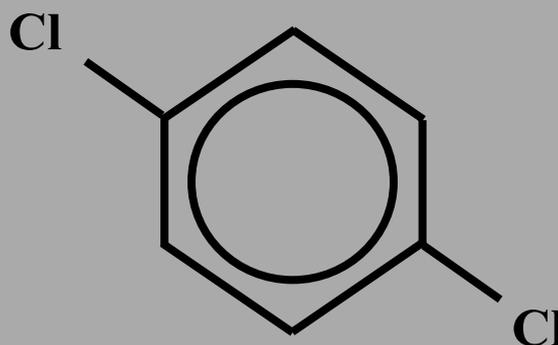# European Union Risk Assessment Report

CAS No: 106-46-7

EINECS No: 203-400-5

1,4-dichlorobenzene

1st Priority List

Volume: 48

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European Union Risk Assessment Report

1,4-DICHLOROBENZENE

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RISK ASSESSMENT

1,4-DICHLOROBENZENE

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RISK ASSESSMENT

Final Report, 2004

France

The French rapporteur for the risk evaluation of 1,4-dichlorobenzene is the Ministry of the Environment with the Ministry of Health and the Ministry of Work.

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Final report: 2004

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.

The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, this is then presented at a Meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) which gives its opinion to the European Commission on the quality of the risk assessment.

If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.

The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992.

This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.
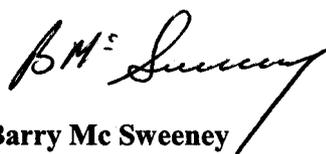
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[1] O.J. No L 084, 05/04/199 p.0001 – 0075

[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011

[3] Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0   OVERALL RESULTS OF THE RISK ASSESSMENT

CAS No: 106-46-7
EINECS No: 203-400-5
IUPAC Name: 1,4-Dichlorobenzene

Environment

Conclusion (ii)   There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

This conclusion is reached for the exposure of the aquatic compartment (including the sediment), the atmosphere, the terrestrial compartment, as well as for predators.

Human health

Human health (toxicity)

Workers

Conclusion (iii)   There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Taking into account the currently available toxicological data and the estimated occupational exposure, this conclusion is reached because of:

- nasal and ocular irritation due to vapour exposure during use for formulation of products containing the substance and production of grinding wheels,

- general systemic toxicity, carcinogenicity and reproductive toxicity due to exposure mainly via inhalation and dermal, during manufacture and use (intermediate, formulation of products containing the substance and production of grinding wheels).

Consumers

Conclusion (iii)   There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Taking into account the currently available toxicological data and the estimated consumer exposure, this conclusion is reached because of:

- carcinogenicity due to inhalation exposure arising from use of moth repellents, air fresheners and toilet blocks.

Humans exposed via the environment

Conclusion (ii)   There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Human health (risks from physicochemical properties)

Conclusion (ii)   There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

CONTENTS

Euses Calculations can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it

TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS No: 106-46-7
EINECS No: 203-400-5
IUPAC-Name: 1,4-Dichlorobenzene
Synonyms: p-Dichlorobenzene, Paradichlorobenzene,
                     p-chlorophenyl chloride, Dichlorocide
Molecular formula: $C_6H_4Cl_2$
Molecular weight: 147.01
Structural formula:
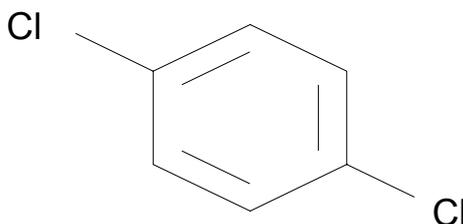
1.2 PURITY/IMPURITIES, ADDITIVES

Degree of purity of the produced/imported products within the EU: 99.7-99.9%

Impurities: 1,2-dichlorobenzene   <= 0.1%
                1,3-dichlorobenzene   <= 0.1%
                chlorobenzene   <= 0.05%
                trichlorobenzene   <= 0.05%

1.3 PHYSICO-CHEMICAL PROPERTIES

In **Table 1.1** the physico-chemical properties are summarised.

Table 1.1   Physico-chemical properties of 1,4-dichlorobenzene

Property	Result
Physical state	solid, colourless or white crystals (flakes/granular)
Melting point	52.8-3.5°C
Boiling point	173-174°C
Density	1.25-1.46 g/cm³ at 20°C 1.23 g/cm³ at 70°C
Bulk density	0.65 g/cm³ (granular form) 0.788 g/cm³ (scale form)
Vapour pressure	160 170 Pa at 2°C * 1,330 Pa at 54.8°C *
Water solubility	60-70 mg/l at 20°C **

Table 1.1 continued overleaf

Table 1.1 continued Physico-chemical properties of 1,4-dichlorobenzene

Property	Result
Henry's law constant	240-262 Pa · m³/mol (at 20°C) ***
Partition coefficient *n*-octanol/water	log Pow = 3.37-3.39 (experimental) ****
Flash point	65- 66°C (closed cup)
Flammability limits in air at 20°C, 101 k Pa	lower = 1.7 (%V) upper = 5.9 (%V)
Autoflammability	no autoflammability up to 500°C

*   Only handbook data or values from MSDSs are available. As the values differ only slightly from each other, they seem to confirm each other.

**   Only handbook data or values from MSDSs are available. As the values differ only slightly from each other, they seem to confirm each other.

*** The value of 262 Pa · m³/mol appears to be the most reliable as some data on the test method is available (Ashworth et al., 1988)

**** Only the value of 3.37 is validated. For the further assessment, a rounded value of 3.4 will   be used.

A test on flammability according to Method A10 (Annex V of Directive 67/548/EEC) was negative.

These data are mainly derived from Auer-Technikum (1985), Rathjen (1975), Mackay (1981), CRC Handbook of Chemistry and Physics (1971), Wasik (1983), Leo (1971), Amoore (1983), Yalkowsky (1979), McNally (1983), Brown (1997) and from the Safety Data Sheet of producers/importers.

1.4   CLASSIFICATION

Classification and labelling according to the 29th ATP of directive 67/548/EEC:

Classification

Xi; R36	Irritating to eyes
Carc. Cat 3; R40	Limited evidence of a carcinogenic effect
N; R50-53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Labelling

Xn; N
R: 36-40-50/53
S: (2-)36/37-46-60-61

The classification of the substance is established by Commission Directive 2001/59/EC of April 2004 adapting to technical progress for the 29th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (OJ L216/30, 16.06.2004, p.1).

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION, IMPORT, EXPORT AND CONSUMPTION VOLUMES

Data from 5 producers/importers are included in the IUCLID-database. These are listed in **Table 2.1**.

Table 2.1 Producers/importers having submitted a HEDSET diskette

Bayer AG, DE
Elf Atochem, F
Enichem Synthesis, IT
Esar, F
Hoechst, DE

In Europe, for 1987 to 1988, a total 1,4-dichlorobenzene production of about 33,000 to 35,000 tonnes/year was reported. The import volume was 4,500 tonnes/year in 1985, whereas 16,500 tonnes/year were exported. The consumption in Europe calculated with these values would have been 22,950 tonnes/year in 1985. In 1987, the 1,4-dichlorobenzene consumption decreased to 20,500 tonnes/year, and 16,400 tonnes/year were used in 1991 (BUA, 1994). Of the last quantity mentioned, 7,000 tonnes/year were processed to 2,5-dichloronitrobenzene (precursor for dyes and pigments). The remaining 1,4-dichlorobenzene was formulated to air fresheners or toilet blocks (3,500 tonnes/year) and moth repellents (4,500 tonnes/year). Since 1992 1,4-dichlorobenzene has no longer been processed to polyphenylenesulfide in Western Europe. Hoechst discontinued 1,4-dichlorobenzene production in 1992.

The consumption quantities world-wide for the three most important manufacturing regions, Western Europe, the USA and Japan, were reported to be 113,000 tonnes/year in 1989 (BUA, 1994). In general a decreasing consumption of 1,4-dichlorobenzene has been observed during the last few years.

The total production/import quantities reported by the EU-producers/importers having submitted a HEDSET diskette is between 22,500 and 30,500 tonnes/year for 1994. For reasons of confidentiality, no specific producer-related quantities are reported here.

In CEFIC - Eurochlor (1995), an export quantity outside Europe is given: 14,835 tonnes/year in 1994. In conclusion, the overall 1,4-dichlorobenzene consumption in the EU is estimated to be at most 15,000 tonnes/year in 1994.

2.1.1 Production process

1,4-Dichlorobenzene is produced by direct chlorination according to a continuous method where liquid benzene is converted with gaseous chlorine in the presence of a catalyst. Through the choice of molar ratio between benzene and chlorine the isomeric ratio of 1,2- to 1,4-dichlorobenzene can be influenced. The chlorination products are separated by distillation (BUA, 1994). This production process is considered in the main category Ib, closed system,

isolated intermediates. After crystallisation, the final product can be packaged and transported in solid or liquid form; the corresponding operations are performed in closed systems.

2.1.2   Uses

Most of the amount produced is processed to 1,4-dichloro-2-nitrobenzene, a precursor for dyes and pigments. 1,4-Dichloro-2-nitrobenzene is synthesised in a continuous procedure by nitration of 1,4-dichlorobenzene with nitrating acid (nitric acid/sulphuric acid). After separation of the sulphuric acid and the remaining nitric acid, the raw product is washed with sodium hydroxide and water and is subsequently purified by fractionating crystallisation (BUA, 1991).

This procedure is considered in the industrial category 3, chemical industry: chemicals used in synthesis and use category 33, intermediates.

Otherwise, 1,4-dichlorobenzene is formulated to moth repellents (industry category 5, personal/domestic use; use category 39, biocides, non-agricultural), air fresheners and toilet blocks (industry category 5, personal/domestic use; use category 36, odour agents). 1,4-Dichlorobenzene acts mainly to disguise odours. The toilet blocks are used in standing urinals and urinal drains and are not hung in flushing tanks or toilet bowls (BUA, 1994).

The consumption in The Netherlands for these uses is estimated by TNO (1995) to be 100 tonnes/year for 1990.

A minor use of 1,4-dichlorobenzene is as a processing aid in the production of grinding wheels (industry category 2: chemical industry, basic chemicals; use category 43: process regulators) (personal communication, Arbeidstilsynet, Norway, 1997; personal communication, Bayer AG, 1997). For the production of porous grinding material, a so-called burnout substance is mixed with the grinding material (aluminium oxide, silicium carbide etc.). Material such as cork, naphthalene or 1,4-dichlorobenzene can be used.

After mixing and shaping, the grinding wheels are dried and then heated to temperatures of 1,100-1,300°C. 1,4-Dichlorobenzene can be recovered during the drying process or is thermally destroyed during the heating process. The European consumption is estimated at 100 tonnes/year (personal communication, Bayer AG, 1997).

1,4-Dichlorobenzene has been detected in wastewater effluents of paint producers and textile dyeing companies in France (INERIS, 1994). According to FET (1995), 1,4-dichlorobenzene can be used as carrier for textile dyes mainly polyester and wool dyes but is more and more replaced by alkylnaphthalenes.

Polyphenylenesulphide (PPS) contain 1,4-dichlorobenzene as an impurity of ca. 0.01%. PPS are not produced within the EU but imported. The use of PPS could contribute to the total releases of 1,4-dichlorobenzene in the environment (personal communication, KEMI, Sweden).

In **Table 2.2**, the quantitative use pattern as estimated by CEFIC-Eurochlor (1995) is reported for 1994.

Table 2.2   Use pattern of 1,4-dichlorobenzene in Europe

Use	Quantity [tonnes/year]	[%]	IC	UC
Intermediate	7,154	49.3	3	33
Toilet blocks / air fresheners	3,170	21.9	5	36
Moth repellents	4,070	28.1	5	39
Grinding wheels	100	0.7	2	43
Total	14,494	100		

Size and number of processing and formulation sites are not given. Production sites may process and formulate at the same site; however, as no accurate data are available, each step in the life cycle of 1,4-dichlorobenzene will be considered separately.

Releases to the environment could also be possible from the degradation of higher chlorinated homologues. Due to lack of quantitative information, this has not been taken into account in this risk assessment.

2.1.3   Legislative controls

1,4-Dichlorobenzene is one of the chemicals identified by the Commission of the European Communities as being a list 1 compound under the Dangerous Substances directive (76/464/EEC). A daughter directive (86/280/EEC) has set limit values for the emission of 1,4-dichlorobenzene from industrial plants. An average monthly limit value of 1.5 mg/l was fixed.

The Swedish Pesticide Ordinance (1985:836) has banned the use of 1,4-dichlorobenzene as a pesticide. The Swedish National Chemical Inspectorate prescribes the following: Chemical products which contain 1,4-dichlorobenzene (paradichlorobenzene, para-chlorophenylchloride, para-dichlorobenzol) and which are intended to disguise odours may not be offered for sale, transferred or used on a professional scale. These regulations entered into force on January 1st, 1990.

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

There are no known natural sources of 1,4-dichlorobenzene. On the basis of its volatility and the disperse nature of its uses, it is expected that most of the volume used in the public sector is released into the environment.

The release of 1,4-dichlorobenzene will be estimated by order of preference from specific information (e.g. from producers/users, use category document) and emission factors.

3.1.2 Environmental fate

3.1.2.1 Distribution

An equilibrium partitioning can be calculated according to the Mackay Model I at 20 °C, with a vapour pressure of 170 Pa and a water solubility of 70 mg/l (Mackay, 1981):

Air: 98.9%
Water: 0.79%
Soil: 0.15%
Sediment: 0.16%

3.1.2.2 Degradation

Wahner and Zetsch (1983) carried out measurements to determine rate constants for the addition of OH-radicals to aromatics in the atmosphere. The OH rate constant obtained with 1,4-dichlorobenzene was determined to be $3.2 \cdot 10^{-13}$ cm^3/molecules.

Klöpffer et al. (1986) realised OH-rate measurements in smog-chambers. This time, an OH rate constant of $4.8 \cdot 10^{-13}$ cm^3/molecules was determined.

Assuming a hydroxyl radical concentration of $5 \cdot 10^5$ molecules/cm^3, half-lives of between 33 and 50 days can be calculated.

No experimental data on hydrolysis is available. Based on its molecular structure, no hydrolysis is expected though.

Biodegradation

Only very few results from standard test systems, where mineralisation is determined are available. Calamari et al. (1982) tested the degradation of 8 and 40 mg 1,4-dichlorobenzene/l by primary sludge in a Sapromat E. The test was comparable to a MITI (I) test. The degradation for the 8 mg/l test concentration reached 0% after 14 days and 80% after 28 days (100% primary degradation); 40 mg/l probably had a toxic effect and degradation rates of 0 and 30% were observed after 28 days. The publication does not state whether the 10-day window criterion was

respected or not. By plotting the results on a graph though, it can clearly be seen that more than 60% were degraded 10 days after reaching 10% biodegradation.

Topping (1987) tested 1,4-dichlorobenzene (1.9 mg/l) for ready biodegradability in a Closed Bottle Test (OECD guideline D): 1.4% was degraded after 8 days, 49.5% after 15 days and 67% after 28 days. Disappearance of the substance was confirmed by specific analysis of 1,4-dichlorobenzene, 1,5-dichlorphenol and 4-chlorophenol. The 60% threshold prescribed by the guideline was passed. Due to the lack of intermediate results, it is not possible to conclude whether the 10-day window was respected or not.

In both test systems, the 60% level was reached whereas the 10-day window criterion was unequivocally fulfilled in only one of the tests. Nevertheless, considering both test results together, 1,4-dichlorobenzene can be classified as "readily biodegradable".

There are no simulation tests on biodegradation of 1,4-dichlorobenzene in surface water available.

For soil and sediment, there are no results from standardised biodegradation systems available. Several tests with flooded soil columns indicating that 1,4-dichlorobenzene is degraded to a high extent in soil are available (Kuhn et al., 1985; Hutchins et al., 1984), but no degradation rate can be extrapolated from those tests.

The biodegradation rates for surface water, soil and sediment are therefore estimated according to the procedure outlined in the Technical Guidance Document (TGD, EC, 1996) (see **Table 3.1**).

Table 3.1 Estimation of biodegradation rate constants in the different compartments

Compartment / medium	Biodegradation rate
Surface water	ksw = 0.046 d^{-1}
Sediment 1)	ksed = 0.002 d^{-1}
Soil 1)	ksoil = 0.023 d^{-1}

1) The biodegradation rates in sediment and soil take account of adsorption to solid matter
Koc = 450 l/kg, see below)

3.1.2.3 Elimination in sewage treatment plants (STPs)

Based on the above cited physical chemical properties (log H = 2.4; log Pow = 3.4), as well as the biodegradation rate of 1 h^{-1} in a STP, the elimination through biodegradation and distribution can be estimated with the model SIMPLETREAT:

Table 3.2 Estimation of removal of 1,4-dichlorobenzene in STPs according to SIMPLETREAT

% to air	37
% to water	6
% to sludge	6
% degraded	50
% removal	94

On the other hand, simulation tests and STP monitoring data are available, providing a more realistic description of the behaviour of 1,4-dichlorobenzene in STPs.

Topping (1987) investigated the biodegradability of 1,4-dichlorobenzene in an OECD Confirmatory Test. After a 15-day adaptation period, the average elimination rate was 97% (15 measurements over 21 days, influent concentration 1 mg/l; primary degradation). The removal of TOC was ca. 88%. Considering elimination through volatilisation (ca. 67%) and adsorption to sewage sludge (< 0.01%), the elimination by biodegradation is ca. 30%.

To confirm the potential biodegradation, a porous pot activated sludge test was performed. Primary settled sewage was dosed into the pots to give a sewage retention time of 6 hours. Sludge retention time (SRT) was adjusted to 6 or 3 days. The temperature was 20, 15 and 8°C 1,4-Dichlorobenzene was monitored in influent, effluent, waste sludge and exhaust air. The removal was mainly due to biodegradation at 15 and 20°C, even at low sludge retention times:

SRT	removal	due to stripping
6 d	95-100%	7-28%
3 d	96-100%	22-29%

At SRT = 3 days and at a low temperature of 8°C, biodegradation diminished seriously, as the part due to stripping rose to 63%, without effect upon the overall removal rate. Little or no 1,4-dichlorobenzene was found on waste activated sludge.

Furthermore the elimination of 1,4-dichlorobenzene was monitored in pilot plants and full scale STPs.

In pilot plants, the removal was respectively ca. 95% (Hannah et al., 1988) and 90% (Parker et al., 1993). In the second plant, the elimination processes were monitored and were respectively: stripping 68%; degradation 22%; adsorption 0.8%.

The elimination in full scale STPs varies from 60-69% (Melcer et al., 1988) to 74% (Parker et al., 1993). The most reliable data are those from Melcer et al. (1988), with ca. 100 paired measurements for 3 STPs each. Unfortunately there is not enough data available on operating parameters to compare the results with those from the pilot plants or from the SIMPLETREAT estimations.

Comparing the data from the Confirmatory Test as well as the pilot plants with the SIMPLETREAT estimation, it becomes clear that the contribution to removal by degradation and adsorption is overestimated while the stripped part is underestimated. Generally also, the total removal in the full scale STPs is well below the removal in the pilot plants the Confirmatory Test or the SIMPLETREAT estimation. This difference could be explained by the very low influent concentrations in the full-scale STPs (2.2-4.2 µg/l) compared to the spiked influents in the pilot plants and the Confirmatory Test (17.7–1,000 µg/l).

For the present assessment, the results from the full scale STPs will be used to estimate environmental concentrations. A removal of 70% seems to be most appropriate. The importance of the different removal processes can be estimated based on the data from the Confirmatory Test as well as from the pilot plants, which are in good agreement with each other: stripping: ca. 50%; degradation, ca. 19% and adsorption ca. 1%.

