human breath was conducted by Conkle *et al.*, (1975). The condensate from the breaths of 8 male volunteers at an airforce base were collected by cryogenic trapping and subjected to GC/MS analysis. Morning samples were collected for 60 minutes after a nine-hour fast prior to analysis. A total of ten determinations were made and samples were corrected for supply air contaminants. Of the ten samples analysed, seven contained traces of dichlorobenzene (mean 4.6 μg/hr, range 0.001 to 26.0 μg/hr; isomer not stated). The authors concluded that the dichlorobenzene was *p*-DCB and was present as a result of the men using the toilet facilities, where it was present in block form, prior to the commencement of the experiment. The airborne levels of *p*-DCB in public toilet facilities have been discussed previously in Section 8.2.4.

Studies in Japan measured airborne concentrations and personal exposure levels by means of passive samplers while the inhabitants lived their daily lives. Maximal *p*-DCB concentrations were 5.9 mg/m^3 (1 ppm) for airborne concentration and 0.5 ppm (3.3 mg/m^3) for personal exposure (SIAR, 1999).

Occupational exposure to vapour can cause irritation to the nose and upper respiratory tract at levels at or above 50 ppm (300 mg/m^3), including such symptoms as coughing, chest pains and difficulty in breathing at around 160 ppm (962 mg/m^3). NOAEL's from chronic, repeat-exposure inhalation studies in rats and mice of 75 ppm (451 mg/m^3) are well in excess of measured personal exposure levels from household products. Medical examinations of 58 workers exposed for up to 25 years (average exposure approximately 5 years) to average airborne concentrations of 45-380 ppm, measured in 3 separate surveys, showed no evidence of *p*-DCB-related effects on health, except for eye and nose irritation. However, the study was not controlled for such things as concentration and type of chemical exposure and pre-existing medical conditions (Hollingsworth *et al.*, 1956). Consequently, the risk from

the use of *p*-DCB products in the household and public toilets is considered to be low.

It should be noted that public exposure could occur during non-industrial use of *p*-DCB, that is, when used as an insect repellent or a pharmaceutical.

Experiments were conducted to determine the air-borne levels of *p*-DCB in two unfurnished rooms where the chemical was used as an insect repellent in two identically sized wardrobes (volume 0.58 m^3). Room 1 had a volume of 25.66 m^3 and room 2 was 30.28 m^3. Approximately 90 g of *p*-DCB were placed between clothes in each wardrobe. The wardrobe in room 1 was never opened while the other was opened for 2 minutes each day. The rooms were aerated daily, with the exception of weekends, for 15 minutes after air samples were taken. Room temperature was maintained at 20°C. Complete dissipation of the *p*-DCB took 80 days. Maximum airborne levels of *p*-DCB in rooms 1 and 2 over 80 days and before aeration were 1.7 ppm (10.0 mg/m^3) and 1.3 ppm (8.0 mg/m^3) respectively. The average levels over 73 days before aeration were 1 ppm (5.8 mg/m^3) for room 1 and 0.7 ppm (4.0 mg/m^3) for room 2 (Globol GmbH, 1986).

Pharmaceutical exposure will principally arise from the use of *p*-DCB in a finished product (Cerumol Ear Drops containing 2% w/v *p*-DCB). Exposure will be via the dermal route, with the possibility of accidental oral and ocular exposure. The hazards associated with the intended use of this product are likely to be low for the following reasons. This product is only available in a small volume (10 ml dropper bottle), and if used as directed, a very small volume (5 drops) should be administered up to twice per day for a few days. It exists in Schedule 2 (S2) of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP), and therefore it is available from pharmacies without prescription. The National Drugs and Poisons Schedule Committee Guidelines (NDPSCG, 1997) describe S2 chemicals as "substantially safe in use but where advice or counselling is available if necessary" and "for minor ailments or symptoms which can be easily recognised by the consumer and/or do not require medical diagnosis or management". Characteristics of preparations in S2 include an "extremely low abuse potential" and "a low potential for harm from inappropriate use".

17. Risk Management

In this section, measures currently employed in the management of human health risks from occupational and consumer exposure to *p*-DCB are discussed. The information reviewed includes national and international standards, together with relevant guidance material, MSDS and labels. Due to the low environmental risk associated with current patterns of use, there are no specific environmental regulatory controls for *p*-DCB.

Relevant information was provided by importers of *p*-DCB and formulators of products in which *p*-DCB is an ingredient. Information was also obtained from site visits.

The key elements in the management of risks discussed in this section include:

- workplace control measures;
- hazard communication (including training and education);
- monitoring and regulatory controls; and
- emergency procedures.

17.1 Workplace control measures

According to the NOHSC *National Model Regulations for the Control of Workplace Substances* (NOHSC, 1994c), exposure to hazardous substances should be prevented or, where this is not practicable, adequately controlled, so as to minimise risks to health and safety. *p*-DCB is classified as a hazardous substance in accordance with the NOHSC Approved Criteria. A *National Code of Practice for the Control of Workplace Hazardous Substances*, lists the hierarchy of controls measures, in priority order, that should be implemented to eliminate or minimise exposure to hazardous substances. These are:

- elimination;
- substitution;
- isolation;
- engineering controls;
- safe work practices; and
- personal protective equipment.

Control measures are not mutually exclusive and effective control usually requires a combination of these measures. Particular attention needs to be given to control measures that minimise inhalation of *p*-DCB.