3.1.2.4 Adsorption-Accumulation in soil

In **Table 3.3** the results from soil sorption experiments with 1,4-dichlorobenzene are summarised.

Table 3.3 Sorption of 1,4-dichlorobenzene in different soils

Soil	Sand %	Silt %	Clay %	org C %	pH	Kf l/kg	1/n	Kp l/kg	Koc l/kg	Reference
Sandy agricultural soil (KOBG)	86.5	7.5	1.4	2.2	4.8	8	0.96		364	van Gestel et al. (1991)
OECD	72.1	7.4	8.1	4.7	5.9	18	0.65		383	van Gestel et al. (1991)
Silty loam	9	68	21	1.1					273	Chiou et al. (1983)
Parabraun soil	12.9	64.3	19.6	0.76	7.45	5.5	1.07		724	Frische et al. (1981)
Podsol	81.5	10	7.2	3.56	3.88	26.5	0.76		744	Frische et al. (1981)
Rendzina	8.5	68.3	20.6	1.11	7.9	8.3	0.87		748	Frische et al. (1981)
Dormont	2	38	60	1.2	4.2			3.36	280	Southworth and Keller (1986)
Apison	4	10	86	0.11	4.5			0.73	665	Southworth and Keller (1986)
Fullerton	11	21	68	0.05	4.4			0.43	850	Southworth and Keller (1986)
Peat soil				17		73.6-76	0.94-0.97	71	423	Friesel et al. (1984) [1]
Sandy soil	90	8	2	2.6		4.05	0.62		155	Uchrin and Lewis (1988) [2]
Aquifer				0.024	5.3			0.143	595	Miller et al. (1989)
Sediment (Ø < 125 μm)				0.73				4.4	602	Schwarzenbach and Westall (1981)
sediment (63 < Ø < 125 μm)				0.08				1.1	1375	Schwarzenbach and Westall (1981)

1) Saturation of adsorption not obtained
2) No indication about redox potential

The obtained results vary from 155 to 1,375 l/kg. The test conditions are not available in detail for all the tests. Some tests were performed with very low contents of organic carbon thereby increasing the possible error of the result (the OECD Guideline 106 suggests an organic carbon content of 0.6-3.5%). By eliminating the results with soils outside this range, the remaining Koc values range between 155 and 748 l/kg. For the assessment, an average Koc value of 450 l/kg will therefore be used.

For the different media, using the standard organic carbon contents proposed in the TGD, the water - solids and total compartments - water partition coefficient can be estimated. The results are presented in **Table 3.4**.

Table 3.4 Partition coefficients between different compartments

Compartments	OC-content (%) of solid phase	Solids-water partition coefficient	Total compartment - water partition coefficient
Soil-water	2	K_{p_soil} = 9 l/kg	K_{soil_water} = 13.7 m³/m³
Sediment - water	5	K_{p_sed} = 22.5 l/kg	K_{sed_water} = 12.1 m³/m³
Suspended matter - water	10	K_{p_susp} = 45 l/kg	K_{susp_water} = 12.2 m³/m³

3.1.2.5   **Bioaccumulation**

In **Table 3.5** all the results from fish-bioaccumulation experiments with 1,4-dichlorobenzene are summarised.

The obtained results vary from 55 to 1,400. The test conditions are not available in detail for all the tests. While the result seems to be independent of the water concentration, there seems to be a clear relationship with the fat content of the fish. The highest BCF of 1,400 has only been observed for the 23-day development stage in *Oncorhynchus mykiss* (i.e. at hatching) (Calamari et al., 1982). As this is probably irrelevant for indirect human exposure assessment, this result will not be taken into account. For the assessment, a worst-case BCF-value of 296 will be used. On the other hand, for secondary poisoning, these findings should be taken into account, as there are numerous predators of fish alevins.

The calculated BCF based on the Kow and according to the TGD would amount to 154.

Table 3.5 Bioaccumulation of 1,4-dichlorobenzene in fish

Species	System	Fat %	Exposure [day]	Water conc [µg/l]	Elimin. DT50 [day]	BCF	Reference
Oncorhynchus mykiss (eggs)	flow-through	4.3	2	13.4 [1]	> 1	55	Galassi et al. (1982)
Oncorhynchus mykiss (eyed egg)	flow-through	8.1	2	13.4 [1]	> 1	128	Galassi et al. (1982)
Oncorhynchus mykiss (hatchlings)	flow-through	7.6	2	13.4 [1]	> 1	152	Galassi et al. (1982)
Oncorhynchus mykiss (adsorbed half yolk)	flow-through	-	2	13.4 [1]	< 1	220	Galassi et al. (1982)
Oncorhynchus mykiss (not completely adsorbed yolk)	flow-through	3.6	2	13.4 [1]	< 1	155	Galassi et al. (1982)
Oncorhynchus mykiss (alevins)	flow-through	2.3	2	13.4 [1]	< 1	45-63	Galassi et al. (1982)
Lepomis macrochirus	flow-through	-	14	10.1	< 1	60	Barrows et al. (1980)
Oncorhynchus mykiss (larvae)	-	-	7	3	< 1	112	Calamari et al. (1982)

Table 3.5 continued overleaf

Table 3.5 continued Bioaccumulation of 1,4-dichlorobenzene in fish

Species	System	Fat %	Exposure [day]	Water conc [µg/l]	Elimin. DT50 [day]	BCF	Reference
Oncorhynchus mykiss (larvae)	-	-	7	15	< 1	40	Calamari et al. (1982)
Oncorhynchus mykiss (larvae)	-	-	7	73	< 1	85	Calamari et al. (1982)
Pimephales promelas	flow-through	3.2-4.1	28	570–1,000	-	110	Carlson and Kosian (1987)
Jordanella floridae	flow-through	8.5	28	5	0.7	296	Smith et al. (1990)
Poecilia reticulata	flow-through	6.5	20	-	-	98	Gobas et al. (1991)
Oncorhynchus mykiss (egg to alevin)	flow-through	-	60	3	-	100-1,400 [2]	Calamari et al. (1982)

1) The results at 4.5 and 65.6 µg/l were lower: BCF 24–188
2) The high BCF was only observed at hatching. It fell to ca. 100 at the end of the test:
   15 days after fertilisation: BCF = ca. 400
   23 days after fertilisation: BCF = ca. 1,400
   30 days after fertilisation: BCF = ca. 300
   60 days after fertilisation: BCF = ca. 100

A further test was performed with *Oncorhynchus mykiss* in Rhine water; (aerated flow-through system, three fish) (LWA, 1989). 100 µg 1,4-dichlorobenzene/kg FW (FW = fresh weight) was detected in intestines, 10 µg 1,4-dichlorobenzene in muscle meat. The trout were 10 weeks old and kept in an open flow-through system for 10 months. All analytical values of the Rhine water were below the detection limit of 0.5 µg/l. The concentrations detected in the fish may origin from the 1,4-dichlorobenzene concentration not detected analytically or from possible metabolism of higher chlorinated benzenes. The concentration in the whole fish is not reported. The concentrations detected indicate a BCF of > 20 for muscle meat and a BCF of > 200 for the intestines. Assuming that the intestines amount to 1/3 of the fresh weight of fish, a BCF of > 80 can be estimated for the whole fish. This is still consistent with the above chosen BCF value of 296.

No data on bioconcentration in earthworms is available. A BCF value can be estimated according to the method described in the TGD.

The partition coefficient earthworm-porewater can be estimated as:

$$K_{worm-porewater} = 0.25 \cdot 0.16 \cdot K_{ow} = 100.5 \text{ l/kg (wet earthworm)}$$

The BCF is estimated as:

$$BCF_{earthworm} = K_{worm-porewater} \cdot RHO_{soil}/K_{soil-water} = 12.5 \text{ kg/kg (wet earthworm)}$$

3.1.3   Aquatic compartment

3.1.3.1   Releases to surface water

The following release estimates, based on the emissions reported from the producers and some processors, can be established.

3.1.3.1.1   Release from production

Producer 1

Production	Use as an intermediate	Release due to Production and Use	%	Year
18,000 tonnes/year	1,440 tonnes/year	< 256 kg/year	< 0.0013	1991

In fact, the 1,4-dichlorobenzene released into receiving water at this plant could not be quantified, since for all 54 measurements the concentrations of the treated effluent were below the analytical determination limit of 5 µg/l in 1991.

Based on the detection limit and a mean water volume in the water outlet of 130,000 to 140,000 m^3/day, a maximum introduction can be calculated. The industrial wastewaters (60,000 to 70,000 m^3/day) are further treated in a second purification step along with 70,000 m^3/day municipal water.

Producer 2

Production	Use as an Intermediate	Release due to Production and Use	%	Year
2,000 tonnes/year	1,060 tonnes/year	120 kg/year	0.006	1994

Concentrations in wastewaters are measured after stripping treatment.

Producer 3

Production	Release due to Production	%	Year
5,500 tonnes/year	3.2 tonnes/year	0.06	1994

At this site no wastewater treatment occurs. The results are based on daily monitoring. This represents the worst-case and will be used in the risk assessment for the calculation of local concentration due to production.

The fourth HEDSET-submitting company is importing and selling only.

As it cannot be distinguished between the releases due to production and use at the same site, it is assumed that the total releases are due to production. As the number of production sites is very low, the regional releases due to production are supposed equal to the highest local release. In summary, the releases to surface water due to production are:

- local: 3.2 tonnes/year,

- regional: 3.2 tonnes/year,

- continental: 3.6 tonnes/year.

3.1.3.1.2   Release from use as an intermediate

The releases indicated by Producer 1 and Producer 2 include those from the on-site chemical transformation. The contributions from the production or transformation processes are not known.

Further data are available from a different site (personal communication), where about 1,200 tonnes/year of 1,4-dichlorobenzene are converted to 1,4-dichloro-2-nitrobenzene. The resulting wastewaters are treated by adsorption on active carbon followed by a biological treatment plant. The releases into surface water are indicated to be less than 2.5 kg/year. The sewage sludge resulting from the STP is incinerated.

No data is available on the missing 3,450 tonnes/year which are also supposed to be used as a chemical intermediate. A default exposure assessment according to the TGD can be performed with the missing 3,450 tonnes/year (cf. Appendix A, EUSES-output).

A realistic release factor can be deduced based on the data submitted from 3 producers of 2,5-dichloronitrobenzene (one of them has stopped production and is not taken into account for overall releases). Based on measurements in the raw wastewater from the production process (monthly measurements over at least two years), average release factors of 0.1 kg/tonne into biological sewage treatment plants can be deduced at two production sites. Higher release factors of 0.2-1 kg/tonne were measured at the beginning of a campaign, i.e. the first month of the production. At a third site, a release into raw wastewater of up to 6.4 kg/tonne is expected, but the raw wastewater is primarily treated in an activated coal column before release into a biological sewage treatment plant, reducing the input to 0.02 kg/tonne. It has to be noted though that measurements are available only from sewage after passage through the activated coal column (trimestrial measurements over 4 years) and that the release from the process is estimated based on the removal efficiency of the activated coal column.

In summary, based on data from three sites, the release factor into biological sewage treatment plants vary from 0.002-0.1%, with an average of 0.002-0.01%.

Given that extensive data is available from only a reduced amount of sites, a cautious value of 0.1% will be used in the default scenario. Applied to the missing 3,450 tonnes/year, a total amount of 3.45 tonnes/year is estimated to be released to the wastewater.

At a local level, the fraction of main source is estimated at 0.3, i.e. the local release to wastewater would amount to 1.15 tonnes/year, over a duration of 207 days. For the regional estimation, the total release from the missing 3,450 tonnes/year is used as no information on the site locations is available.

In summary, the releases to wastewater due to the use as an intermediate are:

- local: 1.15 tonnes/year,

- regional: 3.45 tonnes/year,

- continental: 3.45 tonnes/year.

3.1.3.1.3   Release from formulation of air fresheners or moth repellents

Three formulation sites have been visited by the producers for gathering data on exposure (personal communications). Aqueous waste does usually not occur. Cleaning operations are also usually mechanical and do not involve liquid residues. Releases to water occur through experimental toilets though, in which the finished products are tested.

More specific data are available from one of the largest formulator of 1,4-dichlorobenzene containing air fresheners and moth repellents, with an annual processing of ca. 2,000 tonnes/year (personal communication). 1,4-Dichlorobenzene in its solid form is ground, possibly mixed with perfumes and surfactants, and compacted to small blocks. These operations take place in a confined area with air extraction. The blocks of 1,4-dichlorobenzene are then transferred for packaging. The machinery for grinding, mixing and compacting is cleaned with compressed air only. No water is involved. The recovered 1,4-dichlorobenzene dust is either recycled or transferred for incineration. The amount of 1,4-dichlorobenzene ending up in solid waste is estimated at 600 kg/year.

Ca. 1 tonne/year of 1,4-dichlorobenzene is processed differently though. It is mixed with surfactants, perfumes, colouring agents and fillers in its melted form and subsequently cast in a mould. The moulds are cleaned 3 times a year. An estimated maximum of 300 g of 1,4-dichlorobenzene is released with the washing waters with every cleaning operation.

On the whole, the releases to water due to formulation of air fresheners or moth repellents can be considered to be negligible compared to the releases due to its use as an intermediate and its direct use as air freshener in toilet blocks.

3.1.3.1.4   Release from use as air fresheners or moth repellents

1,4-Dichlorobenzene used as moth repellent is mainly emitted into air. The release into wastewater is considered to be negligible.

A quantity of 3,170 tonnes/year is formulated in Europe to air fresheners or toilet blocks. In Germany, 40% are used as toilet blocks and 60% as air fresheners (BUA, 1994). Assuming the same ratio for a European consumption, a volume of 1,270 tonnes/year of 1,4-dichlorobenzene is used as toilet blocks.

Ware and West (1987) indicate that some 1,4-dichlorobenzene may enter the atmosphere through this usage, but that most would enter the wastewater stream. No further details underlining this estimation were presented. Given the fact that 1,4-dichlorobenzene-containing toilet blocks are not hung into flushing tanks or toilet bowls, but are mainly used in standing urinals and urinal drains, it does not appear very probable that all of the 1,4-dichlorobenzene of this use enters the waste stream.

Further estimations have been performed by Frische et al. (1981). The loss of weight of compacted 1,4-dichlorobenzene toilet stones was determined. The weight loss from stones which were in contact with water (flow: 60 ml/min) was 20 to 30% higher compared to stones which were exposed to air only.

As a worst-case assumption it can therefore be estimated that ca. 1/3 of the toilet block is ending up in wastewater (the remaining having evaporated). This amounts to ca. 420 tonnes/year. 10% of this amount are supposed to contribute to the regional concentration. For the local estimation, a fraction of main source of 0.002 is proposed, applied to the regional quantity, as this is a

diffuse release. The local release to wastewater is therefore estimated at 84 kg/year over 365 days.

In summary, the releases to wastewater due to the use as toilet blocks are:

- local: 0.084 tonne/year,

- regional: 42 tonnes/year,

- continental: 420 tonnes/year.

3.1.3.1.5   Release from use in the production of grinding wheels

It can be supposed that for the production of grinding wheels, the release is comparable to the release during the formulation of toilet stones or moth repellents. The release will be mainly to air and is probably negligible to wastewater.

3.1.3.2   Estimation of local aquatic concentrations

3.1.3.2.1   PEClocal for production

The highest release of 1,4-dichlorobenzene from one of the 3 European production sites was indicated to be 3.2 tonnes/year. There is no wastewater treatment plant. The release at the other production sites is considerably lower. The volume of wastewater discharged in the receiving waters is 72,000 m^3/day. The flow rate of the receiving water is 9.95 m^3/s (INERIS, 1994), which is based on a continuous measured annual minimum (summer) during 30 days consecutively.

Amount released:	3.2 tonnes/year
No. of days of operation (Main category Ib, Appendix B):	300 days
Amount released daily:	11 kg/day
Volume of wastewater:	72,000 m^3/day
Concentration in wastewater:	153 µg/l
River flow rate:	9.95 m^3/s
	859,680 m^3/day
Volume of diluting water:	931,680 m^3/day
Concentration in receiving water (PEClocal$_{aqua}$):	$1.2 \cdot 10^{-5}$ kg/m^3
	12 µg/l

The estimations provided by the producer are confirmed by measurements performed by local authorities and reported in INERIS (1994). As at this specific site, 1,4-dichlorobenzene has been measured in the wastewater effluent (INERIS, 1994):

Amount released daily (measured):	4.667 kg/day
Volume of wastewater (measured):	64,681 m^3/day
Concentration in wastewater:	72 µg/l
River flow rate:	9.95 m^3/s; 859,680 m^3/day
Volume of diluting water:	924,361m^3/day
Concentration in receiving water (PEClocal$_{aqua}$):	5.10^{-6} kg/m^3
	5 µg/l

The concentration in freshly deposited sediment is taken as the PEC for sediment, therefore, the properties of suspended matter are used:

$$PEClocal_{sediment} = K_{susp\_water}/RHOsusp \cdot PEClocal_{aqua} \text{ (wet weight)}$$

$$= K_{p\_susp} \cdot PEClocal_{aqua} \text{ (dry weight)}$$

The PEClocal$_{sediment}$ for the production site is estimated to be 127 µg/kg wet weight (i.e. 540 µg/kg dw) calculated for a concentration of 12 µg/l, and 53 µg/kg wet weight (i.e. 225 µg/kg dw) calculated for a PEClocal$_{aqua}$ of 5 µg/l.

3.1.3.2.2   PEClocal for use as an intermediate

The local release to wastewater for the use as an intermediate is estimated to be 1.15 tonnes/year. The scenario proposed in the release category document "intermediates" can be used. A default value is used for the flow through the STP. The release duration is estimated at 207 days (TGD, Table B 3.2).

Amount released to wastewater:	1.15 tonnes/year
No. of days of operation:	207 days
Amount released daily:	5.5 kg/day
1,4-dichlorobenzene in STP outlet: 30%:	1.6 kg/day
STP flow rate:	2,000 m^3/day
Effluent concentration (PEC$_{STP}$):	0.83 mg/l
River flow rate:	60 m^3/s
Concentration in receiving waters (PEClocal$_{aqua}$):	0.32.10-6 kg/m^3
	0.32 µg/l

Using the same values as above, the PEClocal$_{sediment}$ due to the use of 1,4-dichlorobenzene as an intermediate is estimated to be 3.3 µg/kg wet weight (i.e. 14.4 µg/kg dw).

3.1.3.2.3   PEClocal for formulation

As seen above, the quantities of 1,4-dichlorobenzene release to surface water due to the formulation of air fresheners or toilet blocks are negligible. Releases due to the cleaning of material 3 times a year for the formulation of speciality products of up to 300 g per cleaning operation have nevertheless been reported and can be used for a tentative PEC-estimation using a default scenario:

Amount released to wastewaters:	0.3 kg/day
Flow rate of wastewater:	2,000 m^3/day
Concentration in the STP influent:	150 µg/l
Concentration in the STP outlet: 30% (PEC$_{STP}$):	45 µg/l
Dilution factor:	10
Concentration in receiving waters (PEClocal$_{aqua}$):	4.5 µg/l (3 times a year)

The resulting concentration in sediment would be PEClocal$_{sediment}$ = 48 µg/kg wet weight (i.e. 202 µg/kg dw). Given the intermittent nature of the release, it would be more appropriate to use an annual average value of 0.39 µg/kg wet weight (i.e. 1.66 µg/kg dw).

3.1.3.2.4   PEClocal for use of 1,4-dichlorobenzene in toilet blocks

As seen above (Section 3.1.2.1.4), the local release to wastewater is 84 kg/year over 365 days. The following local concentration can be estimated:

Amount released to wastewaters:	84 kg/year
Release duration:	365 days/year
Amount released daily:	0.23 kg/day
Flow rate of wastewater:	2,000 m^3/day
Concentration in the STP influent:	114 µg/l
Concentration in the STP outlet: 30% (PEC$_{STP}$):	34.2 µg/l
Dilution factor:	10
Concentration in receiving waters (PEClocal$_{aqua}$):	.4 µg/l

If the domestic wastewater is directly released to surface water, the resulting PEClocal$_{aqua}$ is 11.3 µg/l.

The resulting concentration in sediment would be PEClocal$_{sediment}$ = 36 µg/kg wet weight (i.e. 153 µg/kg dw) respectively 170 µg/kg wet weight (i.e. 508 µg/kg dw).

3.1.3.2.5   PEClocal for other releases

As in wastewater effluents of paint manufacturers and textile dyeing companies 1,4-dichlorobenzene has been detected in France (INERIS, 1994) a PEClocal due to these uses is estimated. No further wastewater treatment is assumed. This is only an indicative calculation as no specific information about these uses has been supplied and no data on general release are available. Furthermore, it is based on a single 24-hour mixing sample.

Textile dyeing

1,4-Dichlorobenzene was detected in one out of 16 effluents from textile dye activities monitored in this study.

It is not clear whether 1,4-dichlorobenzene is contained in the dye formulation or whether the textiles were previously treated with 1,4-dichlorobenzene for moth protection during storage.

Amount released daily (measured):	0.099 kg/day
Flow rate of wastewater (measured):	430 m^3/day
Concentration in wastewater (PEC$_{STP}$):	230 µg/l
River flow rate:	0.122 m^3/s
	10,540.8 m^3/day
Volume of diluting water:	10,970.8 m^3/day
Concentration in receiving water (PEClocal$_{aqua}$):	9.10-6 kg/m^3
	9 µg/l

The resulting concentration in sediment would be PEClocal$_{sediment}$ = 95 µg/kg wet weight (i.e. 405 µg/kg dw).

Paint manufacturing

1,4-Dichlorobenzene was detected in 2 out of 12 effluents from paint manufacturing sites monitored in this study.

Site 1:

Amount released daily (measured):	0.008 kg/day
Flow rate of wastewater (measured):	535.4 m^3/day
Concentration in wastewater (PEC$_{STP}$):	15 µg/l
River flow rate:	3 m^3/s
	259,200 m^3/day
Volume of diluting water:	259,735.4 m^3/day
Concentration in receiving water (PEClocal$_{aqua}$):	.10-8 kg/m^3
	0.03 µg/l

The resulting concentration in sediment would be PEClocal$_{sediment}$ = 0.32 µg/kg wet weight (i.e. 1.35 µg/kg dw).

Site 2:

Amount released daily (measured):	0.006 kg/day
Flow rate of wastewater (measured):	7.8 m^3/day
Concentration in wastewater (PEC$_{STP}$):	756 µg/l
River flow rate:	20 m^3/s
	1,728,000 m^3/day
Volume of diluting water:	1,728,008 m^3/day
Concentration in receiving water (PEClocal$_{aqua}$):	3.10-9 kg/m^3
	0.003 µg/l

The resulting concentration in sediment would be PEClocal$_{sediment}$ = 0.03 µg/kg wet weight (i.e. 0.13 µg/kg dw).

In another monitoring study of industrial effluents involving 46 sites in the Franche-Comté region in France (DRIRE Franche-Comté, 1996), one effluent from a paint manufacturing industry was monitored (24-hour mixing sample). 1,4-Dichlorobenzene was not detected.

3.1.3.3 Regional concentration in surface water and sediment

The steady state concentration in a region, taking all releases to all compartments into account is estimated to be (cf. Appendix A, EUSES-output):

PECregional$_{surface\ water}$ = 0.04 µg/l

PECregional$_{sediment}$ = 0.4 µg/kg wet weight = 1.0 µg/kg dw

3.1.3.4 Monitoring data in wastewater, surface water and sediment

3.1.3.4.1 Wastewater

In **Table 3.6**, the concentrations measured in STP effluents of industrial and domestic origin are summarised.

Table 3.6 Measured levels in STP-effluents

Location	Year	Concentration range [µg/l]	Comments	Reference
France: Rhône-Alpes region	1993	n.d.-756	6 positive samples out of 114 from industrial effluents; positive in effluents from chemical industry, textile dyeing and paint manufacturing	INERIS (1994)
France: Languedoc-Roussillon region	1992	< 5 µg/l	n.d. in effluents from 11 industrial sites	DRIRE Languedoc-Roussillon (1995)
France: Franche-Comté region	1994-1995	n.d.	effluents from 46 industrial sites	DRIRE Franche-Comté (1996)
USA: New Jersey	1991	n.d.- 53 µg/l [1]	effluents from 3 STPs; industrial contribution: 0-27%	Clark et al. (1991)
Sweden: Göteborg	1989-1991	< 0.5 µg/l	n.d. in 7 samples	Paxeus et al. (1992)
Canada: Ontario	1988	0.9-1.4	effluents from 3 STPs with varying industrial contribution	Melcer et al. (1988)

1) Results for « dichlorobenzene isomer »; it is not clear whether the concentration reflects the sum of the isomers

Very high effluent concentrations have only been found by INERIS (1994). The results have therefore been used directly for PEC derivations as described above. All other measurements are in agreement with the predicted effluent concentrations due to private use of toilet stones.

3.1.3.4.2 Surface water

Table 3.7 demonstrates recent measurements of 1,4-dichlorobenzene concentrations obtained in different German, French, Dutch and Japanese rivers.

Table 3.7 Measured levels in surface waters

Location: river	Year	Concentration range [µg/l]	Comments	Reference
Germany: Rhine and Main	1989-1991	0.02-0.44	mean max. concentration of 0.44 µg/l was measured close to a production site	Engler-Bunte-Institut (1992)
Germany: Rhine	1990-1991	0.02-0.13	90-percentiles from 9 locations; n = 444 (total); max.: 0.06-0.46 µg/l	Umweltbundesamt (1996)
Germany: Rhine	1990-1994	< 0.01-0.06	4 locations; n = 257 (total)	DVGW (1995)
Germany: Neckar	1990-1994	< 0.01-0.09	3 locations; n = 98 (total)	DVGW (1995)

Table 3.7 continued overleaf

Table 3.7 continued Measured levels in surface waters

Location: river	Year	Concentration range [µg/l]	Comments	Reference
Germany: Donau	1990-1994	< 0.01-0.08	1 location; n = 62	DVGW (1995)
Germany: Main	1992-1994	< 0.01-0.30	1 location; n = 130	DVGW (1995)
Germany: Main	1992/1993	0.02-0.04	n = 48	ARW (1993)
Germany: Rhine, Wupper, Sieg, Erft, Ruhr, Lippe	1988/1989	< 0.5	no. of samples not indicated	ARW (1989)
Germany: Mouth of Emscher	1988	0.5-1.5	n = 6	LWA (1992a, b)
Germany: Wupper	1990	< 0.5-0.8	n = 31: 30 samples < 0.5 µg/l, 1 sample = 0.8 µg/l	LWA (1992a, b)
Germany: Aller, Weser, Elbe, Mulde, Schwarze Elster	1993	< 0.1-2	n = 108 (total); 16 locations	Umweltbundesamt (1996)
Germany, Elbe	1992/1993	0.006-0.049	n = 40 (total); 2 locations	Goetz et al. (1990)
The Netherlands: Rhine and Meuse	1988-1990	< 0.01-0.25	n = 72 (total); 2 locations	RIZA (1991)
Denmark, river	1997	< 0.01	n = 4 (total); 2 locations	Danish EPA (1998)
France: Rhine	1993/1996	< 0.01-4.05	n = 77 (total); 2 locations highest conc.: 0.66; 1.04; 1.17; 4.05 µg/l	Agence de l'eau Rhin-Meuse (1997)
France: Meuse	1993/1996	< 0.01-0.38	n = 65 (total); 3 locations	Agence de l'eau Rhin-Meuse (1997)
France: Moselle	1993/1996	< 0.01-0.34	n = 66 (total); 5 locations	Agence de l'eau Rhin-Meuse (1997)
France: Meurthe	1993/1996	< 0.01-0.26	n = 41 (total); 3 locations	Agence de l'eau Rhin-Meuse (1997)
France: Ill	1993/1996	< 0.01-0.35	n = 28 (total); 2 locations	Agence de l'eau Rhin-Meuse (1997)
France: Thur, Moder, Chiers	1993/1996	< 0.01-0.29	n = 42 (total); 1 location on each river	Agence de l'eau Rhin-Meuse (1997)
France: Rhône and affluents	1993/1996	< 50	n = 379 (total); 98 locations on 53 rivers; detection limit 5 µg/l (n = 85), 10 µg/l (n = 137) or 50 µg/l (n = 157)	Agence de l'eau Rhône-Méditerranée-Corse (1997)
Japan: rivers, sea and lakes	1986-1994	0.18-2.5	max. values for the respective years; 19 locations; 16-22 samples per year	Environment Agency Japan (1996)

The measured concentrations of 1,4-dichlorobenzene in surface water are often higher than the estimated regional concentrations and sometimes even approach the estimated local concentrations. The highest estimated local concentrations and the highest measured concentrations are less than one order of magnitude apart. This would tend to prove that the estimations are rather realistic.

The amount of 1,4-dichlorobenzene formed from higher chlorinated homologues has not been quantified. Given the extensive amount of monitoring data in surface water confirming the

estimated PECs, it can though be considered that the contribution from higher chlorinated homologues to the environmental burden of 1,4-dichlorobenzene is low.

3.1.3.4.3 Sediment and suspended matter

Table 3.8 presents recent measurements of 1,4-dichlorobenzene concentrations in sediment and suspended matter.

Table 3.8   Measured levels in sediments

Location	Year	Concentration range [µg/kg dw*]	Comments	Reference
Germany, Rhine	9/1987-3/1990	< 10-960 mean: 41 90-percentile: 70 highest single values: 100, 200, 210, 960	sediment; 69 samples at different locations	LWA (1988) LWA (1989) LWA (1992a; b)
Germany, Rhine	1989/1990	< 10-48	suspended matter; 18 samples; either single or 14-day mixing samples	LWA (1992a; b)
Germany, Rhine	1992/1993	13.4-47.5	suspended matter, average values from two locations; maximum 77 µg/kg dw	Umweltbundesamt (1996)
Germany, rivers of the region of Hessen including river Main	1994-1996	< 10-180	suspended matter, 13 rivers sampled; highest concentrations in the river Main: 45-180 µg/kg dw	HLfU (1997)
Germany, Niers	9/1987 12/1988 12/1989 10/1991	650/210 850/120 1,500 / < 10 360 / -	sediment; 1 sample each at 2 locations	LWA (1989) LWA (1992a; b)
Germany, Lippe	1989	< 10	sediment; 2 samples	LWA (1989)
Germany, surface waters other than river Elbe around Hamburg	1983-1985	< 3–2,980 mean: 147 highest single values: 173, 242, 2,918	sediment; 25 samples at 25 sampling sites	Environment Agency of the City of Hamburg (1988)
Germany, Elbe (Hamburg harbour)	1983-1985	< 3–10,540 mean: 539 highest single values: 467, 851, 1,500, 10,540	sediment; 32 samples at 32 sampling sites	Goetz et al. (1990)
Germany, Elbe	1990	location 1: 55-1,670, highest single values: 250, 450, 816, 1,090, 1,670; location 2: 17-708 highest single values: 126, 155, 708	suspended matter: 12 samples for each location	ARGE Elbe (1993); Umweltbundesamt (1996)
Germany, Elbe	1994	< 5-58	suspended matter: n = 76; 8 locations; monthly mixing samples	ARGE Elbe (1996)

Table 3.8 continued overleaf

Table 3.8 continued Measured levels in sediments

Location	Year	Concentration range [µg/kg dw*]	Comments	Reference
Germany, Schwarze Elster	1994	< 5	suspended matter: n = 9; 1 location; monthly mixing samples	ARGE Elbe (1996)
Germany, Mulde	1994	23-430 highest single values: 150, 190, 430	suspended matter: n = 7; 1 location; monthly mixing samples	ARGE Elbe (1996)
Germany, Saale	1994	< 5	suspended matter: n = 8; 1 location; monthly mixing samples	ARGE Elbe (1996)
Denmark, marine sediment, inner Danish waters	1996/1997	0.001-0.074	Sediment n = 78	Danish EPA (1998)
Japan: rivers, sea and lakes	1986-1994	27-150	sediment; max. values for the respective years; 19 locations; 16-22 samples per year	Environment Agency Japan (1996)
France: Rhône and affluents	1993-1997	< 200	sediment; 76 samples from 48 locations in 20 rivers; detection limit was either 50 µg/kg dw (n = 29) or 200 µg/kg dw (n = 47)	Agence de l'eau Rhône-Méditerranée-Corse (1997)
France: Rhône and affluents	1993-1997	< 200	suspended matter; 128 samples from 88 locations in 39 rivers; detection limit was either 50 µg/kg dw (n = 89) or 200 µg/kg dw (n = 39)	Agence de l'eau Rhône-Méditerranée-Corse (1997)

*   dw = dry weight

The measured concentrations in sediment and suspended matter and the respective local estimated concentrations are in total agreement. Some measured values are even higher than the respective estimations. This might be due to the lack of wastewater treatment, or an underestimation of the releases or the partition coefficient as well to the fact that the use of 1,4-dichlorobenzene has strongly diminished over the last few years.

As some measured concentrations are higher than the estimated concentrations, it is necessary to derive a representative value, which will be used together with the estimated ones in the risk characterisation.

It is very difficult to interpret the monitoring results, as it is not always clear whether they reflect regional or local conditions. Nevertheless, by comparison with the concentrations measured in sewage sludge (see Section 3.1.4.4), it can be assumed that the high concentrations in sediment and suspended are due to local releases of 1,4-dichlorobenzene, perhaps even without biological sewage treatment, as approximately the same maximum concentrations have been measured in sewage sludge from STPs.

Furthermore, the extensive monitoring campaigns in Southern France (Agence de l'eau Rhône-Méditerranée-Corse, 1997) as well as in Japan (Environment Agency Japan, 1996), tend to prove

that the high measured concentrations in some German rivers are due to local releases. Recent measurements in the river Main and its affluents seem to confirm this situation (HLfU, 1997).

The highest measured concentrations can be commented as follows:

- of the concentrations measured in the Rhine (9/1987-3/1990), the highest measured concentration out of 69 is 960 µg/kg dw. The next highest concentration is 210 µg/kg and the 90 percentile value is 70 µg/kg dw. The high concentrations could not be confirmed by measurements in 1992/1993,

- the concentrations measured at one location in the Niers are consistently high (up to 1,500 µg/kg dw). Only 4 measurement are available from that location though and the most recent (1991) yields a concentration of 360 µg/kg dw. It has to be noted though that at this location, about 70% of the flow of the Niers originates from the domestic wastewater treatment plant of the city of Moenchengladbach. Indeed, upstream of Moenchengladbach, the average flow of the Niers is ca. 0.25 m^3/s, while the STP-flow is ca. 1.4 m^3/s. The rest (ca. 0.35 m^3/s) is due to rain-water and smaller affluents (Umweltamt Krefeld, 1997; Dierke Weltatlas, 1988). At the second location (ca. 56 km downstream), the concentrations have significantly dropped (< 10-210 µg/kg), probably due to further dilution by other affluents,

- of the concentrations measured in 1983-1985 in sediments from the Hamburg area, the highest concentration of 2,918 µg/kg dw seems to be clearly either an outlier or representing an extreme worst-case situation, as the second highest value only amounts to 242 µg/kg dw,

- the high concentrations in the sediment from the river Elbe are confirmed by the high concentrations in suspended matter in 1990 (ARGE Elbe, 1993). By 1994, the concentrations in the Elbe had nevertheless dropped to a maximum of 58 µg/kg dw. High concentrations were only measured in one of the affluents, the Mulde, with concentrations of up to 430 µg/kg,

- due to the lower concentrations in the Elbe in 1994 compared to 1990 (the maximum values dropped by a factor of almost 30), it can be assumed that also the concentrations in the suspended matter of the Hamburg harbour, which is located on the Elbe, have also dropped considerably between 1984 and 1994.

Given the above considerations, only the concentrations at one location of the river Niers remain consistently high. As the flow of the river at that location is mostly (70%) due to treated wastewater, this situation cannot be considered representative.

Given the important decrease of use of 1,4-dichlorobenzene over the last years, the more recent values are to be preferred. Among the recent values, the highest concentration has been measured in the river Mulde with 430 µg/kg dw. This value will be used in the risk characterisation.

3.1.4   Atmosphere

3.1.4.1   Releases to the atmosphere

3.1.4.1.1   Release from production

The following emissions to the air compartment are reported by the European producers.

Producer	Production	Release due to Production	%	Year
1	18,000 tonnes/year	0	0	1992
2	2,000 tonnes/year	14 tonnes/year	0.7	1994
3	5,500 tonnes/year	36.5 tonnes/year	0.7	1994

The releases to air due to stripping in the STPs at the production sites are not known.

The fourth HEDSET-submitting company is importing and selling only.

For Producer 1, all of the exhaust from production, and most of that of processing is collected and disposed of in a thermal purification plant, followed by flue-gas scrubbing. This is the reason why no introduction into the atmosphere occurs.

The worst case of 1,4-dichlorobenzene release due to production into the air compartment is occurring at the production site of Producer 3. The results are based on daily monitoring. This site is going to be the support (worst-case) for the calculation of PEClocal$_{air}$ due to production. There is no contribution from stripping in the STP, as there is no treatment on this site. As the number of production sites is very low, the regional releases due to production are supposed equal to the highest local release.

In summary, the releases to air due to production are:

- local: 36.5 tonnes/year,
- regional: 36.5 tonnes/year,
- continental: 50.5 tonnes/year.

3.1.4.1.2   Release from use as an intermediate

The following emissions to the air compartment are reported by some European processors.

Producer	Processing	Release due to Processing	%	Year
1	1,440 tonnes/year	200.5 kg/year	0.014	1992
2	1,060 tonnes/year	100 kg/year	0.009	1994

Further data are available from a different site (personal communication), where about 1,200 tonnes/year of 1,4-dichlorobenzene are converted to 1,4-dichloro-2-nitrobenzene. The releases into atmosphere are indicated to be 700 kg/year in 1994 i.e. a release factor of 0.058%.

No data is available on the missing 3,450 tonnes/year which are also supposed to be used as a chemical intermediate. A release factor of 0.001% is suggested in the TGD. This is considerably lower than the above reported release factors and therefore the highest of the above reported release factor of 0.058% (i.e. 2 tonnes/year) will also be applied here. Furthermore of the releases to wastewater, 50% (i.e. 1.7 tonnes/year) are supposed to be stripped to air during sewage treatment. The fraction of main source is estimated at 0.3 i.e. the local release to air is 0.6 tonne/year directly and 0.5 tonne/year indirectly by stripping.

For the regional estimation, the total releases from the missing 3,450 tonnes/year are used as no information on the site locations is available.

In summary, the releases to air due to the use as an intermediate are:

- local: 0.6 tonne/year directly + 0.5 tonne/year via the STP,
- regional: 2 tonnes/year directly + 2 tonnes/year via the STP,
- continental: 2.3 tonnes/year directly + 2 tonnes/year via the STP.

3.1.4.1.3   Release from formulation to air fresheners or moth repellents

No specific data on releases to the atmosphere during formulation of air fresheners and moth repellents is available. The TGD proposes to use a default release factor of 1%. For a total volume of 7,240 tonnes/year processed to air fresheners and moth repellents, the release would amount to 72.4 tonnes/year. For the local level, it has to be taken into account that the number of formulators is very low (probably less than 10) and that the different products are formulated on the same sites. It is therefore appropriate to apply a high fraction of main source of 0.4 (TGD, Table B 2.1) to the continental release to derive a local release i.e. 29 tonnes/year. This amount will also be used for the estimation of the regional concentration. As seen above, the releases to wastewater are negligible and therefore the contribution by stripping in the STP is also neglected here.