17.1.1 Elimination and substitution

Elimination is the removal of a chemical from a process and should be the first option considered when minimising risks to health.

In situations where it is not feasible or practicable to eliminate the use of a chemical, substitution should be considered. Substitution includes replacing the chemical with a less hazardous substance or the same substance in a less hazardous form.

17.1.2 Isolation

Isolation as a control measure aims to separate employees, as far as practicable, from the chemical hazard. This can be achieved by distance or enclosure.

The formulation of *p*-DCB, by the addition of additives in a melting or mixing process, is predominantly an open process. For example, at one site visited, melting of *p*-DCB is achieved by loading manually the raw material into a tank which is sealed during the melting process. Liquid *p*-DCB is then transferred from the tank to the processing area by a system of pipes. Prior to spreading the molten *p*-DCB onto a stainless steel conveyer belt a small quantity of dye and perfume are added. All subsequent operations which include crystallisation of molten material on the conveyer belt, flaking into a hopper and transfer into bags take place under open conditions.

The pressing and wrapping of *p*-DCB products is a simple and open process and generally the equipment is of older manufacture. The operation of hoppers and presses results in the formation of dust particles composed of *p*-DCB. This dust was observed at all sites visited. The survey and site visits revealed that open containers, for temporary storage of *p*-DCB, were common to many companies.

17.1.3 Engineering controls

Two of 3 formulators have exhaust ventilation during formulation. But on one site visit an air extraction system used to reduce airborne levels of *p*-DCB was of uncertain efficiency due to tears in the ducting material and the presence of *p*-DCB dust on the ducting itself. One company pressing and wrapping end products reported use of ventilation.

17.1.4 Safe work practices

No safe work practices were identified that can be characterised as unique to *p*-DCB. Common safe work practices employed include storage in closed containers in well ventilated areas, and away from incompatible materials and immediate clean-up of spills.

17.1.5 Personal protective equipment

Where other control measures are not practicable or adequate to control exposure, personal protective equipment (PPE) should be used. In practice, PPE used for handling *p*-DCB includes the following:

- overalls;
- safety glasses; and
- gloves

Three companies (2 formulators and 1 pressing end products) reported that workers used respirators during processing of *p*-DCB.

17.2 Emergency procedures

The availability of an emergency response plan to deal with unexpected releases of *p*-DCB, such as large spills, is good practice. All employees need to be trained in accident and emergency procedures.

All plans/procedures should be fully documented and available to all workers. Local emergency services should be consulted on the appropriateness of emergency procedures developed. No emergency plans for *p*-DCB were submitted for assessment.

17.3 Hazard communication

17.3.1 Assessment of material safety data sheets (MSDS)

MSDS are the primary source of information for workers involved in the handling of chemical substances. Under the NOHSC *National Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994c) and the corresponding State and Territory legislation, manufactures and suppliers are obliged to provide an MSDS to their customers for all hazardous substances. Due to its inclusion on the *List of Designated Hazardous Substances* (NOHSC, 1999b), *p*-DCB is considered to be a hazardous substance.

A sample MSDS for *p*-DCB, prepared in accordance with the MSDS Code, is provided at Appendix 3. The sample MSDS, prepared from information obtained for the assessment of *p*-DCB, is for guidance purposes only. Under the National Model Regulations, manufactures and suppliers have the responsibility to compile their own MSDS and ensure that the information is up-to-date and accurate.

Of 20 companies handling *p*-DCB to which survey forms were sent, 5 did not submit a MSDS for assessment. A total of 7 MSDS for commercial grade *p*-DCB (purity 99%) were provided for assessment and an additional 8 for *p*-DCB-containing products (purity > 95%) intended for industrial use. The 15 MSDS were assessed against the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994d). The results of the MSDS assessment are presented in Table 18.

A significant number of MSDS were deficient on information which adequately describes the health hazards associated with *p*-DCB. Particularly noticeable was the lack of information pertaining to the effects associated with ingestion of the chemical and effects on the central nervous system. Several MSDS contained advice that the

administration of adrenaline is contra-indicated in the event of *p*-DCB exposure, however, this assessment found no evidence to support this direction.

Table 18 - Findings of the MSDS Assessment

Type of Information	Number of MSDS
Statement of hazardous nature	12/15
Product Identification	
Correct CAS number	13/15
Physical description/properties	15/15
Health Hazard Information	
Acute effects	
Harmful if swallowed	14/15
Ingestion effects (headache, nausea, vomiting, anaemia)	5/15
Irritating to the eye	15/15
Irritating to the upper respiratory tract	15/15
Effects on central nervous system (confusion, incoordination, narcosis, paresthesia)	7/15
Chronic effects	
Ingestion effects (anaemia)	5/15
MSDS stated 'no data available' for health effects	1/15
First Aid Advice[1]	
If more than 15 minutes from medical attention induce vomiting	12/15
Do not give milk or oils	11/15
Advice to physician	14/15
If poisoning occurs, contact a doctor or Poisons Information Centre	15/15
Instruction that adrenaline is contra-indicated	9/15
Precautions For Use	
Correct value for TWA and STEL exposure standard	10/15
Adequate ventilation	14/15
Eye protection	14/15
Gloves protection	12/15
Respirator	11/15
Safe Handling Information	
Statement of combustible nature	13/15
Statement that hydrogen chloride or phosgene form on combustion	12/15
Adequate information on extinguishing media (CO_2, foam, dry chemical, water fog)	14/15
Contact Point	
Contact person nominated	10/15
Direct phone number for contact person	9/15
Emergency telephone number provided	6/15

[1]First aid statement as recommended by SUSDP; TWA, time weighted average; STEL = short term exposure limit.

One third of companies did not provide full exposure standard values, in particular, the STEL value was not reported by these companies. A number of MSDS did not convey sufficient information for the contact point in the event of an emergency. A general deficiency was the absence of an emergency or direct contact telephone number for an appropriate contact person.

The MSDS supplied by two major importers were the least comprehensive of all submitted. Of the 6 MSDS issued by suppliers, 2 were of recent issue date (1998), 2 issued in 1997 and 1 each issued in 1996 and 1994. All companies, with the exception of one, provided good advice concerning personal protective equipment.

17.3.2 Assessment of labels

Under the NOHSC *National Model Regulations and Code of Practice for the Control of Workplace Hazardous Substances* (NOHSC, 1994c) and the corresponding State and Territory legislation, suppliers of industrial chemicals are obliged to provide labels in accordance with the NOHSC *Code of Practice for the Labelling of Hazardous Substances* (Labelling Code) (NOHSC, 1994e).

The information needed on labels for containers with a capacity of more than 500 g of *p*-DCB include:

- Signal word 'Hazardous';
- Identification information
- product name
- chemical name
- Directions for use (where appropriate);
- Safety phrases;
- First aid instructions;
- Emergency procedures;
- Supplier details; and
- Reference to MSDS.

Nine labels were provided for assessment, comprising 3 labels for industrial grade *p*-DCB and 6 for products containing *p*-DCB (greater than 90% *p*-DCB) for industrial use. As all the products are intended for industrial use they should be labelled in accordance with the Labelling Code (NOHSC, 1994e). The findings of the assessment of labels issued for commercial grade material are summarised in Table 19.

The risk phrase required prior to this Assessment (from the List of Designated Hazardous Substances [NOHSC: 10005(1994)]) was 'Harmful if swallowed' (R22).

Of the 9 labels submitted for assessment only 3 contained reference to the harmful nature of the product.

Generally, the labels assessed failed to convey the necessary information required in the event of an emergency. Specifically, information relating to health risks and first aid procedures were either absent or insufficient.

Table 19 - Findings of the label assessment

Information provided	Number of Labels
Signal word	
HAZARDOUS	3/9
Identification information	
Chemical name	8/9
Safety phrases	
Avoid contact with skin and eyes (S24/25)	6/9
First aid instructions (or similar statement)	
If poisoning occurs contact a doctor or Poisons Information Centre	7/9
In case of contact with eyes, rinse immediately with plenty of water for 15 minutes. Contact a doctor or Poisons Information Centre if irritation persists	4/9
If swallowed, and more than 15 minutes from medical attention induce vomiting, preferably using Ipecac Syrup APF	6/9
Do not give milk or oils	6/9
Information on emergency procedures	3/9
Reference to MSDS	4/9

17.3.3 Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP)

Where products containing *p*-DCB are intended for domestic end-use, they need only comply with the SUSDP labelling requirements (Australian Health Ministers' Advisory Council, 1997). *p*-DCB is listed (as 'Paradichlorobenzene') in Schedule 5 of the Drugs and Poisons Schedule (SUSDP, Australian Health Ministers' Advisory Council, 1997). Its availability is not restricted, but it must be labelled with the signal words 'KEEP OUT OF THE REACH OF CHILDREN' together with the following safety directions (SD) and first aid instructions, if it is likely to be used in the public domain:

Safety directions:

- Avoid contact with eyes (SD1);
- Avoid contact with skin (SD4).

First aid instructions:

- If poisoning occurs, contact a doctor or Poisons Information Centre;
- If swallowed and more than 15 minutes from a hospital, induce vomiting, preferably using Ipecac Syrup AFP;
- Avoid giving milk or oils.

Of 13 labels from 2 companies assessed for compliance with the SUSDP Code, 2 did not contain the general safety precautions.

17.3.4 Education and training

Guidelines for the induction and training of workers potentially exposed to hazardous substances are provided in the NOHSC *Model Regulations and Code of Practice for the Control of Workplace Hazardous Substances* (NOHSC, 1994c). Specifically, matters that need to be addressed for *p*-DCB include:

- the potential adverse health effects of *p*-DCB;
- specific protective equipment to be worn; and
- explanation of data contained in MSDS and labels.

17.4 Other regulatory controls

The following sections comprise regulations/standards promulgated with the aim of protecting workers from adverse exposures to *p*-DCB in Australia.

17.4.1 Atmospheric monitoring

Under the NOHSC *Model Regulations and Code of Practice for the Control of Workplace Hazardous Substances* (NOHSC, 1994c), employees are required to carry out an assessment of the workplace for all hazardous substances, the methodology of which is provided in the NOHSC *Guidance Note for the Assessment of Health Risks Arising from the Use of Hazardous Substances in the Workplace* (NOHSC, 1994f). When the assessment indicates that the risk of exposure via inhalation is significant, atmospheric monitoring should be conducted to measure *p*-DCB levels in the workplace as a precursor to the implementation of suitable control measures to reduce exposure. Subsequent monitoring will be required to ensure that such measures are effective.