In summary, the releases to air due to the formulation of air fresheners and moth repellents:

- local: 29 tonnes/year,
- regional: 29 tonnes/year,
- continental: 72.4 tonnes/year.

3.1.4.1.4   Release from use as air fresheners or moth repellents

1,4-Dichlorobenzene used as a moth repellent i.e. 4,070 tonnes/year is mainly emitted into air. A further quantity of 3,170 tonnes/year is formulated in Europe to air fresheners or toilet blocks. In Germany, 40% (1,270 tonnes/year) are used as toilet blocks and 60% (1,900 tonnes/year) as air fresheners (BUA, 1994). Assuming the same ratio for a European consumption, a volume of 1,270 tonnes/year of 1,4-dichlorobenzene is used as toilet blocks. An estimated 67% of the toilet block are evaporating during use (Frische et al., 1981). This amounts to ca. 851 tonnes/year. The 1,4-dichlorobenzene used in air fresheners is assumed to be released integrally to air (i.e. 1,900 tonnes/year). The total release to air is therefore 6,821 tonnes/year.

Furthermore, of the 420 tonnes/year ending up in wastewater, ca. 50% (i.e. 210 tonnes/year) are supposed to be stripped to air during sewage treatment. The total release into air is therefore

estimated to be 7,031 tonnes/year. 10% of this amount is supposed to contribute to the regional concentration. As these releases are diffuse, no local release estimations are performed.

In summary, the direct and indirect releases to air due to the use as moth repellents, air fresheners or toilet blocks are:

- local: -,
- regional: 682 tonnes/year directly + 21 tonnes/year via the STP,
- continental: 6,821 tonnes/year directly + 210 tonnes/year via the STP.

3.1.4.1.5   Release from use in the production of grinding wheels

The use of 1,4-dichlorobenzene in the production of grinding wheels will mainly give rise to releases to air. According to Bayer AG (1997, personal communication), the burn-out substance which gives the grinding material its porous aspect is either lost by evaporation before the material is heated or it is destroyed in the oven at temperatures of 1,100–1,300°C. In the absence of more precise information, it is supposed that all of the 100 tonnes/year of 1,4-dichlorobenzene is released to air. On a regional scale an amount of 10 tonnes/year is used. As the number of sites using 1,4-dichlorobenzene for the production of grinding wheels, as well as their location is not known, the regional amount of 10 tonnes/year is supposed to be released on a single site over a duration of 10 days (TGD, Appendix 1, Table B 3.2). This appears to be an extremely worst-case situation, but for modelling purposes, these figures will be used in the assessment.

In summary, the releases to air due to the use as a processing aid in the production of grinding wheels are:

- local: 10 tonnes/year,
- regional: 10 tonnes/year,
- continental: 100 tonnes/year.

3.1.4.2   Estimation of local air concentrations and deposition rates

The concentration in air at 100 m from a point source can be estimated as follows:

$$PEClocal_{air} \ (mg/m^3) = Elocal_{air} \cdot Cstd_{air}$$

Where $Elocal_{air}$ (kg/day) = local direct emission rate to air
         $Cstd_{air}$ = standard concentration in air at source strength of 1 kg/day
         $= 2.78 \cdot 10^{-4}$ mg/m^3.

Based on its vapour pressure, 1,4-dichlorobenzene is integrally present in vapour form in the atmosphere. The gaseous deposition over a radius of 1,000 m around the source can therefore be estimated as:

$$DEPtotal = (Elocal_{air} + Estp_{air}) \ \cdot DEPstd_{gas}$$

Where $Estp_{air}$ (kg/day) = local indirect emission to air from the STP
         $DEPstd_{gas}$ = deposition flux of gaseous compounds (log H > 2) at
         source strength of 1 kg/day = $3 \cdot 10^{-4}$ mg/m^2/day.

3.1.4.2.1   PEClocal for production

The worst-case release to air from a production site is 36.5 tonnes/year. No sewage treatment takes place at this site therefore no additional release to air occurs due to stripping in a STP. Assuming 300 production days, the daily release would be 122 kg/day. Therefore:

$$\text{PEClocal}_{air} = 0.034 \text{ mg/m}^3 = 34 \text{ µg/m}^3$$

The resulting average deposition rate over 365 days is:

$$\text{DEPtotal} = 0.03 \text{ mg/m}^2/\text{day}$$

3.1.4.2.2   PEClocal for use as an intermediate

The worst-case release to air at a processing site is estimated to be 0.6 tonne/year directly and 0.5 tonne/year through the STP. Assuming the same parameters as above, and using the highest release rate of 0.6 tonne/year with a release duration of 207 days per year:

$$\text{PEClocal}_{air} = 0.0008 \text{ mg/m}^3 = 0.8 \text{ µg/m}^3$$

With a total release of 1.1 tonnes over 365 days, the deposition flux is:

$$\text{DEPtotal} = 0.0009 \text{ mg/m}^2/\text{day}$$

3.1.4.2.3   PEClocal for formulation of moth repellents and air fresheners

The worst-case release to air at a formulation site is estimated to be 29 tonnes/year over a duration of 300 days. Therefore:

$$\text{PEClocal}_{air} = 0.027 \text{ mg/m}^3 = 27 \text{ µg/m}^3$$

The resulting average deposition flux over 365 days is:

$$\text{DEPtotal} = 0.024 \text{ mg/m}^2/\text{day}$$

3.1.4.2.4   PEClocal for use as moth repellent and air freshener

The release of 1,4-dichlorobenzene due to its use as moth repellent and air freshener is diffuse and no local PEC calculation is necessary.

3.1.4.2.5   PEClocal for use in the production of grinding wheels

The highest local release is estimated to be 10 tonnes/year over 10 days (worst-case). Therefore:

$$\text{PEClocal}_{air} = 0.278 \text{ mg/m}^3 = 278 \text{ µg/m}^3$$

The resulting average deposition flux over 365 days is:

$$\text{DEPtotal} = 0.008 \text{ mg/m}^2/\text{day}$$

3.1.4.3   Regional concentration in air

The steady state concentration in a region, taking all releases to all compartments into account is estimated at (cf. EUSES output[4]):

$$PECregional_{air} = 0.074 \text{ μg/m}^3$$

3.1.4.4   Monitoring data in air and precipitation

The following table sums up the measurements of 1,4-dichlorobenzene concentrations in air performed in Germany.

Table 3.9   Measured levels in air

Location	Year of measurement	Concentration range	Comments	Reference
3 unnamed garbage incineration plants	not mentioned	0.03-0.15 μg/Nm^3 mean: 0.066 μg/Nm^3 *	each garbage incineration plant has been sampled twice	Ballschmitter et al. (1988)
Gaseous effluents from garbage dump, Berlin, Germany	not mentioned	0.440 mg/Nm^3	anaerobic dump gas with wastes of unknown origin	Höfler et al. (1986)
Urban air, Hamburg	measurements were carried out during period of one year	mean monthly values: 0.08-0.3 μg/m^3 mean annual values: 0.14 μg/m^3	12 different sites; some measures below detection limit of 0.04 μg/m^3	Bruckmann et al. (1988)
Metropolitan Essen	12/1987-11/1988	0.147-0.269 μg/m^3; mean value: 0.199 μg/m^3	every sample was taken during 0.5 hour at 16 sampling sites	Beier et al. (1989)
Hoechst factory Frankfurt am Main	11/1987-6/1988	max. value: up to 4 μg/m^3	n = 224; 97% below detection limit of 1 μg/m^3	Hoechst AG (1988)

*   Nm^3 = standard m^3, calculated for 1,013 hPa and 20°C

Rain samples (n = 7, 5-27 litres) in spring 1984 in Portland, Oregon, showed a mean concentration of 0.120 μg/m^3 in air and 0.0048 μg/l in rain at about 8°C (Ligocki et al., 1985).

However, in 119 rain water samples from different locations in Hessen, Germany, collected between 12/1988 and 11/1989, all 1,4-dichlorobenzene concentration were below the 45 ng/l detection limit (Schleyer et al., 1991).

The concentrations measured in urban air are in close agreement with the estimated regional concentration while the highest measured value of 4 μg/m^3, related to a chemical activity is close to the estimated local concentration.

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[4] **Euses Calculations** can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it

3.1.5   Terrestrial compartment

3.1.5.1   Releases to soil

1,4-Dichlorobenzene can reach agricultural soil through two exposure routes:

- application of sewage sludge in agriculture,
- dry and wet deposition from the atmosphere.

The highest local atmospheric deposition rates and transfer rates to sewage sludge as determined above are:

	Transfer to sewage sludge	Average deposition rate
Production	-	0.03 mg/m^2/day
Use as a chemical intermediate	0.05 kg/day	0.0009 mg/m^2/day

3.1.5.2   Estimation of local soil concentrations

3.1.5.2.1   PEClocal for production

With the local atmospheric deposition rate of 0.03 mg/m^2/day, the resulting worst-case concentration in agricultural soil can be estimated, as presented in Appendix 1 (EUSES calculation):

$$PEClocal_{soil} = 2.1 \ \mu g/kg \ (wet \ soil) = 2.3 \ \mu g/kg \ dw$$

Concentration in groundwater

In a first approach, the resulting pore-water concentration in soil can be used to approximate the resulting groundwater concentration (see Appendix 1): Cgrw = 0.26 µg/l

3.1.5.2.2   PEClocal for use as an intermediate

With the local transfer to sewage sludge of 0.05 kg/day, the resulting concentration in sewage sludge can be estimated using the default values proposed in the TGD, as presented below:

Capacity of the STP	CAPACITY$_{stp}$	10,000 eq.
Sewage flow per inhabitant	WASTEWinhab	0.2 m^3/day/eq
Effluent discharge rate of STP	EFFLUENT$_{stp}$	2,000 m^3/day
Concentration of suspended matter in influent	SUSPCONC$_{inf}$	0.45 kg/m^3
Surplus sludge per inhabitant equivalent	SURPLUS$_{sludge}$	0.011 kg/day/eq
Rate of sewage sludge production	SLUDGERATE	710 kg/day
Local emission rate to water during episode	Elocal$_{water}$	5.5 kg/day
Fraction of emission directed to sludge by STP	Fstp$_{sludge}$	0.01 -
Concentration in dry sewage sludge	C$_{sludge}$	77 mg/kg dw

With the resulting sludge concentration as well as the local atmospheric deposition rate of 0.0009 mg/m^2/day, the resulting worst-case concentration in agricultural soil can be estimated, as presented Appendix A (EUSES calculation):

PEClocal$_{soil}$ = 64 µg/kg (wet soil) = 72.5 µg/kg dw

Using the highest measured concentration in sewage sludge of 15 mg/kg (see Section 3.1.4.4) or a more representative value of ca. 4 mg/kg, the resulting concentration in soil would be at least one order of magnitude lower.

Concentration in groundwater

In a first approach, the resulting pore-water concentration in soil can be used to approximate the resulting groundwater concentration (cf. Appendix 1, EUSES calculation):

Cgrw = 1.8 µg/l

3.1.5.3   Regional concentration in agricultural soil

The steady state concentration in a region, taking all releases to all compartments into account is estimated at (cf. Appendix 1, EUSES calculation):

PECregional$_{soil}$ = 0.008 µg/kg wet weight = 0.01 µg/kg dw

3.1.5.4   Monitoring data in sewage sludge and soil

In 1989, the 1,4-dichlorobenzene content of digested sludge from two different sewage plants in North Rhine-Westphalia was determined. In the sewage plant receiving domestic and industrial wastewater (mainly from textile and leather industry), the concentration in digested sludge was 15,000 µg/kg dw (dw = dry weight). In sludge from the second treatment plant (treating mainly household waste waters the concentration was 340 µg/kg dw (Friege et al., 1989).

In Hessen, Germany, a survey of 12 treatment plants in 1985, 1987 and 1988 showed a decreasing 1,4-dichlorobenzene content in sewage sludge:

- 1985: in 11 samples, 1,4-dichlorobenzene concentrations from n.d. (not detected) up to 4,800 µg/kg dw were determined, (with peak values: 3,600, 4,200, 4,800 µg/kg dw),

- 1987: in 11 samples, 1,4-dichlorobenzene concentrations from n.d. up to 1,710 µg/kg dw were determined (peak values: 770, 890, 1,710 µg/kg dw),

- 1988: in 11 samples 1,4-dichlorobenzene concentrations from n.d. up to 580 µg/kg dw were reported (peak values: 450, 550, 580 µg/kg dw).

The reason for this reduction of 1,4-dichlorobenzene concentration in sewage sludge is uncertain, a systematic error in the analysis of 1985 as well as a decline in toilet block usage is possible (Kroeber B. and Haeckl M., 1989).

In 1996, 2 samples from domestic sewage sludge of sewage treatment plants from the Hessen region in Germany revealed 1,4-dichlorobenzene concentrations of 65-120 µg/kg dw (HLfU, 1997).

1,4-Dichlorobenzene was monitored in 1994 in sewage sludge from domestic sewage treatment plants in the Rhine and Meuse area in France. 1,4-dichlorobenzene was detected in only 25% out of 50 samples. The highest measured concentration was 1,700 µg/kg dw. Unfortunately the detection limit was very variable and mostly ranged from 100 to 5,000 µg/kg dw (Agence de l'eau Rhin-Meuse, 1997).

In 1995, 1,4-dichlorobenzene was monitored in sewage sludge of 19 STPs in Denmark. The industrial load of these STPs varied between 0 and 70%. Of the 20 samples taken, only 4 were above the detection limit of 1 µg/kg dw, the highest concentration being 39 µg/kg dw. The concentration in the water extracts (liquid/solid ratio = 10) was 0.52 µg/l at maximum (Danish environmental protection agency, 1998).

During 1984 the Environmental Agency of the City of Hamburg investigated the soil pollution with chlorinated hydrocarbons through single random samples at 24 sites throughout the municipal area. No dichlorobenzene were detected at a detection limit of 3 µg/kg (Freie und Hansestadt Hamburg, 1988).

The estimated maximum local concentration based on modelling is 464 µg/kg dw while the regional concentration is estimated at 0.012 µg/kg dw. It seems obvious that the estimated local concentration is a worst-case. On the other hand the available monitoring data is not very extensive and does not allow a clear conclusion.

3.1.6 Secondary poisoning

The highest local surface-water concentration is estimated at 12 µg/l, the regional concentration being 0.06 µg/l. Assuming that 50% of the diet of fish-eating birds comes from a source contaminated at 12 µg/l and 50% from a source at a concentration of 0.06 µg/l, the level in food can be estimated with the BCF for fish of 296:

$$PEC_{oral} = 1.78 \text{ mg/kg (wet fish)}$$

Using the annual average surface water concentration (cf. Appendix 1, EUSES calculation), the exposure becomes $PEC_{oral} = 1.44$ mg/kg (wet fish).

As seen above, the BCF in newly hatched alevins might be up to 1,400. Supposing a consumption of alevins only:

$$PEC_{oral} = 8.4 \text{ mg/kg (wet fish)}$$

The highest local concentration in agricultural soil is estimated at 64 µg/kg wet weight, the regional concentration being 0.008 µg/kg wet weight. Assuming that 50% of the diet of worm-eating birds or mammals comes from a source contaminated at 64 µg/kg and 50% from a source at a concentration of 0.008 µg/kg, the level in food can be estimated with the BCF for earthworms of 12.4:

$$PEC_{oral} = 0.4 \text{ mg/kg (wet earthworm)}$$

Using a concentration averaged out over 180 days after application of sewage sludge (cf. Appendix 1), the exposure becomes $PEC_{oral} = 0.09$ mg/kg (wet earthworm).

Monitoring data in biota

The available data about 1,4-dichlorobenzene in biota is very limited:

- *Oncorhynchus mykiss*: three fish were kept in Rhine water; (aerated flow through system). 100 µg 1,4-dichlorobenzene/kg FW (FW = fresh weight) was detected in intestines, 10 µg 1,4-dichlorobenzene in muscle meat. The trout were 10 weeks old and kept in an open flow-through system for 10 months. All analytical values of the Rhine water were below the detection limit of 0,5 µg/l. The concentrations detected in the fish may origin from the 1,4-dichlorobenzene concentration not detected analytically or from possible metabolism of higher-chlorinated benzenes the detection limit of 0.5 µg/l. The concentrations detected in the fish may originate from the 1,4-dichlorobenzene concentration not detected analytically or from possible metabolism of higher-chlorinated benzenes. In rainbow trout kept in drinking water, no 1,4-dichlorobenzene was detected (LWA, 1989),

- in a wildlife monitoring program in Japan, 1,4-dichlorobenzene was detected in 1990, 1992 and 1993 in fish, shellfish and birds at concentrations between 0.01 and 0.21 ppm (total number of samples: 315). Most values refer to fish. 1,4-Dichlorobenzene was only found in one shellfish-sample and one bird-sample (Environment Agency Japan, 1993, 1995 and 1996). It is not clear whether the results refer to wet weight or dry weight,

- honey samples: 55 samples, the analysis revealed no 1,4-dichlorobenzene in 35 samples, in 10 samples the 1,4-dichlorobenzene content was between 0.0005 and 0.01 mg/kg, and in 10 other samples between 0.011 and 0.036 mg/kg (Hamann et al., 1990),

- honey samples: 98 domestic honeys, < 0.01 mg 1,4-dichlorobenzene/kg honey was detected in 14 samples and 4 samples contained 0.05-0.06 mg 1,4-dichlorobenzene/kg. Higher concentrations were detected in imported honey in 3 samples out of 32 with 0.05-0.11 mg/kg (Landesuntersuchungsamt für das Gesundheitswesen Südbayern Oberschleissheim, 1993).

The monitoring data in fish exposed to Rhine-water are about one order of magnitude lower than the estimated concentrations in fish, but still consistent as the 1,4-dichlorobenzene-concentration in the Rhine water where the fish were held was < 0.5 µg/l.

The data from the wildlife-monitoring programme correspond well with the estimated concentrations, given that the maximum concentrations measured in surface water during the same time were 0.18-2.5 µg/l.

3.2   EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT) ASSESSMENT

3.2.1   Aquatic compartment (incl. sediment)

3.2.1.1   Toxicity test results

3.2.1.1.1   Fish

Acute tests

The most relevant acute test results are:

Brachydanio rerio (zebra fish):   96-hour LC50:   2.1 mg/l
                                       48-hour LC50:   4.8 mg/l

(Method: OECD Guideline 203; flow-through system; analytical concentrations; Röderer, 1990)

Pimephales promelas (fathead minnow):

                        96-hour LC50:   3.6 mg/l [larvae]
                        96-hour LC50:   14.2 mg/l [juveniles]
                        96-hour LC50:   11.7 mg/l [subadults]

(Method: ASTM Guideline 1980; static acute toxicity test; closed system; nominal concentrations; Mayes et al., 1983)

Pimephales promelas   96-hour LC50:   4.2 mg/l

(Method: US EPA 1975; flow-through system; 30-day-old animals; analytical concentrations, Carlson and Kosian, 1987)

Jordanella floridae (American flagfish):

                        96-hour LC50:   4.5 mg/l (semi-static)
                        96-hour LC50:   2.1 mg/l (flow through)

(Method: US EPA 1975; it is not stated whether the aquaria were covered in the semi-static test; analytical concentrations; Smith et al., 1991)

Cyprinodon variegatus (sheepshead minnow):

                        96-hour LC 50:   7.4 mg/l
                        96-hour NOEC: 5.6 mg/l

(Method US EPA 1975; nominal concentrations; static system, brackish water, Heitmüller et al., 1981)

Oncorhynchus mykiss (rainbow trout): 96-hour LC50:   1.12 mg/l
(flow-through system, analytical concentrations; Call et al., 1983)

Long-term tests

Brachydanio rerio   14-day NOEC: 0.44 mg/l
                             14-day LOEC: 0.7 mg/l

(Method: OECD guideline 204; flow-through system, effects: death, weight and behaviour; analytical concentrations; Röderer, 1990)

Early life stage testing:

Pimephales promelas   28-day NOEC: 0.570 mg/l
                             28-day LOEC: 1 mg/l

(Method: flow-through system; development from egg to larvae, each test was initiated in placing 30 embryos (4 to 12 hours old), into the aquarium; the percentage of normal larvae hatching and surviving (abnormal developing fish included) were used as endpoints; analytical concentrations; Carlson and Kosian, 1987)

Jordanella floridae   14-16-day NOEC: 0.20-0.23 mg/l (1)
                             28-day NOEC: ≥ 0.35 mg/l (2)

(Method: early life stage toxicity test similar to test developed for fathead minnow; flow through system; analytical concentrations; water samples were analysed 5 days per week during a 28-day exposure; two age groups were used simultaneously: (1) eggs/embryo/larval fish, with data collected on hatching success (4-6 days after exposure) and 10-day larval survival (2 test series); (2) one week old fry (young fish) with data generated on survival and growth over 28 days; with test concentrations of 0.042 to 0.835 mg/l, no influence was demonstrated on hatching rate; significant effects with respect to survival of larvae up to 10 days of age in the two parallel test series were observed at respectively 0.31 and 0.32 mg/l (LOECs); Smith et al., 1991)

Oncorhynchus mykiss   60-day NOEC: ≥ 0.1 mg/l

(Method: development test, flow through system, 1,000 eggs per test concentration; no morphological and histological effects at any tested concentration (25 embryos per test concentration examined), 30% cumulative mortality in all the treatments as well as the controls; analytical concentrations; Calamari et al., 1983)

3.2.1.1.2   Invertebrates

Acute effects

Daphnia magna   24-hour EC50:   1.6 mg/l

(Method: AFNOR 1974, immobilisation, analytical concentrations; Calamari et al., 1982)

Daphnia magna   24-hour EC50:   3.2 mg/l

(Method: DIN 38412 part 11; nominal concentrations; Kühn et al., 1989)

Daphnia magna   48-hour EC50:   0.7 mg/l
                             48-hour LC50:   2.2 mg/l

(Method: OECD proposal 1979; analytical concentrations; EC50 refers to immobilisation; LC50 refers to additional parameters e.g. activity of thoracic appendages; Canton et al., 1985)

The 48-hour EC50 value of 0.7 mg/l will be retained for the environmental classification, as this is the only result available after 48 hours of exposure.

A 96-hour LC50 value of 1.99 mg/l for *Mysidopsis bahia* is cited in US EPA (1978), but no details on test conditions are reported, so that the result could not be validated.

Long-term effects

Daphnia magna   21-day NOEC: 0.4 mg/l

(Most sensitive parameter: time of first birth; analytical concentrations; nominal concentration: 0.50 mg/l; animals were transferred every 2nd day to new, closed test bottles; the test concentration decreased from 0.5 to 0.3 mg/l before renewal; the mean concentration of 0.4 mg/l can be used here as a NOEC; Kühn et al., 1989)

Daphnia magna   28-day NOEC: 0.22 mg/l

(Method: fertility tests; 25 new-born animals were exposed to different 1,4-dichlorobenzene concentrations for 28 days; toxic et 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 initial concentration; Calamari et al., 1982)

3.2.1.1.3   Aquatic plants

Scenedesmus pannonicus   72-hour EC50:   31 mg/l

(Method: OECD proposal 1979: growth inhibition test, analytical concentrations; Canton et al., 1985)

Selenastrum capricornutum   96-hour EC50:   1.6 mg/l
                                  96-hour EC0:   0.57 mg/l

(Method: US-EPA 1971, closed system, 1,4-dichlorobenzene concentration confirmed analytically at the beginning and end of the test; Calamari et al., 1983)

Scenedesmus subspicatus   48-hour EbC50:   28 mg/l
                                  48-hour EbC10   13 mg/l
                                  48-hour EµC50:   38 mg/l
                                  48-hour EµC10   16 mg/l

(Method: DIN regulation 38,412, part 9, cell proliferation inhibition test; bottles were sealed, no analytical control, Kühn and Pattard, 1990)

Cyclotella meneghiniana   48-hour EC 50:   34.3 mg/l

(Method: Growth inhibition and DNA decrease; analytical control only before dilution experiment; Figueroa and Simmons, 1991)

3.2.1.1.4   Microorganisms

Sewage sludge   12-hour EC50:   330 mg/l

(Method: Inhibition O_2 uptake; closed system, 35°C, pH 7, bacterial density 200 mg/l, Blum and Speece, 1991)

Nitrosomonas spec.   12-hour EC50:   86 mg/l

(Method: Inhibition of NH_4 uptake, closed system, 25°C, pH 6.5-8; bacterial density 450 mg/l, Blum and Speece, 1991)

Methanogenic sewage sludge   48-hour EC50:   86 mg/l

(Method: Inhibition of gas production, closed system, 35°C, pH 7, bacterial density 900 mg/l, Blum and Speece, 1991)

Sewage sludge at 100 mg/l: saturation, no significant effect

(Anaerobic fermentation of sewage sludge: amount of gas produced is used as test criterion; closed system; duration: 35 days: aerobic processes plus digestive processes in anaerobic conditions; at 1,000 mg/l gas in digester reduced to 40%, batches 5–1,000 mg/l; Schefer, 1981)

A further test with anaerobic bacteria from domestic activated sludge was performed by Hoechst (1982), yielding a 24-hour NOEC of 15 mg/l. No data on the test conditions was available, so that the study could not be validated.

3.2.1.2   Comparison with (Q)SAR data

Using the (Q)SAR-relationships proposed in the TGD for base-line toxicity, the following effect concentrations can be estimated:

Fish

Pimephales promelas	96-hour LC50	7.7 mg/l
	28-32-day NOEC	0.6 mg/l
Daphnia magna	48-hour EC50	4.1 mg/l
	16-day NOEC	0.55 mg/l

Algae

| *Selenastrum capricornutum* | 72-96-hour EC50 | 3.4 mg/l |

These estimations are very consistent with the actual determined concentrations and only differ by a factor 2-6, the acute/long-term ratio being closely respected.

3.2.1.3 Calculation of Predicted No Effect Concentration (PNEC)

PNEC for the aquatic compartment

To determine the aquatic PNEC, the results from long-term tests can be used:

Fish: 10-day NOEC: 0.20 mg/l (*Jordanella floridae*, Smith et al., 1991)

This is the lowest long-term NOEC derived from a concentration-effects curve. The 60-day NOEC of ≥ 0.1 mg/l with *Oncorhynchus mykiss* is not considered, as no effects were observed up to the highest test concentration of 0.1 mg/l.

Invertebrates: 28-day NOEC: 0.22 mg/l (*Daphnia magna*, Calamari et al., 1982)

Algae: 96-hour EC 0: 0.57 mg/l (*Selenastrum capricornutum*; Calamari et al., 1983)

The 96-hour EC0 with *Selenastrum capricornutum* can be used as a NOEC. As long-term NOECs are available for all 3 trophic levels, an assessment factor of 10 can be used, applied to the lowest of the NOEC values, 0.20 mg/l. Therefore:

$PNEC_{aqua}$ = 200/10 = 20 µg/l

PNEC for sewage treatment plants

A reliable test was realised with Nitrosomonas, nitrifying bacteria, in which a 12-hour EC50 of 86 mg/l was derived (Blum and Speece, 1991).

A lower effect value (24-hour NOEC of 15 mg/l) was found with anaerobic bacteria from domestic sewage sludge in Hoechst (1982). No further data on test conditions were provided, so that the test result could not be validated. Furthermore, in a long-term test (35 days) with anaerobic bacteria from domestic sewage sludge, the gas production was not inhibited at 100 mg/l (Schefer, 1981). The lower NOEC-value in the acute test can therefore be ignored for the PNEC derivation.

An inhibition of the biodegradation of 1,4-dichlorobenzene was observed at 40 mg/l by Calamari et al. (1982), while no inhibition was observed at 8 mg/l. As no concentration effect curve is available, no NOEC or EC50 value can be derived from these findings. Furthermore, no effect on the respiration of activated sludge was detected at saturation by Blum and Speece (1991).

As effect data are available with specific bacterial populations (e.g. *Nitrosomas sp.*) as well as anaerobic bacteria, which would be exposed to the highest concentration if a denitrifying tank is present near the influent of the STP, a safety factor of 10 applied to the lowest effect concentration seems to be sufficient.

$PNEC_{stp}$ = 86/10 = 8.6 mg/l

This PNEC would respect the findings from the biodegradation study performed by Calamari et al. (1982).

PNEC for sediment

In the absence of any toxicological data for sediment dwelling organisms, the PNEC is calculated using the equilibrium partitioning method with the PNEC for aquatic organisms and the

sediment-water partitioning coefficient. As for the PEC-calculation, the properties of suspended matter are used:

$$PNEC_{sed} = Ksusp_{water}/RHO_{susp} \cdot PNEC_{aqua} = 212 \text{ μg/kg (wet weight)}$$

or

$$PNEC_{sed} = Kp_{susp} \cdot PNEC_{aqua} = 900 \text{ μg/kg (dry weight)}$$

One test has been performed with a sediment dweller (*Tanytarsus dissimilis* 48-hour EC50 = 13 mg/l) in an aqueous system with a layer of sand at the bottom of the vessel (Call et al., 1983). The organic carbon content of the sand is not indicated. Using each of the different compartment-water partition coefficients for soil, sediment and suspended matter, a concentration in the sand layer of 157-178 mg/kg wet weight can be estimated. This result tends to validate the PNEC for sediment. The value obtained is a factor of 10^3 lower than the experimental LC50 value. The PNEC for aquatic organisms is more like a factor 300 less than the acute toxicity results for fish. This might indicate that the PNEC sediment is over-estimating the toxicity to some degree.

3.2.2   Atmosphere

Only older results regarding the efficacy of 1,4-dichlorobenzene is available in the literature. Anonymous (1953) tested the effects of vapours of 1,4-dichlorobenzene on 3 insect species, the confused flour beetle (*Tribolum confusum*), the webbing clothes moth (*Tincola bisselliella*) and the black carpet beetle (*Attagenus piceus*). Weighted amounts of fine crystals were placed in the bottom of Strand flasks, in which the insects were suspended in bolting silk. The flasks were placed on a warm surface for 5-10 minutes to vaporise the crystals. The exposure duration was 24 hours. The corresponding LC50 for the 3 test species were:

Tribolum confusum   24-hour LC50 = 2,140 mg/m^3
Tincola bisselliella   24-hour LC50 = 5,620 mg/m^3
Attagenus piceus   24-hour LC50 = 8,040 mg/m^3

These results could not be validated though due to the lack of data regarding test conditions. It is therefore not opportune to derive a PNEC based on the above results.

3.2.3   Terrestrial compartment

3.2.3.1   Toxicity tests results

Soil dwelling organisms

Short-term tests have been performed with two earthworm species in two types of soil (Van Gestel et al., 1991). According to the TGD, the results should be normalised to an organic matter content of 3.4%. The observed and recalculated results are indicated below:

		Reported (mg/kg dw)		Recalculated (mg/kg dw)	
		KOBG-soil	OECD-soil	KOBG-soil	OECD-soil
Eisenia andre	14-day LC50	128	229	118	96
Lumbricus rubellus	14-day LC50	184	615	169	258

KOBG: natural, sandy agricultural soil; o.m. = 3.7%
OECD: laboratory soil mixed according to OECD guideline; o.m. = 8.1%

The tests were performed in an open static system. 1,4-Dichlorobenzene was first combined with a small amount of dry soil, and this mixture was then combined with the remaining soil.

Terrestrial plants

Only one test has been performed in soil (Method: OECD guideline 208; Hulzebos et al., 1993). The reported result as well as the normalised result to an organic matter content of 3.4% is indicated below:

	Reported (mg/kg dw)	Recalculated (mg/kg dw)
Lactuca sativa 14-day EC50	213-248	453-527

o.m.   = 1.6%

3.2.3.2   Calculation of Predicted No Effect Concentration (PNEC)

PNEC for soil

Only short-term toxicity tests are available for 1,4-dichlorobenzene. The lowest value is 96 mg/kg dw soil (*Eisenia andrei*). As results are available with plants and invertebrates only, an assessment factor of 1,000 can be used. Therefore:

$PNEC_{soil}$ = 96/1,000 = 0.096 mg/kg = 96 µg/kg dw (i.e. 84.7 µg/kg wet weight)

3.2.4   Secondary poisoning

The most relevant NOAEL of 10 mg/kg/day was found in a chronic study with dogs (oral administration) (cf. Section 4.1.2.6 and 4.1.2.8). An oral NOAEL of 30 mg/kg/day was found for developmental effects in rats. It is therefore necessary to perform a risk assessment for secondary poisoning.

Using a conversion factor to food of 10 and a safety factor of 10, as applicable to chronic studies, the PNEC for secondary poisoning becomes:

$PNEC_{oral}$ = 10 mg/kg (food)

3.3   RISK CHARACTERISATION

3.3.1   Aquatic compartment (incl. sediment)

Sewage treatment plants

The highest value estimated in a STP outlet, PEC_{stp}, is 0.83 mg/l, for a worst-case site using 1,4 dichlorobenzene as an intermediate.

With a $PNEC_{stp}$ microorganism of 8.6 mg/l, the PEC/PNEC ratio amounts to 0.1, and therefore no risks to microorganisms in the STP are to be expected.

Conclusion (ii)   There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Surface waters

In **Table 3.10** the comparison between the total surface water concentrations and the aquatic PNEC for the different exposure scenarios is presented. The regional concentrations being negligible compared to the local concentrations, only the latter are retained for the risk characterisation.

Table 3.10 Highest estimated PEC/PNEC-ratios for the surface water

Scenario	PEC (µg/l)	PEC/PNEC
Production *	12	0.60
Use as an intermediate **	0.32	0.02
Formulation of moth repellents and air fresheners ***	4.5	0.22
Use of toilet blocks	3.4	0.17
Textile dyeing ****	9	0.45
Paint manufacturing ****	0.03	< 0.01

*	the measured concentrations at the two other European production sites are significantly lower
**	estimated worst-case concentration, assuming a use of 3,450 tonnes/year of 1,4-dichlorobenzene. The concentrations at three other identified sites, based on measurements are significantly lower
***	intermittent release, 3 times a year
****	single measured concentration

With the highest single measured concentration in a monitoring programme of 4.05 µg/l, a PEC/PNEC-ratio of 0.2 can be deduced. As all the above-calculated PEC/PNEC ratios are below 1, it can be concluded that there is no risk to aquatic organisms through 1,4-dichlorobenzene: **conclusion (ii).**

Sediment

In **Table 3.11** the comparison between the estimated sediment concentrations and the sediment PNEC for the different exposure scenarios is presented. The regional concentrations being negligible compared to the local concentrations, only the latter are retained for the risk characterisation.

Table 3.11 Highest estimated PEC/PNEC ratios for the sediment

Scenario	PEC (µg/kg dw)	PEC/PNEC
Production *	540	0.60
Use as an intermediate **	14.4	0.02
Formulation of air fresheners and moth repellents ***	1.66	< 0.01
Use of toilet blocks	153	0.17
Textile dyeing ****	405	0.45
Paint manufacturing ****	1.35	< 0.01

*	the estimated concentrations at the two other European production sites are significantly lower
**	estimated worst-case concentration, assuming a use of 3,450 tonnes/year of 1,4-dichlorobenzene. The estimated concentrations at three other identified sites are significantly lower
***	intermittent release, 3 times a year; use of annual average concentration
****	based on single measured concentrations in the effluent

Based on the monitoring data in sediment and suspended matter, a reasonable worst-case concentration of 430 µg/kg dw was derived. Based upon this value, a PEC/PNEC ratio of 0.5 can be derived.

As all the above calculated PEC/PNEC-ratios are below 1, it can be concluded that there is no risk to sediment dwelling organisms through 1,4-dichlorobenzene: **conclusion (ii)**.

3.3.2 Atmosphere

Based on the physical properties of 1,4-dichlorobenzene, the air compartment is the preferred target compartment. It is however so far not possible to realise a biotic assessment in the same way as described for other compartments, as the available experimental data on environmental organisms exposed through the gas phase could not be validated. Further data on the efficacy of 1,4-dichlorobenzene will probably be generated for the authorisation process under the biocides directive (directive 98/8/CE). A quantitative biotic risk assessment for the atmosphere might then be performed.

For the evaluation of an atmospheric risk, abiotic effects can be considered. The atmospheric life-time of 50 days indicates no risk for stratospheric ozone and for global warming, although 1,4-dichlorobenzene contains two Cl substituents: **conclusion (ii)**.

3.3.3 Terrestrial compartment

The highest local concentrations in agricultural soil have been estimated during production and during the use of 1,4-dichlorobenzene as an intermediate. The regional concentration is negligible. The resulting PEC/PNEC ratios are:

Scenario	PEC (µg/kg dw)	PEC/PNEC
Production	2.3	0.024
Use as an intermediate	72.5	0.75

As all the above-calculated PEC/PNEC-ratios are below 1, it can be concluded that there is no risk to the terrestrial compartment through 1,4-dichlorobenzene: **conclusion (ii)**.

3.3.4 Secondary poisoning

The worst-case exposure to fish eating birds has been estimated as being 8.4 mg/kg (wet fish). With a $PNEC_{oral}$ of 10 mg/kg, the PEC/PNEC ratio becomes 0.84.

The worst-case exposure to earthworm-eating birds or mammals has been estimated as being 0.4 mg/kg (wet earthworm). With a $PNEC_{oral}$ of 10 mg/kg, the PEC/PNEC ratio becomes 0.04.

No risks to predators are therefore to be expected: **conclusion (ii)**.

4   HUMAN HEALTH

4.1   HUMAN HEALTH (TOXICITY)

4.1.1   Exposure assessment

4.1.1.1   General discussion

Exposure to 1,4-dichlorobenzene may arise during its manufacture, its use in synthesis, its incorporation into various products and from their use: workers and consumers may be exposed to 1,4-dichlorobenzene. The main source of exposure is likely to be vapour emitted by the solid (consumers, workers) or from molten material (workers).

4.1.1.2   Occupational exposure

Occupational exposure may occur by inhalation, skin contact and oral route. Inhalation of vapour is the predominant route of exposure. Dust exposure may arise but is likely to be minimal because of the physical state of the substance (flake).

Dermal exposure from the solid (flake) is expected to be low. It is also expected to be low when the substance is used as a hot molten material.

Oral exposure is not considered to be a significant route under normal working practices.

Current occupational exposure limits in EU member states and in the US are reported in the following table.

Table 4.1   Current occupational exposure limits

Country	8-hour TWA value		STEL value	
	ppm	mg/m³	ppm	mg/m³
Belgium	75	450	110	660
Denmark	10	60		
Finland	75	450	115	690
France	75	450	110	675
Germany	50	300	100/30 min	600
Netherlands	75	450		
United Kingdom	25	150	50	300
United States (ACGIH)	10	60		
Norway	40	240		
Sweden	75	450	110	660

Conversion factor: 1 ppm = 6.01 mg/m³ at standard temperature (25°C) and pressure.

4.1.1.2.1   Occupational exposure during manufacture

1,4-Dichlorobenzene is produced in three plants in the EU, in closed system processes. Control measures like local exhaust ventilation are usually applied in the workplace.

Inhalation exposure

Literature exposure data

Few data are available on the levels of 1,4-dichlorobenzene in the workplace.