17.4.2 Occupational exposure standard

The current national occupational exposure standard for *p*-DCB is 75 ppm (451 mg/m^3) TWA, 110 ppm (661 mg/m^3) STEL (NOHSC, 1995g).

Overseas occupational exposure limits for *p*-DCB are listed in Table 20.

The adopted Australian occupational exposure standard of 75 ppm is consistent with the standard in several other countries, however, some countries have a lower

exposure standard, including the United Kingdom, Germany, Ireland, the Netherlands and Japan.

The current occupational exposure standard for *p*-DCB in Australia was adopted from the ACGIH standard of 1961 to 1992. This standard was set on the basis that *p*-DCB is less toxic than the ortho isomer (which had a ceiling limit value of 50 ppm) and that the TLV and STEL should be 'sufficiently low to prevent acute and chronic poisoning'.

Table 20 - Occupational exposure limits for *p*-DCB (ACGIH, 1998)

Country	TWA		STEL		Year adopted
	ppm	mg/m³	ppm	mg/m³	
Belgium	75	450	110	660	1993
Denmark	75	450	-	-	1993
Finland	75	450	115	690	1993
France	75	450	110	675	1993
Germany	50	300	100	600	1995
Ireland	25	150	50	300	1997
Japan	50	300	-	-	1998
Netherlands	25	153	50	300	1997
United Kingdom	25	153	50	306	1995
United States (ACGIH)	10	60	-	-	1996
United States (OSHA)	75	450	-	-	1996
Sweden	75	450	110	660	1993
Switzerland	75	450	150	900	1993

TWA = time weighted average; STEL = short term exposure limit.

In 1993, the ACGIH adopted a TWA (8 hrs) of 10 ppm (plus A3, animal carcinogen). The TWA was aimed to protect against eye irritation reported in humans (effects observed at 17 ppm in an unpublished study) and renal toxicity in rats (LOAEL of 25 ppm in male rats (NTP, 1987)).

The UK Health and Safety Executive adopted occupational exposure standards of 25 ppm TWA and 50 ppm STEL (15 minutes) in 1993. The STEL is aimed to prevent nose and eye irritation which occurs in humans at about 50 ppm or above. The TWA was set at 25 ppm (8 hrs) to 'allow for possible differences in response between animals and man' (based on a NOAEL of about 100 ppm for repeated exposures in animals).

17.4.3 Health surveillance

In accordance with NOHSC *Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994c), employers have a responsibility to provide

health surveillance in those workplaces where the workplace assessment indicates that exposure to a hazardous substance may lead to an identifiable substance-related disease or adverse health effect. *p*-DCB is not listed in Schedule 3 (list of substances requiring health surveillance) and as such there are no formal requirements for health surveillance programs for exposed workers.

17.4.4 Australian Code for the Transport of Dangerous Goods by Road and Rail

Currently, *p*-DCB is not classified in the 6th Edition of the Australian Code for the Transport of Dangerous Goods (ADG) (FORS 1998). However, the current International Maritime Dangerous Goods code classifies *p*-DCB as a UN3077 Class 9 Environmentally Hazardous Substance, Solid, N.O.S. and this classification is expected to be adopted in the 7th Edition of the ADG code.

18. Discussion and Conclusions

The manufacture of *p*-DCB does not occur in Australia and up to 1000 tonnes are imported annually. Almost all of this material is used in the production of toilet deodorant (approximately 85%) and air freshener blocks (approximately 13%). The remaining *p*-DCB is used in non-industrial activities, that is, pharmaceutical and agricultural uses.

p-DCB is used extensively in the public and private sectors mostly as an air freshener/deodoriser and predominantly in toilet facilities. Due to the high vapour pressure of *p*-DCB it slowly sublimates to the atmosphere. Use of this property is made by wrapping disks of the material (typically 99% *p*-DCB) with cellophane which, when punctured, allows the vapour to slowly disperse where it can act to disguise odours. Alternatively, and because of its insoluble nature, where use in urinals is required the blocks or disks are unwrapped and placed directly in the urinal.

Health effects

Animal studies have shown *p*-DCB to be of low acute toxicity by either oral, dermal or the inhalation route. Acute toxic effects reported in animals are nephrotoxicity in male rats and hepatotoxicity in both sexes of mice. Limited data indicate that *p*-DCB is not corrosive or irritating to the skin but can cause eye and respiratory tract irritation in animals and humans.

The systemic health effects of *p*-DCB in humans are poorly characterised and based on cases of accidental or intentional exposure. The effects reported from case studies include the development of neurological and haematological symptoms, all of which appear to be reversible following cessation of exposure. There have been two deaths attributed to *p*-DCB exposure, however, other factors which may have contributed to mortality were not discussed in the case report and a causal relationship can not be established. In humans, ingestion of *p*-DCB or inhalation of its vapour, by accidental or intentional means, may result in drowsiness, nausea, incoordination, unconscious, hypotension and anaemia. Exposure to vapour may cause coughing, chest pains and difficulty in breathing.

There have been no adverse reports of reproductive effects in humans or animals described in the literature and investigations of the genotoxic effect of *p*-DCB using several test systems have yielded negative results.

p-DCB has been found to be carcinogenic in the rat and mouse in two well-conducted studies. In an oral study, renal tumours were found in the kidneys of male rats and in oral and inhalation studies livers tumours were observed in both sexes of mice. Due to the negative results of genotoxicity testing the tumours are considered to be epigenetic in nature. The renal tumours in the male rat are not considered to be relevant to humans. There have been no well conducted epidemiological studies of the effects of *p*-DCB on humans.