A study (Pagnotto and Walkley, 1965) was made in a 1,4-dichlorobenzene plant (USA): the substance was prepared in a closed vessel. Workroom ventilation was provided in part by blowers, but mostly by opened doors and windows. The results are summarised in **Table 4.2**.

Table 4.2   Manufacture exposure data   (Pagnotto and Walkley, 1965)

Production plant	Range (ppm)	Average (ppm)
Washing	7-48	34
Shovelling and centrifuging	10-49	|33
Crushing and sizing	8-46	24

However, these data are too old to be taken into consideration because work conditions have been improved.

Another study (Hollingsworth, 1956) was made in a company where 58 men were working (generally 8 hours per day, 5 days a week) continually on operations involving the handling of 1,4-dichlorobenzene. Spot samples of the workroom atmosphere were collected and analysed for vapour concentrations of 1,4-dichlorobenzene. The results of analyses of 62 air samples showed concentrations ranging from 10 to 550 ppm with an average of 85 ppm. In this case, no data are available on control measures. These data are also too old to be taken into consideration for the same reasons as above.

NIOSH reported that a worker at a 1,4-dichlorobenzene drumming operation was exposed to 5 ppm (8-hour TWA) and that some workers were exposed to levels up to 37 ppm. Measurements made by the US Occupational Safety and Health Administration (OSHA) between 1981 and 1986 show numerous instances of workers exposed to concentrations greater than 8 ppm (ATSDR, 1989). For the values 37 ppm and 8 ppm, the type of exposure (exposure during the actual tasks or 8-hour TWA) is not described.

Industry exposure data

The data in **Table 4.3** represent the figures from 3 producers in the EU, covering production and a number of related handling operations.

Table 4.3   Manufacture exposure data (industry source)

	Production/Processing 8-hour TWA (ppm)		Filling 8-hour TWA (ppm)	
	Average	Range	Average	Range
Production 1*	0.54	≤ 0.09-2.15	< 0.05	0.04-0.21
Production 2	3	≥ 1-7	2.5	≥ 1-5
Production 3	< 0.5	-	< 0.5	-

*   Only production

Number of sampling is not always available; for producer 1, the exact number of samples was 35. During production and filling, the 8-hour TWA is ranging from 0.04 to 7 ppm.

Data from model calculation

During manufacture, the use pattern is a closed system; the exposure estimated with the EASE model is between 0 and 0.1 ppm (8-hour TWA) if the control pattern is a full containment.

However in some operations, the system can be breached (discharging from the reactor, packaging, sampling, maintenance and cleaning). In this case, the estimated exposure with local exhaust ventilation is 0.5-3 ppm (8-hour TWA).

Conclusion on inhalation exposure during manufacture

The data provided by industry are consistent with the EASE exposure estimate. For risk characterisation purposes, it seems reasonable to take the higher value provided by industry, i.e. 7 ppm (42 mg/m^3) (8-hour TWA) as a worst-case scenario.

Dermal exposure

Dermal exposure may occur during handling the particulates and from contaminated surfaces during cleaning and maintenance activities. The EASE model predicts dermal exposure of 0.1-1 mg/cm^2/day (non dispersive use with direct handling and intermittent contact). This is likely to be an over estimate, therefore the reasonable worst-case scenario is expected to be at the bottom of the range (expert judgement). It is assumed that during activities, two hands will be exposed (800 cm^2) which results in an estimated exposure of 80 mg/day. Although this exposure will be mitigated significantly by the use of suitable gloves.

4.1.1.2.2   Occupational exposure during use as a synthesis intermediate

Inhalation exposure

Industry exposure data

Exposure data supplied by two producers include data from both manufacture and processing (see above production 2 and 3).

A study (personal communication, 1996) was made in a 2,5-dichloronitrobenzene plant (EU). At this plant 2,5-dichloronitrobenzene is produced in closed system by nitration of 1,4-dichlorobenzene

according to a continuous method where molten 1,4-dichlorobenzene is converted to 2,5-dichloronitrobenzene at a temperature of 65°C. The 1,4-dichlorobenzene is pre-heated and fed in as a liquid; it is piped direct into the reaction vessel.

Workroom ventilation is provided in part by blowers and also by opened doors and windows.

The results of analyses of air samples show concentrations ranging from 0.01 ppm (workroom atmosphere, 8-hour TWA) to 0.1 ppm (cleaning and handling operations, 8-hour TWA).

The available information was not sufficient to evaluate carefully the quality of these measured data and their representativeness for the risk assessment: the exact number of samples for 1,4-dichlorobenzene, the sampling period and the exact locations of the sampling equipment were not provided.

Conclusion on inhalation exposure during use as a synthesis intermediate

These data show that inhalation exposure during processing is likely to be low and in the same order as during manufacture.

Dermal exposure

It is likely to be in the same order as during manufacture (80 mg/day).

4.1.1.2.3 Occupational exposure during formulation of household products

Occupational exposure to the substance may occur during discharging, formulation, cleaning and maintenance activities and packaging. 1,4-Dichlorobenzene is mainly used as a solid: after grinding and blending with other ingredients, the mixture is pressed into balls or blocks. 1,4-Dichlorobenzene may also be mixed in the molten state with other ingredients: this scenario appears to be less usual.

Inhalation exposure

Literature exposure data

Pagnotto and Walkley (1965) investigated 1,4-dichlorobenzene air concentrations in two plants.

Table 4.4   Formulation exposure data   (Pagnotto and Walkley, 1965)

Plant Household product packaging	Range (ppm)	Average (ppm)
Pulverising	18–34	25
Moth cake line	8–12	11
Dumping crystals	7–10	9
Crystal line	8-18	11

No data are available on control measures. These data are too old to be taken into consideration.

In the UK, HSE inspectors have reported on two factories manufacturing toilet blocks. At one of these, eight workers were exposed to 20 to 54 ppm 1,4-dichlorobenzene, although a concentration of 136 ppm was found near one machine. At the other factory four workers were exposed to 5 to

11 ppm, whilst a fifth was exposed to 81 ppm (in both cases the samples covered only part of the shift, for example 1 to 3.5 hours, but the results are likely to be typical of the whole shift). In both factories, improvements in control appeared to be achievable by fairly simple means (HSE, 1994).

Information from the "Berufsgenossenschaftliches Institut für Arbeitssicherheit"(BIA) database.

Occupational exposure data on 1,4-dichlorobenzene were made available by the "Employers' Liability Insurance Association for the Chemical Industry" and extracted from the MEGA database of the BIA (Germany). 55 companies were evaluated; 108 measurements have been gathered from 1991 to 1995 with a duration of exposure superior or equal to 1 hour (TWA). The analytical detection limit for a sampling duration of two hours amounts to 0.5 mg/m^3. Measurement results for all types of operation are: 50% of all concentrations are below detection limit, 90% of all concentrations are below 3.2 mg/m^3 (about 0.5 ppm), 95% of all concentrations are below 6.2 mg/m^3 (about 1 ppm).

Measurement data above the analytical detection limit were determined during the production of air fresheners or air disinfectants (mixing, cold-pressing).

Industry exposure data

A study (personal communication, 1996) was made in a moth repellent and toilet block plant within the EU. At this plant solid 1,4-dichlorobenzene (in granular and/or scale form) is mixed with other components and transferred into a compacting machine and then into a packaging machine. Only natural ventilation is provided. Air sampling was carried out to determine worker exposure to 1,4-dichlorobenzene. In each case the samples covered only part of the shift (2 hours) but the results are likely to be typical of the whole shift. Five samples have been collected. The results of analyses of air samples show concentrations ranging from 13 to 27.5 ppm. The highest occupational exposure level is likely to occur during compacting near the compacting machine. In this factory a new moth repellent and toilet block plant is being built.

Another study (personal communication, 1996) was made in a modern plant where moth repellents, toilet blocks and other household products are manufactured. An effective ventilation is provided in the workrooms where these products are manufactured. All the installations, e.g for filling of raw material, grinding, compacting, packaging, et all conveying belts are in closed system. The number of employees potentially exposed to 1,4-dichlorobenzene in the workrooms is lower than 45. The occupational exposure levels range from 0.1 to 0.6 ppm. The available information was not sufficient to evaluate carefully the quality of these measured data and their representativeness for the risk assessment: the exact number of samples and the sampling period were not provided.

However, these two studies show that exposure during formulation may change significantly from one company to another, depending on available control measures and age of the factory.

Data from model calculation

Handling of molten material (process temperature $\geq$ 60°C): For inhalation exposure (vapour), application of EASE model, using a scenario of non-dispersive use, no full containment, moderate volatility and local exhaust ventilation gives a predicted inhalation exposure ranging from 10 to 50 ppm (8-hour TWA).

Handling of solid material: For inhalation exposure (vapour), application of EASE model gives the following results:

- exposure to vapour using a scenario of non-dispersive use pattern, no full containment, low volatility and local exhaust ventilation is predicted to be 0.5-3 ppm (8-hour TWA),

- exposure to vapour using a scenario of non-dispersive use pattern, no full containment, low volatility and direct handling is predicted to be 10-50 ppm (8-hour TWA).

Conclusion on inhalation exposure during formulation

Although measured data are available, they are insufficient to establish with confidence the level of exposure. Therefore, it is proposed for risk characterisation purposes, to take the higher value provided by the EASE model, 50 ppm (8-hour TWA), as the worst-case scenario. Furthermore, the value of 50 ppm (300 mg/m^3) (8-hour TWA) appears consistent with the highest measured data.

Dermal exposure

It is likely to be in the same order as in manufacture (80 mg/day).

4.1.1.2.4   Occupational exposure during abrasive manufacturing

Inhalation exposure

Literature exposure data

Pagnotto and Walkley (1965) investigated 1,4-dichlorobenzene air concentrations in two plants.

Table 4.5   Abrasive manufacturing exposure data (Pagnoto and Walkley 1965)

Plant Abrasive manufacturing	Range	Average (ppm)
Mixing	8-14.5	11.5
Wheel-forming	7-9	8

No data are available on control measures. These data are too old to be taken into consideration.

Information from database (EXPO-register Norwegian database, Norwegian Institute of Occupational Health, NIOH)

1,4-Dichlorobenzene is used in the production of grinding wheels to make the grinding wheels porous. It is mixed with the grinding material and this mixture is pressed together and then burnt in a furnace. The measurements date back to 1985.

Table 4.6   Exposure data during grinding wheel production (EXPO-register)

Personal sampling	Median (ppm)	Lowest (ppm)	Highest (ppm)	Work operation
6	17.4	9.9	41.6	Mixing the chemicals
6	8.4	5.7	32	Operating the furnace
6	9.6	7.7	13	Pressing the mix
6	15.2	7.1	62.6	Mixing the chemicals

Data from model calculation

Assuming a scenario of non dispersive use pattern, no full containment, low volatility and direct handling of the solid material, the inhalation exposure (vapour) is predicted by the EASE model to be in the range of 10-50 ppm (8-hour TWA).

It is proposed for risk characterisation purposes, to take the higher value provided by the EASE model, 50 ppm (8-hour TWA), as the worst-case scenario. Furthermore, the value of 50 ppm (300 mg/m^3) (8-hour TWA) appears consistent with the highest measured data.

Dermal exposure

It is likely to be in the same order as in manufacture (80 mg/day).

4.1.1.2.5 Summary on occupational exposure

Table 4.7   Summary on occupational exposure

Scenario		Estimated inhalation exposure level (8-hour TWA) (Data industry and Ease)		Estimated skin exposure level (EASE and expert)
		mg/m³	ppm	mg/day
scenario 1	Production of 1,4-dichlorobenzene Synthesis intermediate	42	7	80
scenario 2	Formulation of household products Abrasive manufacturing	300	50	80

4.1.1.3   Consumer exposure

Consumers are mainly exposed by inhalation, both at home and in public lavatories by the use of mothballs, air fresheners and toilet blocks.

4.1.1.3.1   Exposure from uses

Field studies

In the scientific literature, data on the levels of 1,4-dichlorobenzene in indoor air, personal air and biological matrixes are available. Here are the data from these studies organised by regions of the world.

Studies from the USA

US EPA created a database to gather and organise the available data on VOC air concentrations (Shah et al., 1988) in the 80's. The collected data came from different sources (literature searches, direct contacts with individuals and organisations measuring VOC, questionnaires, meetings of experts). Nearly 90% of indoor air data were from California and New Jersey. About 98% of them were measured from 1981 to 1984 and more than 95% were collected with a 1- to 24-hour sampling period.

1,4-dichlorobenzene was detected at 2,121 sites. The mean, median, lower- and upper-quartile were 23.9, 1.7, 0.3 and 5.6 $\mu g/m^3$, respectively.

The mean that is higher than the upper quartile shows that a few measures have a very high value.

The median of 1,650 personal air measures is also reported and is equal to 2.5 $\mu g/m^3$.

From 1979 to 1985, US EPA conducted the TEAM study (Total Exposure Assessment Methodology) to measure exposure to 20 VOC. Several scientific articles report the results of this study. The exposure of 600 people, being seven years old or more, were measured in several phases, at different seasons. The subjects were selected to represent a total population of 650,000 residents of cities in New Jersey, North California, North Dakota and California. Outdoor, indoor, personal air samples, exhaled breath and water samples were collected. A stratified probability selection was implemented to insure the inclusion of potentially highly exposed people. An extensive quality assurance program was carried out to verify the precision and the reproductivity of the measurements.

Each participant carried a personal sampler during normal daily activities for two consecutive 12-hour periods (the daytime period: from 6 a.m. to 6 p.m. and the overnight period: from 6 p.m. to 6 a.m.). An identical sampler was installed in the backyards of one participant's home out of four during the same two 12-hour periods to measure outdoor concentration. Samples were collected by a pump drawing air at 30 ml/min.

Most of the data from this study are reported for the sum of 1,3 dichlorobenzene and 1,4-dichlorobenzene, but as the latter compound is used in much greater quantity, it has been assumed by the authors that the concentrations found could be assimilated to 1,4-dichlorobenzene.

The most comprehensive statistical results have been presented for the first phase of the study, conducted in New Jersey, in fall 1981. The number of measurements is between 339 and 348 for personal air concentrations and between 81 and 90 for outdoor air concentrations.

The range was:

- 0.08-1,500 µg/m^3 (arithmetic mean 56 µg/m^3, 95th percentile: 260 µg/m^3, 99th percentile: 1,200 µg/m^3) for the overnight personal air concentrations,

- 0.11-790 µg/m^3 (arithmetic mean 35.1 µg/m^3, 95th percentile: 210 µg/m^3, 99th percentile: 490 µg/m^3) for the daytime personal air concentrations,

- 0.07-13.0 µg/m^3 (arithmetic mean 1.54 µg/m^3, 95th percentile: 4 µg/m^3, 99th percentile: 13 µg/m^3) for the overnight outdoor air concentrations

- And 0.10-57.0 µg/m^3 (arithmetic mean 1.94 µg/m^3, 95th percentile: 5.4 µg/m^3, 99th percentile: 5 µg/m^3) for the daytime outdoor air concentrations.

Overnight personal air exposures were essentially indoor air measurements, as the monitor was on the bedside table while the participant slept. Comparing the overnight personal air concentrations and the outdoor air concentrations, it appears that the indoor air concentrations are much higher than outdoor air concentrations. This fact is confirmed by many other studies. The distribution of measurements, especially for overnight personal air concentration, is highly stretched. A few measurements have very high value.

The results of the different phases of the study can be compared.

In New Jersey, the average of the arithmetic means of day and night 12-hour personal air samples was:

- in fall 1981: 45 µg/m^3 (number of measurements: 340),

- in summer 1982: 50 µg/m^3 (number of measurements: 150),

- in winter 1983: 71 µg/m^3 (number of measurements: 49).

In Los Angeles, the average of the arithmetic means of day and night 12-hour personal air samples was:

- in February 1984: 18 µg/m^3 (number of measurements: 110),

- in May 1984: 12 µg/m^3 (number of measurements: 50).

In Contra Costa (California), the average of the arithmetic means of day and night 12-hour personal air samples was:

- in June 1984: 5.5 µg/m^3 (number of measurements: 67).

Owing to the cumulative frequency curves presented by Wallace et al. (1988), the 95th and 99th percentiles of the personal air concentration distributions can also be compared.

In Los Angeles:

- in February 1984, the 95th and 99th percentiles of the overnight personal air concentration were 110 µg/m^3 and 300 µg/m^3, respectively, whereas the 95th and 99th percentiles of the daytime personal air concentration were 110 µg/m^3 and 200 µg/m^3, respectively.

- in May 1984, the 95th and 99th percentiles of the overnight personal air concentration were 110 μg/m^3 and 220 μg/m^3, respectively, whereas the 95th and 99th percentiles of the daytime personal air concentration were 60 μg/m^3 and 130 μg/m^3, respectively.

In Contra Costa:

- in June 1984, the 95th percentile of the overnight personal air concentration was 36 μg/m^3, whereas the 95th and 99th percentiles of the daytime personal air concentration were 22 μg/m^3 and 60 μg/m^3, respectively.

In the suburb of Philadelphia, in November 1992, Heavner et al. (1996) measured VOC, included 1,4-dichlorobenzene, in 61 non-smokers houses and in 32 smokers' houses. Samples of 20 litres were collected with pumps during an average time of 14 hours. The sample collection started at approximately 6 p.m. till the following morning. During the waking hours at home, the samplers were worn by participants and during sleeping hours, the samplers were placed in the bedroom near participants.

In the non-smokers houses, the mean was 5.18 μg/m^3 with a range of 0.00 to 121.86 μg/m^3 whereas in the smokers' houses, the mean was 16.77 μg/m^3 with a range of 0.00 to 302.28 μg/m^3.

Pellizari et al. (1999) measured from July 1995 to May 1997, the exposure to VOC and metals of a sample of population living in the region of Great Lakes. Personal air samples were collected for 63 children (from 1 to 14 years old) and for 313 adults (being more than 21 years old). The mean and median concentrations of 1,4-dichlorobenzene was respectively 2.6 μg/m^3 and below the detection limit for children and 3.8 and 0.87 μg/m^3 for adults. The range of values obtained is not presented in this article.

The statistical analysis shows that personal air exposure, defined by the median, is significantly higher in adults than in children.

Hill et al. (1995) measured the level of 2,5-dichlorophenol, the metabolite of 1,4-dichlorobenzene, in urine samples and 1,4 dichlorobenzene in blood samples of 1,000 adults throughout the USA 2,5-dichlorophenol was detected in 98% of urine samples with a mean of 200 μg/l and a 95th percentile of 790 μg/l (maximum value 8,700 μg/l) and 1,4-dichlorobenzene in 96% of blood samples with a mean of 2.1 μg/l and a 95th percentile of 11 μg/l (maximum value 19 μg/l). The statistical results show that exposure to 1,4-dichlorobenzene is very widespread.

Studies from Canada

Chan et al. (1990) measured the levels of VOC in twelve Canadian houses in November and December 1986. The concentration of 1,4-dichlorobenzene was measured in the living or family room in the evening or late afternoon with a 90-minute sampling time. The mean was 15 μg /m^3 with a range from 1 to 107 μg/m^3. In February and March 1987, the measurements were repeated in six of the houses. The minimal value was below the detection limit whereas the maximum value was not quantifiable due to the saturation of the detector of the mass spectrometer. As the number of houses where measurements were made is very limited, the results obtained cannot be viewed as representative of the general situation. However, it appears that the individual concentrations are highly variable.

Fellin et al. (1994) measured the concentration of VOC in 754 houses in Canada, at different seasons of the year. A random selection of the houses from the census was made to ensure an equal probability of sampling in the different regions of the country. 24-hour samples were

collected by a passive monitoring device. Only the mean concentrations measured in each season and according to the external temperature are reported.