Occupational health and safety

In Australia, between 500 and 1000 tonnes of *p*-DCB were processed, formulated or handled by at least 23 companies into products for industrial or domestic use in 1998. The processing of *p*-DCB is generally semi-automated and usually involves 1 or 2 workers per company. Production is intermittent with consumer demand regulating production activities. The duration of exposure varies from 6 to 7 hours/day for approximately 12 to 150 days/year.

The major route for occupational exposure to *p*-DCB is by inhalation. Occupational exposure in Australia can occur during the formulation of products or from use of finished products containing *p*-DCB. Absorption by the oral and dermal routes is unlikely to be a significant source of exposure under normal occupational use.

Occupational exposure is likely to occur due to vapour emitted from the volatile solid or from molten material during reforming of imported material into blocks or tablets. Exposure to vapour and dust can occur during handling of the solid material although once processed into finished products a cellophane wrapping minimises subsequent exposure. Monitoring data for workplace airborne concentrations of *p*-DCB in Australia are inadequate. However, based on data supplied and modelling estimates, exposure levels are likely to range from 5 to 15 ppm giving an expected MOE of 15 to 5. In practice, the MOE will be larger due to intermittent exposure.

There is the potential for workers involved in the hygiene sector, particularly those involved in cleaning toilet facilities, to be exposed to *p*-DCB. Due to the relatively small amount of *p*-DCB used per facility, the use of ventilation and the comparatively short exposure times involved, the risk to these workers is expected to be low.

Based on current knowledge concerning the use of *p*-DCB in Australia, it is concluded that, due to intermittent exposure and its relatively low toxicity, the risk to workers engaged in the manufacture of products containing *p*-DCB or in the use of *p*-DCB products is expected to be low.

A survey of workplace control measures indicated that the provision of adequate ventilation is not a routine procedure. In particular, the provision of exhaust ventilation in areas where *p*-DCB products are re-packaged appeared to be deficient.

An assessment of submitted MSDS and labels revealed a number deficiencies. The most common deficiency for MSDS was generally poor information on human health effects, both acute and chronic. A number of MSDS (60%) did not include an emergency contact number. For labels, the appropriate signal word (66%), safety phrases (33%) and information on emergency procedures (66%) were absent from several labels.

The hazard assessment identified an adverse effect in animals that was observed at a concentration equivalent to the body burden achievable at the current occupational exposure level for *p*-DCB, therefore, the occupational exposure standard should be reviewed.

Under the *National Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994c), a hazardous substance means a substance which is on the *List of Designated Hazadous Substances* (NOHSC, 1999b). Consequently, as *p*-DCB appears on the *List of Designated Hazadous Substances* (NOHSC, 1999b) it must be considered to be a hazardous substance.

Public health

Public exposure will principally arise from the use of *p*-DCB in toilet deodorant blocks and air fresheners. Public exposure will occur principally by inhalation, with the potential for dermal exposure reduced by the containment of *p*-DCB in cellophane wrapping during handling. There have been no confirmed reports of skin irritation or sensitisation in widespread human use. Consequently the risk of dermal irritation or sensitisation is considered to be low.

Several products in tablet and button form are sold as household insect repellents and/or air deodorisers with insect repellent activity and are used to protect clothes in cupboards and wardrobes from silverfish and moths. The sublimation of such *p*-DCB products may lead to a concentration of *p*-DCB vapour in an enclosed space. Investigations of the airborne concentrations resulting from the use of *p*-DCB as an insect repellent in wardrobes or as a household air freshener indicate that concentrations are likely to be well below those where irritation or chronic effects may be observed. Consequently, the risk to the public from the intended use of *p*-DCB blocks or buttons in the household or public toilets is considered to be low.

As the lowest reported oral LD_{50} value for the rat is 2512 mg/kg (Varshavskaja, 1967), a T-value of 250 would be expected. Currently, the T-value listed in the SUSDP for *p*-DCB is 50.

Environment

The chemical is biodegradable and relatively soluble in water. Its removal from aqueous systems occurs significantly from volatilisation, and at equilibrium, over 98% of the chemical would be expected to partition to the atmosphere where it will break down through reaction with hydroxy radicals. Concentrations likely to occur in aquatic systems are expected to be far lower than those of concern, and this expectation is supported by monitoring data from Australia and around the world. A low aquatic risk is predicted.

Additionally, the short atmospheric lifetime of *p*-DCB indicates concentrations will not occur at levels harmful to the atmosphere. While widespread transport within the troposphere is likely, the chemical is not expected to reach the stratosphere and therefore not expected to have an influence on global warming or ozone depletion.

No risks have been identified for the environment due to the use of *p*-DCB. However, there appears to be the potential for accumulation of *p*-DCB in sediments. No Australian data exists for this compartment, and levels should be monitored where possible to determine whether accumulation is a factor. It is noted that use levels of *p*-DCB in Australia have been declining over the last few years, and the trend appears to be for a continuing decline, which may negate this issue.

Data gaps

Generally, the toxicity of *p*-DCB has been extensively investigated using a number of critical endpoints. Consequently, further testing is not required. Within Australia the major data gap is a need for further workplace monitoring data.