According to the authors, moth crystals are normally deployed in a manner that gives reasonably constant emissions during several weeks. Consequently, the average concentration of 1,4-dichlorobenzene follows a trend consistent with the expected ventilation:

- the highest concentration of 1,4-dichlorobenzene is observed during winter or when outdoor temperature is below 0°C, that means when ventilation of the house is expected to be the lowest,

- and the lowest average concentration is obtained in summer or when temperature exceeds 15°C that means when ventilation is expected to be the highest.

Mean indoor concentrations were 35.75 $\mu g/m^3$ in winter, 15 $\mu g/m^3$ in spring, 10.54 $\mu g/m^3$ in summer and 15 $\mu g/m^3$ in autumn. They were 23.64, 22.02, and 11.83 $\mu g/m^3$ when the outdoor temperatures were below 0°C, between 0 and 15°C and above 15°C, respectively.

Studies from Japan

Morita et al. (1975a) measured 1,4-dichlorobenzene in 34 adipose tissue samples coming from general hospitals and a medical examiners office of Tokyo. The mean value was 2.3 $\mu g/g$ (range 0.2-11.7). They also measured the concentration of 1,4-dichlorobenzene in the blood of six healthy individuals being less than 35 years old and living in Tokyo. The mean blood concentration was 9.5 $\mu g/l$ (range 4-16). Three measures of 1,4-dichlorobenzene in different places of a house were also made. The results were 1,700 $\mu g/m^3$ inside wardrobe, 315 $\mu g/m^3$ inside closet and 105 $\mu g/m^3$ in the bedroom.

Matsumura et al. (1992) measured the levels of 1,4-dichlorobenzene in samples of indoor air and personal air. The samples were collected during 24 hours at a rate of 30 ml/min. The indoor air samples were collected from August 1989 to September 1990. The personal air samples were collected from May to September 1990, while the different individuals (housewives, office workers, one student) did their regular activities. Twenty-nine indoor air samples were obtained with 1,4-dichlorobenzene concentrations in the range of 42-5962 $\mu g/m^3$ (median value 607 $\mu g/m^3$; 95th percentile 3,485 $\mu g/m^3$; 99th percentile 5,310 $\mu g/m^3$). The results for the 17 personal exposure measurements were between 24 to 3,275 $\mu g/m^3$ (median value 234 $\mu g/m^3$, 95th percentile 2,660 $\mu g/m^3$, 99th percentile 3,485 $\mu g/m^3$). From January to February 1992, Matsumara et al. (1993) made another series of personal air concentration measurements (housewives, office workers, students and school children), with a passive sampler exposed during 24 hours. The results were in the range 42-1,232 $\mu g/m^3$ (median value 117 $\mu g/m^3$, 95th percentile 716 $\mu g/m^3$, 99th percentile 1,129 $\mu g/m^3$). The results of the two campaigns are comparable, even if the passive sampler gives lower results than those obtained with an active monitor device (11% lower on average).

For the two campaigns, it appears that the housewives, who spend the longest time per day within their home, have the highest personal exposure to 1,4 dichlorobenzene. Pooling the 9 results of the two campaigns concerning the exposure of housewives, their exposure concentrations vary from 102 to 3,275 $\mu g/m^3$, with an average of 1,124 $\mu g/m^3$.

During the summer 1996, Olansandan et al. (1999) measured the concentrations of VOC in indoor air and outdoor air of seventy residences in Shizuoka, owing to a passive sampler exposed during 24 hours. Compared to an active sampler, the collection efficiency of the passive one was at least 96.4%. The geometric mean and 95th percentile were 12.1 and 2,060 $\mu g/m^3$ in 60 living

rooms (upper value 13,800 µg/m^3), 9.35 and 768 µg/m^3 in 70 kitchens (upper value 3,130 µg/m^3), 71.6 and 3,460 µg/m^3 in 70 bedrooms (upper value 16,000 µg/m^3), and 10.4 and 375 µg/m^3 in 70 bathrooms (upper value 1,760 µg/m^3).

The results are very dispersed and some values of indoor air are very high. The geometric mean concentration in bedroom is significantly higher than that those in kitchen, bathroom and living rooms, corresponding to the use of 1,4-dichlorobenzene as an insect repellent in the closets of the bedrooms.

In winter 1998, Nakai et al. measured VOC for four consecutive days with a passive sampler in indoor air of thirty houses. Among these houses, twenty of them were inhabited by allergic children. The difference between the mean concentration of 1,4-dichlorobenzene in the houses inhabited by allergic children and in those inhabited by non-allergic children was not significant. The average for the thirty houses was 90 µg/m^3 with a standard deviation equal to 129 µg/m^3. In the rooms where insect repellent for clothes had been placed in the chest at the measurement room, the mean concentration was 111 µg/m^3, whereas in the others it was 54 µg/m^3. Nevertheless, the difference was not significant.

Study from Hong-Kong

Shun Cheng. et al. (2002) measured the levels of 1,4-dichlorobenzene outdoors, in the living room and the kitchen of six homes from July to October 1999. Sampling lasted 8 hours with a flow rate of 0.011 l/min, from lunchtime to dinner time. The means were 2.6 µg/m^3 (1.2-4.3) in the living room and 3 µg/m^3 (1.2-4.4) in the kitchen.

Studies from Europe

Lebret et al. (1984) measured the levels of VOC in 134 houses in the city of Ede in the Netherlands. Sampling flow was approximately 100 ml/min during a period of five to seven days, from October 1981 to April 1982. 66% of the measurements made were above the detection limit comprised between 2 and 4 µg/m^3. The mean and maximum values were 7.2 and 140 µg/m^3.

Kostiainen (1994) sought VOC in 26 Finnish houses. He detected 1,4-dichlorobenzene in 100% of the cases. Further quantitative measurements were performed in 50 "normal" houses and in 38 "sick" houses (houses where people complained of the odour or had symptoms resembling Sick Building Syndrome). Sampling was made during about thirty minutes. Two hours before and during sampling, no human activity was allowed at the sampling site and the outer door and windows were closed. In the "normal" houses, the mean obtained was 0.65 µg/m^3, with a range from 0 to 8.94 µg/m^3.

The concentrations of 1,4-dichlorobenzene are said to be "at least 5-10 times higher than the median concentration of the normal houses in about every fifth sick houses".

A preliminary study has been conducted in three French regions by Kirchner et al. (2002) to measure the concentration of 1,4-dichlorobenzene in the bedroom of 63 housings. The concentrations were comprised between the detection limit (1µg/m^3) and 293 µg/m^3 (medians between 0,7 and 3,5 µg/m^3 for the 3 regions).

Experimental studies

Some authors have measured the indoor air concentrations following the use or the placement of deodorant or mothballs in a house.

Scuderi (1986) (quoted in ATSDR, 1998) have measured the indoor air concentrations resulting from the use of 1,4-dichlorobenzene in bathroom and closets:

- bathroom with one deodoriser block: 469–757 $\mu g/m^3$,

- bathroom with one toilet deodoriser block in one urinal and one toilet: 697–1,322 $\mu g/m^3$,

- inside closet with moth flakes in closed garment bag: 1,316–3,275 $\mu g/m^3$,

- outside closet with moth flakes in closed garment bag: 78–427 $\mu g/m^3$.

In a German study (Globol Werke GmbH, 1986) the concentration of 1,4-dichlorobenzene was measured in rooms, where moth repellent, air freshener or toilet block were used.

Moth repellent: in two unfurnished rooms with identical wardrobes (0.58 m^3 each), 5 bags of 6 mothballs each (90 g) were laid between clothes in the wardrobe. Wardrobe 1 was never opened; wardrobe 2 was daily opened for 2 minutes. Both rooms were aerated daily, except on weekends, by opening the windows for 15 minutes, about 2 hours after the measurements were carried out. The temperature in the rooms was kept at 20 ± 2°C. The volumes of both rooms were 25.66 and 30.28 m^3. The measurements were performed until the 80th day, when the mothballs were entirely used up.

Airborne concentrations of 1,4-dichlorobenzene were 63-373 mg/m^3 (mean 289 mg/m^3) in wardrobe 1 and 3.7-19 mg/m^3 (mean 11.4 mg/m^3) in wardrobe 2. Before aeration, they were 1.3-10 mg/m^3 (mean 5.8 mg/m^3) in room 1 and 1.7-8 mg/m^3 (mean 4 mg/m^3) in room 2; after aeration, they were 0-6.8 mg/m^3 (mean 1.6 mg/m^3) and 0-2.6 mg/m^3 (mean 1.4 mg/m^3), respectively.

Air freshener: in a lavatory, an air freshening tablet (77.4 g) was attached to the wall, 1.6 m above the urinal. The ventilation was not controlled and depended on the lavatory users. The experimental period was 30 days; during this period, the tablet was not used up completely. The temperature varied between 16 and 22°C. The volume of the room was 15.42 m^3. Airborne concentration of 1,4-dichlorobenzene was 1.7-23 mg/m^3 (mean 3.6 mg/m^3) in the morning, 1.9-22.4 mg/m^3 (mean 4.2 mg/m^3) in the afternoon and 1.5-23.8 mg/m^3 (mean 7.5 mg/m^3) in the evening resulting in a mean concentration of 5.1 mg /m^3.

Toilet block: in two lavatories, containing two urinals (lavatory 1) or only one (lavatory 2), three toilet blocks (41.3 g/block) were placed in each urinal. Ventilation of the rooms was not controlled and depended on the lavatory users. Temperature varied between 16 and 22°C. The volumes of the rooms were 39.56 m^3 and 15.42 m^3. The blocks were used up within 67 and 57 days, respectively. In the morning, airborne concentrations of 1,4-dichlorobenzene were 0.3-5.8 mg/m^3 (mean 1.8 mg/m^3) in lavatory 1 and 0.6-13.3 mg/m^3 (mean 3.5 mg/m^3) in lavatory 2; in the afternoon they were 0.6-10.1 mg/m^3 (mean 3.6 mg/m^3) and 0.6-7.5 mg/m^3 (mean 3.9 mg/m^3), respectively.

At last, Wallace et al. (1989) studied the influence of personal activities on exposure to VOC. During three days, they measured the indoor concentration in four test houses and the personal air concentration of seven volunteers living in these houses. The sampling periods lasted between 5 and 11 hours. After the introduction of a toilet bowl deodorant in one of the four houses, the indoor and personal air concentrations increased by two orders of magnitude, from less than 5 $\mu g/m^3$ to 100-600 $\mu g/m^3$ during the next 48 hours (corresponding to the end of the experiment). In the case of the use of a liquid deodoriser (a drop in 4 locations throughout the house), the indoor and personal concentrations of 1,4 dichlorobenzene increased from non-detectable to 28-30 $\mu g/m^3$. These levels were maintained during 15 hours and then declined.

During the three days of the experiments, the median indoor air concentration houses ranged from 2.2 to 240 µg/m^3 according to the houses, while the maximum concentrations were from 7.2 to 740 µg/m^3. Personal air concentrations of the seven exposed people seemed to show the same variations as the indoor air concentration. The mean personal air concentrations over the three days range from 4 to 240 µg/m^3.

The study also shows that some houses can have a relatively elevated air concentration of 1,4-dichlorobenzene (40-60 µg/m^3 in average) although no known source have been identified.

4.1.1.3.2   Summary of consumer exposure

The results of the studies described above are summed up in the following **Tables 4.8** and **4.9**.

Table 4.8 Indoor air concentrations of 1,4-dichlorobenzene   (µg/m^3)

Study	Country	Number of measurements	Median	Mean	95th perc.	99th perc.	Range
Shah et al. (1988)	USA	2,121	1.7	23.9			
Chan et al. (1990)	Canada	12		15.0			1-107
Fellin et al. (1994)	Canada	754		35.75 in winter 15.00 in spring and fall 10.54 in summer			
Morita et al. (1975a)	Japan	1 1 1	1,700 in wardrobe 315 inside closet 105 in bedroom				
Matsumura et al. (1992)	Japan	29	607	1,088	3,485	5,313	42-5,962
Olansandan et al. (1999)	Japan	60: living room 70: kitchen 70: bedroom 70: bathroom	12.1 * 9.35 * 71.6 * 10.4 *		2,060 768 3,460 375		1.15-13,800 0.961-3,130 1.69-16,000 < 0.21-1,760
Nakai et al.	Japan	30		90			
Shun Cheng et al. (2002)	Hong Kong	6: living room 6: kitchen		2.6 3.0			1.2-4.3 1.2-4.4
Lebret et al. (1984)	The Netherlands	134		7.2			< DL-140
Kostianen et al. (1994)	Finland	50	0.08	0.65			0-8.94
Kirchner (2002)	France	63	0.7-3.5				1-293
Scuderi (1986)		bathroom with one deodoriser block bathroom with one toilet deodoriser block in one urinal and one toilet outside closet with moth flakes in closed garment bag					469–757 697–1,322 78–427

Table 4.8 continued overleaf

Table 4.8 continued Indoor air concentrations of 1,4-dichlorobenzene

Study	Country	Number of measurements	Median	Mean	95th perc.	99th perc.	Range
Globol Werke Gmbh (1986)	Germany	measurements during 80 days in two rooms with a wardrobe containing five bags of six mothballs		5,800 in room 1 before aeration 4,000 in room 2 before aeration 1,600 in room 1 after aeration 1,400 in room 2 after aeration			room 1 before aeration: 1,300-10,000 room 2 before aeration: 1,700-8,000 room 1 after aeration: 0-6,000 room 2 after aeration: 0-2,600
Wallace et al. (1989)	USA	6 with use of a toilet (solid) deodoriser 9 with limited use of a spray deodoriser 3 with limited use of a liquid deodoriser 6 without deodoriser	340 37 25 2.6				Max: 630 Max: 59 Max: 30 Max : 5.2

*   Geometric mean
DL   Detection limit

Table 4.9   Personal air concentrations of 1,4-dichlorobenzene   ($\mu g/m^3$)

Study	Country	Number of measurements	Median	Mean	95th perc.	99th perc.	Range
Shah et al. (1988)	USA	1,650	2.5				
Wallace et al. (1986a)	USA New Jersey	346: overnight 346: daytime	3.80 3.50	56.0 35.1	260 210	1,200 490	0.08–1,500 0.11-790
Wallace et al. (1986b)	USA New Jersey California	340: Fall 150: Summer 49: Winter 110: February 50: May 67: June		45 50 71 18 12 5.5			
Heavner et al. (1996)	USA	61: non- smokers at home 32: smokers at home	0.58 0.91	5.18 16.77			0.00-121.86 0.00-302.28
Pellizari et al. (1999)	USA	63: children 313: adult	< DL 0.87	2.6 3.8			
Matsumara et al. (1992)	Japan	17	234	637	2,660	3,152	24-3,275
Matsumara et al. (1992)	Japan	18	117	235	716	1,129	42-1,232

Table 4.9 continued overleaf

Table 4.9 continued Personal air concentrations of 1,4-dichlorobenzene

Study	Country	Number of measurements	Median	Mean	95th perc.	99th perc.	Range
Wallace et al. (1989)	USA	12 with use of a toilet (solid) deodoriser	330				Max: 500
		16 with limited use of a spray deodoriser	33				Max: 84
		5 with limited use of a liquid deodoriser	12				Max: 28
		10 without deodoriser use	2.4				Max: 6.6

The most relevant data to assess the consumer long-term exposures are the personal exposure measurements since they integrate the different sources and conditions of exposure during the people's daily life.

All the above studies show that the personal exposure has very large statistical distributions, with a few measurements having a very high value. Consequently, to take into account a realistic pessimistic case, a high percentile of the personal exposure distributions or the maximum value measured has to be considered, even if it is much higher than the median or the mean exposure.

On one hand, the indoor and personal air concentrations measured in Japan appear to be systematically higher than those measured in the other studies. As the consumption of 1,4-dichlorobenzene as insecticide and deodorant in Japan is much higher per inhabitant than in the U.S.A and Western Europe, the data from the studies do not seem to be representative for the European consumers.

On the other hand, the largest study on personal exposure is the TEAM study, reported by Wallace et al. and characterised by a large program of quality assurance. By averaging the statistical data of the overnight and daytime personal exposure from the first phase led in New Jersey, we can have a conservative assessment of the distribution of the personal exposure for 24 hours. Even if the process is not mathematically satisfactory, the maximum exposure is

supposed to be equal to 1,145 µg/m^3 ($\dfrac{1500 + 790}{2}$) and the 99th percentile to 845 µg/m^3

($\dfrac{1200 + 490}{2}$).

These values can be compared to the data obtained in the experimental studies.

Using a toilet deodoriser, Wallace et al. (1988) reported a maximum personal exposure of 500 µg/m^3 for the occupants of the corresponding house. But it seems that the experiment was stopped before that the measured concentrations have been completely stabilised. Consequently, if the study had been carried on longer periods, slightly higher exposure might have been measured.

Using the data measured in the Globol Werke GMbH with the mothballs and the air refreshener, the daily exposure of consumers can be assessed with the following scenario:

- daily presence in a room with a wardrobe containing mothballs: 8 hours,

- daily presence in a lavatory or a bathroom containing an air freshener tablet: 1 hour.

In the Globol Werke GMbH, an unrealistic quantity of mothballs had been used in the wardrobes. If only one bag of six mothballs had been used (instead of five), we can assess that the concentrations measured would have been five times lower (since the emitted flux is proportional to the surface of the mothballs, since all the mothballs are assumed to be the same and the saturation concentration was not reached in the wardrobes). Taking into account the highest average concentration measured in the room (5,800 μg/m^3) and dividing it by five, a room concentration equal to 1,160 μg/m^3 is obtained.

Considering the above hypotheses, a daily exposure of 599 μg/m^3 can be calculated. On one hand, this scenario assumes that mothballs and deodorant are used during the whole year, which means that they are replaced when they are used up. It is certainly not true for mothballs. On the other hand, this calculation of exposure does not include the exposure in the other rooms of a house, during the rest of the day, linked to the diffusion of 1,4-dichlorobenzene from the room containing the mothballs or the deodorant towards the whole house. It does not include either the exposure linked to wearing clothes possibly impregnated with 1,4 dichlorobenzene.

Considering all these elements, it seems that a realistic worst-case of exposure level averaged over a day can be defined between 600 and 1,150 μg/m^3, with a preferred value of 850 μg/m^3 (personal air exposure level exceeded by less than 1% of the population studied by Wallace et al., in New Jersey).

For acute exposures, the most elevated concentrations, which have been measured in exposure places, have to be considered. The highest reported value measured in indoor air (23.8 mg/m3 in a lavatory with an air refreshener) will be taken into account to assess acute exposures.

4.1.1.4 Humans exposed via the environment

The estimation of the indirect exposure of humans via the environment is presented in Appendix 1 (EUSES calculation). The total daily intake based on the local environmental concentrations due to the different uses is presented in the **Table 4.10**.

Table 4.10 Total daily intake due to local environmental exposures

Scenario	DOSE tot (mg/kg bw/day)
Production	0.0109
Use as an intermediate	0.00052
Formulation of moth repellents and air fresheners	0.0049
Use of moth repellents and air fresheners	0.00179
Use in the production of grinding wheels	0.00172

Based on the regional concentrations, the total daily intake for humans is 3.8 10^{-5} mg/kg bw/day.

The highest indirect exposure is estimated for production processes. The human intakes via different routes are presented in **Table 4.11**.

Table 4.11 Different routes of intake from human exposure via the environment due to local exposure due to production of 1,4-dichlorobenzene in mg/kg bw/day

Daily dose through intake of drinking water	DOSEdrw	0.00013
Daily dose through intake of fish	DOSEfish	0.0046
Daily dose through intake of above ground plants	DOSEstem	0.00011
Daily dose through intake of below ground plants	DOSEroot	0.00003
Daily dose through intake of meat	DOSEmeat	< 0.00001
Daily dose through intake of milk	DOSEmilk	< 0.00001
Daily dose through intake of air	DOSEair	0.00597

The highest exposures are to be expected through intake of fish and through inhalation.