19. Recommendations

19.1 Hazard classification

The recommended classification for *p*-DCB based on the health hazard assessment of currently available data and in accordance with the National Occupational Health and Safety Commission's *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999a), is:

- Do not breath vapour (Safety phrase S23)
- Avoid contact with skin (Safety phrase S24)
- Avoid contact with eyes (Safety phrase S25)
- Use only in well ventilated areas (Safety phrase S51)

MSDS, labels and training materials should be amended to provide appropriate information.

Due to its inclusion on the *List of Designated Hazardous Substances* (NOHSC, 1999b), *p*-DCB is considered to be a hazardous substance.

19.2 Hazard communication

19.2.1 Material safety data sheets (MSDS)

The NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994d) provides guidance for the preparation of MSDS.

It is recommended that Australian suppliers of *p*-DCB amend their MSDS taking into account the classification recommended in Section 19.1 and, where necessary, rectify the deficiencies identified by this assessment with particular attention being given to the following:

- inclusion of a statement of hazardous nature;
- inclusion of appropriate risk and safety phrases;
- inclusion of acute and chronic health effects;
- inclusion of appropriate first-aid advice;
- inclusion of appropriate advice to doctor;
- inclusion of Australian exposure standard;
- inclusion of appropriate engineering controls, such as exhaust ventilation in areas where vapours are likely to occur; and
- inclusion of an Australian emergency contact number.

A sample MSDS for *p*-DCB is provided at Appendix 3.

19.2.2 Occupational health and safety

The following recommendations are made:

- monitoring data for *p*-DCB are of either poor quality or non-existent. Consequently, it is recommended that quantitative monitoring of workplace *p*-DCB levels should be undertaken to determine actual worker exposure levels and to identify whether improvements in control measures are warranted;
- adequate ventilation should be provided to minimise worker exposure to *p*-DCB vapour;
- containers used for the temporary storage of *p*-DCB should be provided with lids when not in use; and
- regular removal of *p*-DCB dust from work areas should be undertaken.

19.2.3 Occupational exposure standard

It is recommended to NOHSC that the present occupational exposure standard for *p*-DCB of 75 ppm TWA (8 hr) be reviewed noting:

- eye and nose irritation in humans at 50 ppm; and
- the NOAEL of 75 ppm (inhalation) and a LOAEL of 300 ppm (inhalation) for increases in liver weight.

19.3 Public health

The requirements for the first aid instruction and general safety precautions for products in the public domain should be strictly adhered to. There are no changes recommended to the first aid instructions or general safety precautions for *p*-DCB. The lowest oral rat LD_{50} value quoted as a single value is 2512 mg/kg. Consequently, it is recommended that SUSDP consider re-assigning the T-value from 50 to 250.

20. Secondary Notification

Under Section 65 of the Act, the secondary notification of *p*-DCB may be required, where an applicant or other introducer (importer) of *p*-DCB, becomes aware of any circumstances which may warrant a reassessment of its hazards and risks. Specific circumstances include:

a) The function or use of *p*-DCB has increased, or is likely to change, significantly;

b) The amount of *p*-DCB introduced into Australia has increased, or is likely to increase significantly;

c) Manufacture of *p*-DCB has begun in Australia; or

d) Additional information has become available to the applicant/notifier as to the adverse health and/or environmental effects of *p*-DCB.

The Director must be notified within 28 days of the applicant/notifier becoming aware of any of the above circumstances.

Appendix 1

List of products containing *p*-DCB

This appendix provides a list of products containing *p*-DCB (Table 21) that were marketed in Australia in 1998/99. The list includes the trade name of each product, the use and the amount (in % w/w) of *p*-DCB in the product.

This list is not intended to be exhaustive but is considered to be representative of current *p*-DCB usage in Australia as indicated by information provided by manufactures and distributors of *p*-DCB products. Formulations may have changed since the preparation of the list and some products may no longer be commercially available.

Table 21 - List of products containing *p*-DCB

Product	Use	*p*-DCB (% w/w)
Air Freshener Blocks	Air freshener/Deodorant	98.8
Blue Bell Blocks	Deodorant - Toilets	100
Brickettes	Deodorant - Toilets	99.0
Dellas Air Freshener Tablet	Air freshener/Deodorant	99.0
De-Odo-Air	Deodorant - Toilets	99.0
Deodorant Buttons	Deodorant - Toilets	99.0
Fragrasan	Air freshener/Deodorant	99.2
Fresha	Air freshener/Deodorant	99.0
Fresh Air	Air freshener/Deodorant	99.2
Masquerades - Blue	Deodorant - Toilets	99.0
Masquerades - Lemon	Deodorant - Toilets	99.0
Parry's Fresh Air	Air freshener/Deodorant	99.2
Parry's Fresh Guard	Household Fumigant	99.2
1,4-Dichlorobenzene	Chemical reagent	100
Para-dichlorobenzene	Chemical reagent	97.5
Para-dichlorobenzene	Chemical reagent	99.9
Rainbow Blue	Air freshener/Deodorant	98.8

Appendix 2

Excerpt from the Approved Criteria (NOHSC, 1999a)

CARCINOGENIC SUBSTANCES

4.76 Substances are determined to be hazardous due to carcinogenic effects if they fall into one of the following categories:

Category 1 Substances known to be carcinogenic to humans.

Category 2 Substances which should be regarded as if they are carcinogenic to humans.

Category 3 Substances which cause concern for humans owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment.

EXPLANATORY NOTES REGARDING THE CATEGORISATION OF CARCINOGENIC SUBSTANCES

4.77 The placing of a substance into Category 1 is done on the basis of epidemiological data; placing into Categories 2 and 3 is based primarily on animal experiments.

CATEGORY 1

4.78 Substances are determined to be hazardous and classified as Toxic (T) and assigned risk phrase R45 or R49 in accordance with the criteria given below.

R45 MAY CAUSE CANCER

R49 MAY CAUSE CANCER BY INHALATION[2]

[2] For substances which present a carcinogenic risk only when inhaled, for example, dust, vapour or fumes (and where other routes of exposure, for example, by swallowing or in contact with the skin do not present any carcinogenic risk) the specific risk phrase R49 should be used.

4.79 A substance is included in Category 1 if there is sufficient evidence to establish a causal association between human exposure and the development of cancer on the basis of epidemiological data. The existence of a causal relationship would be any of the following:

- an increased incidence of one or more cancer types in an exposed population in comparison with a non-exposed population,
- evidence of dose-time-response relationships, that is, an increased cancer incidence associated with higher exposure levels or with increasing exposure duration,
- an association between exposure and increased risk observed in more than one study,
- demonstration of a decline in risk after reduction of exposure, and
- specificity of any association, defined as an increased occurrence of cancer at one target organ or of one morphological type.

CATEGORY 2

4.80.1 Substances are determined to be hazardous and classified as Toxic (T) and assigned risk phrase R45 or R49 in accordance with the criteria given below.

R45 MAY CAUSE CANCER
R49 MAY CAUSE CANCER BY INHALATION[2]

4.81 A substance is included in Category 2 if there is sufficient evidence, on the basis of appropriate long term animal studies or other relevant information, to provide a strong presumption that human exposure to that substance may result in the development of cancer.

4.82 For classification as a Category 2 carcinogen either positive results in two animal species should be available or clear positive evidence in one species, together with supporting evidence such as genotoxicity data, metabolic or biochemical studies, induction of benign tumours, structural relationship with other known carcinogens, or data from epidemiological studies suggesting an association.

[2] For substances which present a carcinogenic risk only when inhaled, for example, dust, vapour or fumes (and where other routes of exposure, for example, by swallowing or in contact with the skin do not present any carcinogenic risk) the specific risk phrase R49 should be used.

4.83 Human data providing suspicions of carcinogenic potential may warrant a Category 2 classification irrespective of the nature of any animal data. Increased confidence in the credibility of a causal relationship would be provided by evidence of carcinogenicity in animals and/or of genotoxic potential in short term screening tests.

CATEGORY 3

4.84 Substances are determined to be hazardous and classified as Harmful (Xn) and assigned risk phrase R40 in accordance with the criteria given below.

R40 POSSIBLE RISK OF IRREVERSIBLE EFFECTS

4.85 A substance is included in Category 3 if there is some evidence from appropriate animal studies that human exposure can result in the development of cancer, but this evidence is insufficient to place the substance in Category 2.

Category 3 actually comprises 2 sub-categories

(a) substances which are well investigated but for which the evidence of a tumour-inducing effect is insufficient for classification in Category 2. Additional experiments would not be expected to yield further relevant information with respect to classification;

(b) substances which are insufficiently investigated. The available data are inadequate, but they raise concern for humans. This classification is provisional; further experiments are necessary before a final decision can be made.

4.86 For a distinction between Categories 2 and 3 the arguments listed below are relevant which reduce the significance of experimental tumour induction in view of possible human exposure. These arguments especially in combination, would lead in most cases to classification in Category 3, even though tumours have been induced in animals:

- carcinogenic effects only at very high dose levels exceeding the 'maximal tolerated dose'. The maximal tolerated dose is characterised by toxic effects which, although not yet reducing lifespan, go along with physical changes such as about 10% retardation in weight gain,

- appearance of tumours, especially at high dose levels, only in particular organs of certain species known to be susceptible to a high spontaneous tumour formation,

- appearance of tumours, only at the site of application, in very sensitive test systems (e.g. intraperitoneal, or subcutaneous application of certain locally active compounds), if the particular target is not relevant to humans,

- lack of genotoxicity in short-term tests *in vivo and in vitro,*

- existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g. hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation),

- existence of a species-specific mechanism of tumour formation (e.g. by specific metabolic pathways) irrelevant for humans.

NO CARCINOGEN CLASSIFICATION

4.87 For a distinction between Category 3 and no classification arguments are relevant which exclude a concern for humans:

- a substance should not be classified in any of the categories if the mechanism(s) of experimental tumour formation is/are clearly identified, with good evidence that such mechanism(s) cannot be extrapolated to humans for each tumour,

- if the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories,

- particular attention should be paid to cases where the only available tumour data are the occurrence of neoplasms at sites and

in strains where they are well known to occur spontaneously with a high incidence.

Appendix 3

Sample Material Safety Data Sheet for 1,4-Dichlorobenzene

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1,4-Dichlorobenzene is classified as Hazardous according to the National Occupational Health and Safety Commission's *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(1999)]

Physical description and properties

Appearance
White or colourless crystalline solid with penetrating aromatic odour.

Melting point
53.1°C (127.4°F)

Boiling point
174.6°C

Vapour pressure
0.84 hPa at 20°C

Specific gravity
1.248 (water = 1)

Flashpoint
66°C (150°F) (closed cup)

Flammability limits
Not available

Solubility in water
60 to 70 mg/l (at 20°C)

Other properties

Odour: Aromatic odour.

Odour threshold: 0.18 ppm

Density: 1.248 g/cm^3 (at 20°C)

Ignition temperature: >500°C

Ingredients

Chemical Name: 1,4-dichlorobenzene **CAS Number:** 106-46-7 **Proportion:**

Health hazard information

HEALTH EFFECTS

Acute

Inhalation: Low acute inhalation toxicity. Vapour may be irritating to the nose at 50 ppm or greater. May cause headache, dizziness, nausea, vomiting and breathing difficulties. High doses may cause depression of the central nervous system.

Skin: Low acute dermal toxicity in animal studies. May cause burning sensation on prolonged contact with solid material.

Eye: Vapour irritating to the eyes at 50 ppm or greater.

Swallowed: Low acute oral toxicity. Symptoms may include, headache, nausea, vomiting and anaemia.

Chronic

Skin: No evidence of sensitisation in animals or humans.

Systemic: In humans, ingestion over prolonged periods may cause reversible neurological symptoms including unsteady gait, incoordination and paresthesia (tingling) of the limbs. Haematological disorders can include anaemia. Has been shown to cause kidney tumours in rats by ingestion and liver tumours in mice by ingestion and inhalation.

FIRST AID

Inhalation: Remove from exposure to fresh air immediately. Victim may appear intoxicated. Keep warm and at rest until fully recovered. If breathing is laboured and patient cyanotic (bluish colouration of skin and mucus membranes) give oxygen. If the victim is not breathing, clear airway and apply artificial respiration. Call a doctor.

Skin: Remove contaminated clothing. Wash affected area immediately with copious quantities of water and non-abrasive soap (at least 15 minutes). Seek medical attention if irritation develops.

Eye: Irrigate immediately with copious quantities of water or normal saline for at least 15 minutes. Seek medical attention.

Swallowed: Do not give anything by mouth if victim is losing consciousness, unconscious or convulsing. If more than 15 minutes from medical attention induce vomiting, preferable with Ipecac Syrup APF. Do not give milk or oils. Seek medical attention.

Alcohol consumption may accelerate the onset and severity of symptoms caused by ingestion of *p*-DCB.

Contact a *Poisons Information Centre* for further information.

ADVICE TO DOCTOR

Treatment is symptomatic and supportive. No specific antidote.

Precautions for use

EXPOSURE STANDARD

Australian Exposure Standard: 75 ppm (451 mg/m^3) TWA (8 hr), 110 ppm (661 mg/m^3) STEL

ENGINEERING CONTROLS

Control airborne concentrations below the exposure standard.

Use only with adequate ventilation.

Local exhaust ventilation may be necessary for some operations.

PERSONAL PROTECTION

Wear overalls, rubber footwear, safety glasses and gloves in accordance with manufacturer's recommendations. A respirator with full-face protection may be required where engineering controls are inadequate, such as during clean-up of large spills.

An emergency eye wash station should be available in the immediate work area.

Self contained breathing apparatus (SCBA) and complete protective clothing should be worn during fire fighting.

FLAMMABILITY

Combustible solid.

Safe handling information

STORAGE and TRANSPORT

Non-regulated goods. Store in a cool, dry place and out of direct sunlight and away from naked flame and sources of ignition. Ensure adequate ventilation.

Store away from incompatible materials (see FIRE/EXPLOSION HAZARD).

SPILLS and DISPOSAL

Evacuate unprotected personnel from spillage area.

Shut off all possible sources of ignition following spillage.

Increase ventilation in contaminated area.

Clean-up personnel should wear self-contained breathing apparatus and full protective clothing.

Place collected material into metal or plastic containers and dispose of in accordance with all Local, State and Federal regulations at an approved waste disposal facility.

FIRE/EXPLOSION HAZARD

Incompatible materials: organic peroxides and strong oxidising agents.

Vapour is heavier than air.

Toxic and irritant vapours and gases, including oxides of carbon, hydrogen chloride and phosgene, may be formed on combustion.

Fire fighting:

- wear SCBA and complete protective clothing.
- Water fog, foam, alcohol foam, carbon dioxide or dry chemical extinguishing media may be used.

Other information

Animal toxicity data:

Acute (inhalation) LD_{50} (4hr): LC_{50} > 5.07 mg/litre (rat).

Acute (oral) LD_{50} : 3863 to 3790 mg/kg bw (rat).

Acute (dermal) LD_{50}: >6000 mg/kg (rat).

Reproductive and developmental data: Negative results.

Mutagenic data: Negative results for mutagenicity by several test systems.

Environmental data:

1,4-Dichlorobenzene is moderately toxic to aquatic life.

Acute:

Daphnia magna	48h EC_{50}	0.7 mg/L
Mysidopsis bahia	96h EC_{50}	1.99 mg/L
Brachydanio rerio (Zebra fish)	96h LC_{50}	2.1 mg/L
Pimephales promelas (Fathead minnow)	96h LC_{50}	4.2 mg/L
Oncorhynchus mykiss (Rainbow trout)	96h LC_{50}	1.12 mg/L

Further information:

National Industrial Chemicals Notification and Assessment Scheme, *para*-Dichlorobenzene - Priority Existing Chemical Assessment Report No. 13 , NICNAS, Sydney, 2000.

Contact point

Contact name | Telephone number

Position title

Address

State | Postcode | Country

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