Furthermore, 1,4-dichlorobenzene has been detected in honey samples (BUA, 1994). The highest measured concentration was 0.06 mg/kg. Using this concentration and assuming a worst-case consumption of 50 g honey per capita per day, an additional dose of 0.00004 mg/kg bw/day can be calculated. This would represent a negligible addition to the total daily intake.

4.1.1.5   Combined exposure

The indirect exposure via the environment can be considered negligible. Combined exposure is mainly occupational and consumer exposure, taking into account that a person may be daily exposed at work during 8 hours and at home during 16 hours.

Occupational exposure: the relevant values for this section are an estimated (8-hour TWA) inhalation exposure of 50 ppm (300 mg/m^3) and an estimated dermal exposure of 80 mg/day which result in a total body burden of 33.2 mg/kg/day.

Consumer exposure: considering an exposure concentration of 0.142 ppm (850 μg/m^3) for 16 hours per day (with a breathing rate of 0.7 m^3/hour, and a body weight of 60 kg) is equivalent to an internal dose of 0.119 mg/kg/day.

Combined exposure corresponds to 33.3 mg/kg/day. This result confirms that combined exposure results mainly from occupational exposure.

4.1.2   Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.1.2.1   Toxicokinetics, metabolism and distribution

4.1.2.1.1   Studies in animals

The toxicokinetics of 1,4-dichlorobenzene was studied in F344 and SD rats and B6C3F1 mice (via oral and respiratory pathways), in Wistar rats and in rabbits (via the oral pathway) and in SD rats (via subcutaneous route) (Hawkins, 1980; Azouz, 1955; Kimura, 1979; Wilson, 1990; Hissink, 1996b; HRC, 1976). The major differences in toxicokinetics, biotransformation and distribution of 1,4-dichlorobenzene via oral and inhalation exposure in F344 rats and B6C3F1 mice, were the absorption percentage.

The distribution and kinetics of ^{14}C-1,4-dichlorobenzene was investigated in male and female F344 rats and B6C3F1 mice following both oral and inhalation exposure; in rats, oral exposures were conducted at single doses of 149 and 305 mg/kg/day and repeated oral exposures at 309 mg/kg/day; inhalation exposures were conducted in male rats at 160 and 502 ppm and in female rats at 161 and 496 ppm; in mice, single oral exposures of 310 and 638 mg/kg/day were conducted as were inhalation exposures at 158 and 501 ppm; intravenous dosing was performed in male rats at doses of 216 and 217 mg/kg/day for blood kinetics study (Wilson, 1990); daily subcutaneous injections were done at 250 mg/kg/day for 10 days in SD rats for distribution study, daily oral administration at 250 mg/kg/day for 10 days in SD rats and 3 hours per day inhalation exposures at 1,000 ppm in SD rats for metabolism study (HRC 1976); oral administration of ^{14}C-1,4-dichlorobenzene at 250 mg/kg/day for 5 days in CFY female rats or whole body inhalation exposure (1,000 ppm, 3 hours/day, for 10 days in CFY female rats (Hawkins, 1980).

Absorption takes place through the digestive and respiratory tracts rapidly but not completely, and subcutaneously. Absorption after inhalation exposure was poor compared to oral exposure. B6C3F1 mice demonstrated increased absorption relative to F344 rats after inhalation (59% in mice versus 25-33% in rats); absorption was similar via oral route in F344 rats and B6C3F1 mice (after single dose: 72% in rats and 71% in mice; after repeated exposure: 62% in rats) (specimen collected during 7 days after end of exposure) (Wilson, 1990). Dose level, multiple dosing and sex have little effects on the extent of absorption. No documentation is available on percutaneous absorption in animals: it can be stated that significant dermal absorption cannot be excluded (see Section 4.1.2.2. and 4.1.2.6.1.).

Following gavage in F344 rats and B6C3F1 mice and intravenous administration in male F344 rats, peak blood levels were observed at one hour after oral dosing with a distribution half-life of 4 minutes in intravenously dosed rats and 3.5 hours in orally dosed rats; peak tissue level appeared at 6 hours after oral and inhalation exposure (Wilson, 1990). Plasma concentrations 24 hours after oral or subcutaneous administrations were similar (HRC, 1976).

1,4-Dichlorobenzene is distributed primarily in the fatty tissues, the kidneys, the liver, the lungs, the gonads and muscle tissues; tissue distribution was similar for oral, inhalation and subcutaneous routes of exposures (HRC, 1976), with higher concentration of 1,4-dichlorobenzene in the fat (Hawkins, 1980). Concentrations in tissues were found similar after either inhalation, oral and subcutaneous exposures in SD rats (HRC, 1976). Female F344 rats show higher concentrations in the liver than males, but male F344 rats have higher concentrations than females in the kidneys following whole body inhalation exposure at 500 ppm for 24 hours (Umemura et al.,

1990; 1992); a similar difference in kidney tissue levels in male F344 rats was reported after 6 hours inhalation exposure at 160 or 502 ppm with tissue to blood ratio in the male rat kidney increased at 1-3 days after dosing (this was not observed in mice or female rats) (Wilson, 1990). Tissue levels of 1,4-dichlorobenzene are similar when female SD rats inhale 1,000 ppm (almost 6 mg/l), 3 hours/day, 10 days or receive 250 mg/kg/day, 10 days via oral or subcutaneous route (Hawkins, 1980).

Regardless of the penetration, 1,4-dichlorobenzene is mainly metabolised through hydroxylation to the sulphate and glucuronide conjugates of 2,5-dichlorophenol but also to free 2,5-dichlorophenol (2,5-DCP) and 2,5-dichlorohydroquinone (2,5-DCHQ). There are some species differences in metabolism of 1,4-dichlorobenzene (results are in percentage of recovered activities):

- in F344 rats after oral exposure, the sulphate conjugate of 2,5-DCP was the major urinary metabolite via oral and inhalation route (30% and 20% for sulphate, 6% for glucuronide, < 4% for free 2,5-DCP) with induction of glucuronidation (3 times) and decrease of free 2,5-DCP after repeated oral exposure (but not after inhalation exposure); after inhalation exposure, the major percent of dose was eliminated as sulfate conjugate (20%), glucuronide (2 to 6%) and free 2,5-DCP (0.6 to 2.5%) (Wilson, 1990). In F344 rats, minor amounts of 2,5-DCHQ (1.1 to 1.4%) and of 1,4-dichlorobenzene mercapturic acids [(2-(N-acetylcysteine-S-yl)-1,4-dichlorobenzene (0.4 to 1.4%) and (N-acetylcysteine-S-yl)-2,3-dihydro-3-hydroxy-1,3-hydroxy-1,4-dichlorobenzene] and mercapturic acid of monochlorophenol (via 3,4-epoxide) were found in urine after an unique oral exposure (Klos, 1994),

- in SD rats after oral and inhalation exposure, urinary metabolites were 50% for sulphate, 30% for glucuronide, traces of 2,5-dichlorobenzene mercapturic acids and 2,5-DCHQ (Hawkins, 1980); dichlorocatechol and free 2,5-DCP were also found (HRC, 1976),

- in Wistar rats after single oral exposure, urinary metabolites were 50%-60% for sulphate, 20%-30% for glucuronide, 5%-10% of free 2,5-DCP, 10% for epoxide derived 2,5-dichlorobenzene mercapturic acids but no 2,5-DCHQ (Hissink, 1996b); small amounts of 2,5-dichlorophenyl-methylsulphoxide and -methylsulphone were found after single oral exposure (Kimura, 1979),

- in B6C3F1 mice, the production of sulphate and glucuronide conjugates of 2,5-DCP were equivalent in urine (25 to 30% of each), 6-9% of free 2,5-DCP, with induction of glucuronidation after high dose oral and inhalation exposures (with increase in 2,5-DCP via inhalation route) (Wilson, 1990).

Additional peaks, nature of the peaks not precisely identified (suggested to be 2,5-dichlorophenyl-methylsulphoxide and -methylsulphone), were observed in F344 rats and B6C3F1 mice (chromatograms similar) but not quantified (collectively they represent about 5-21% of the dose but individually less than 5% of the dose) (Wilson, 1990; Kimura, 1979).

In rabbits, conjugates of 2,5-DCP are the major metabolites (60%) but also free 2,5-DCP (35%) and 2,5-DCHQ (6%) are formed but no mercapturic acid and no catechol (Azouz, 1955).

SD rats exhibit an enterohepatic cycle: about 50% of each dose (respectively 48, 63 and 46%) were eliminated in the bile during 24 hours versus (respectively < 0.1, 9, < 0.1% in the faeces) after single dose by inhalation (1,000 ppm), oral (250 mg/kg) or subcutaneous (250 mg/kg) administration (HRC, 1976). At 250 mg/kg, single dose by gavage in Wistar rats 10 to 30% of total radiolabelled appeared in the bile with less than 5% in the faeces suggesting a significant enterohepatic cycle (Hissink, 1996b). No effect of 1,4-dichlorobenzene on bile duct pancreatic fluid was noted (Holtzman rat, ip, 5 mmol/kg) (Yang, 1979).

Table 4.12 Species specific metabolism of 1,4-dichlorobenzene (results in percentage of recovered activities)

Strains	Sulphoconjugates of 2,5-DCP	Glucuroconjugates of 2,5-DCP	Free DCP	DCHQ	Mercapturic acids	Methylsulphone methylsulphoxide
F344 rats	20-30%	6%	< 4%	<1,4%	< 1,4%	traces?
SD rats	50%	30%	traces	traces	traces	?
Wistar rats	50-60%	20-30%	5-10%	0	10%	traces?
B6C3F1 mice	25-30%	25-30%	6-9%	?	?	traces?

Routes of elimination and percentage of an administered dose of 1,4-dichlorobenzene eliminated are similar among F344 rats and B6C3F1 mice.

In SD rats, 97.4%, 97.1% and 90.5% (means) of the material excreted during 5 days were found in the urine after inhalation, oral and subcutaneous administration (HRC, 1976).

Elimination of ^{14}C 1,4-dichlorobenzene absorbed dose is more complete after oral exposure (after 7 days, mean cumulative total excretion is 80%-99% of the dose in F344 rats and male B6C3F1 mice, in which 55 to 70% were in urine and 8% to 15% in the faeces and 10 to 12% in the expired air for Wilson (1990) and 38-42% in the urine 72 hours after dosing for Klos (1994) versus inhalation exposure (after 7 days, mean cumulative total excretion is 35% in F344 rats and 55% in male B6C3F1 mice, in which 18 to 32% in rat and 32 to 47% in mice were in urine and 2% in rat and 6 to 19% in mice in the faeces); dose did not affect significantly the percentage of ^{14}C 1,4-dichlorobenzene excreted in the urine; qualitatively the routes of elimination are comparable after oral and inhalation exposures. Elimination is principally via the urine (> 80%), and most likely via the faecal (3-11%) and biliary pathways as well (HRC, 1976; Wilson, 1990; Hissink, 1996b; Hawkins, 1980). In SD rats, 87% via oral, 73% via inhalation and 41% via subcutaneous routes were eliminated in the urine versus only 1.9%, 2.5% and 0.1% in the feces via the same routes, respectively (HRC, 1976). Pulmonary elimination after gavage administration accounted for less than 1% of all doses in 2 studies [10 to 250 mg/kg/day in Wistar rats (Hissink, 1997a), 250 mg/kg/day in CFY female rats (Hawkins, 1980)], but up to 12% of the orally administered dose in the study of Wilson (1990) (single doses in F344 rats at 149 and 305 mg/kg and in B6C3F1 mice at 638 mg/kg).

A majority of 1,4-dichlorobenzene is eliminated in the urine and faeces by 48 hours, by a biexponential kinetic with an elimination half-life stated of 0.4 days after oral administration for the alpha elimination phase and of 10 days for the beta elimination phase and an elimination half-life stated of 0.67 days after intravenous administration. Elimination kinetic appears similar in the kidney and the other tissues following both oral administration of ^{14}C-1,4-dichlorobenzene at 250 mg/kg/day for 5 days in CFY rats and inhalation exposures 1,000 ppm, 3 hours/day, for 10 days, but only CFY female rats were studied (Hawkins, 1980). Elimination kinetic appears similar irrespective of the dose level, the route of administration and the species except in the kidney of dosed male rats versus female rats or male mice where elimination is slower and except at the 6th hour where urinary excretion was 2 times higher in mice via inhalation but similar at the 24th hour (Wilson, 1990). Seven days after dosing, less than 0.1% of the applied dose was associated with tissue or blood samples (Wilson, 1990). Tissue concentration of 1,4-dichlorobenzene in F344 rats fell 90% in the 24 hours following dosing (vapour, 500 ppm, for 6, 12, 24 hours) (Umemura et al., 1990). After repeated daily administration (250 mg/kg/day) during 10 days via oral route in SD rats, tissue concentration of 1,4- dichlorobenzene was below the limit of detection at 120 hours (4 days); after repeated daily administration (250 mg/kg/day) during 10 days via subcutaneous route in SD rats, tissue concentration of 1,4- dichlorobenzene

was below the limit of detection at 192 hours (8 days); after repeated inhalation administration (1,000 ppm; 3 hours/day) during 10 days in SD rats, tissue concentration of 1,4-dichlorobenzene was below the limit of detection after 96 hours (5 days) (HRC, 1976). Total elimination occurs within 5 days: in Wistar rat, 4 days after a single oral administration, only traces of 1,4-dichlorobenzene and 2,5-dichlorophenol were detectable in plasma and tissue; after repeated oral administration in food (28 days), no residue of 1,4-dichlorobenzene or 2,5-DCP were found in plasma, liver, kidney and fat after 35 days (Schmidt, 1977a). Accumulation of 1,4-dichlorobenzene in tissue is unlikely after inhalation or oral exposure in Wistar rats (Schmidt, 1977a,b; HRC, 1976).

In vitro studies

1,4-Dichlorobenzene was quantitatively and qualitatively metabolised equally in both F344 and SD rats and human liver slices with glutathione/cysteine conjugates as major metabolite and also glucuronide and sulphate conjugates (Fisher, 1995, 1991b, 1990).

In Wistar rat liver microsomes, 1,4-dichlorobenzene is metabolised in 2,5-DCP (and to a lesser extent 2,4-dichlorophenol), which are readily oxidised to its hydroquinone derivative: 2,5-DCHQ and by a subsequent oxidation to dichlorobenzoquinone species (and 3,5-dichlorocatechol and little 1-dichlorobenzoquinone) (Den Besten et al., 1992).

In vitro, conversion of 1,4-dichlorobenzene was much higher in B6C3F1 mouse microsomes (16%) than in F344, Wistar or SD rats or human microsomes (1.3% in F344 and Wistar rats, 0.6% in SD rats, 0.3% in human) (Hissink, 1997b, 1996a). The GSH conjugate of the epoxide of 1,4-dichlorobenzene (derived from endogenous glutathion) accounts with exogenous glutathion for 40 to 50% of total conversion in rat liver microsomes, 2% in mice and 6% in human (with endogenous glutathion: for 5 to 15% of total conversion in rat liver microsomes, not detected in mice and human); in rats and mice, addition of cytosol had a marginal effect on formation of this GSH conjugate whereas in human a major increase of this GSH conjugate was seen with cytosol (from 6 to 43%). Comparing the different strains, production of hydroquinone metabolites (HQ) (as chlorohydroquinone) was similar (in percentage of total conversion) in B6C3F1 mice and human liver microsomes (16%), higher in F344 rats (27%) and lower in SD and Wistar rats (10%). Addition of the reducing agent ascorbic acid AA (which inhibits oxidation of hydroquinones to benzoquinones), increased the recovery of hydroquinone metabolites in all liver microsomes with a most pronounced formation in B6C3F1 mice (55% of total conversion) than in human (28% of total conversion) while decreasing covalent binding (from 21% to 1.7% in mice, and from 5.8 to 4.4% in human (this supposes that benzoquinone is probably formed in human but at very low level).

Relatively more glutathione conjugates of quinones are produced by human and especially B6C3F1 mice (26% and 39%, respectively) microsomes compared to rats (3% to 22%); comparing the three rat strains, the F344 rats produce the highest amount of glutathione conjugates of quinones.

In all cases, 2,5-DCP accounted for more than 60% of total conversion of 1,4-dichlorobenzene: human microsomes showed the highest conversion into 2,5-DCP (62%) in comparison with rats and mice (27 to 35%) (Hissink, 1997b).

4.1.2.1.2   Studies in humans

Absorption occurs via the digestive and respiratory tracts. No data were available on cutaneous absorption (Pagnotto, 1965; Ghittori, 1985). 1,4-Dichlorobenzene is essentially distributed to the fatty tissues, but also to the liver and milk (Jan, 1983; Sumino, 1988). Elimination occurs essentially through the urine in the form of 2,5-DCP (but also 2,5-dichloroquinol after accidental ingestion in a child (Hallowell, 1959). It also occurs via the respiratory tract from study in volunteered persons (Wallace, 1989; Hill, 1989). In humans occupationally exposed (manufacturing, packaging) to 1,4-dichlorobenzene, with measurements of 2,5-dichlorophenol in spot samples collected at the end of the workshift, excretion begins with starting of exposure, attains a maximum level at approximatively the 8th hour, and continues for several days; an average of 33 ppm of 1,4-dichlorobenzene concentration in air corresponds to a mean of 100 mg/l in the urine at the end of the workshift (Pagnotto, 1965).

In the case of occupational exposures, the quantity of 2,5-DCP excreted between the beginning and the end of the work shift is well correlated with the intensity of the exposure. For exposures on the order of 10 ppm, the concentration of 2,5-DCP excreted in the urine at the end of the shift is approximatively 45 mg/l (Ghittori, 1985).

Detectable levels of 2,5-DCP in urine (up to 8.7 mg/l, mean 0.2 mg/l) and blood (up to 49 µg/l, mean 2.1 µg/l) were found in a sample of 1,000 adults who lived in the United States (Hill, 1995).

Average concentrations of 2.3 µg/g in adipose tissue and of 9.5 ng/ml in blood (obtained from general hospital admission) have been found in subjects exposed to 1,4-dichlorobenzene as residents in the Tokyo metropolitan area with environmental exposure (possible inhalation and digestive through food (34 adipose tissues, 6 blood samples, environmental exposure, 13 to 80 years old, levels of exposure: outdoors (1.5 to 4.2 µg/m^3) indoors (105 to 1,700 µg/m^3), sampling time unknown) (Morita, 1975a, b). These concentrations in fat tissue samples are in women (0.13 µg/g) and in men (0.11 µg/g), and in subjects between the ages of 15 and 44 years (0.13 µg/g) (Anonym 1990 (chemical regulation reporter 421)). Mean levels of 1,4-dichlorobenzene in adipose tissue was 0.146 µg/g in Yugoslavian subjects (died from traffic accident in 1979-1980) (Jan, 1983). The same values were observed in others studies (Sumino, 1988).

4.1.2.1.3   Mechanisms of action of 1,4-dichlorobenzene

In vitro studies

In vitro, in human liver slices 1,4-dichlorobenzene isomer was less toxic; pre-treatment with P 450 inhibitors does not change hepatotoxicity (Fisher, 1991a).

A study in Wistar rat liver microsomes demonstrates that metabolites of 1,4-dichlorobenzene (reactive quinone) covalently binds with proteins and only to a small extent with DNA (of the added foetal veal serum); the protein binding is nearly completely inhibited by the addition of the reducing agent ascorbic acid with a concommitant increase in the formation of chlorohydroquinones and chlorocatechols (increase from 35 to 61%) (Den Besten et al., 1992).

Covalent binding to liver microsomal proteins was 21% of total conversion for the B6C3F1 mice, 10% for SD rats, 8% for Wistar and F344 rats, 6% for human liver microsomes (percentage of total metabolites formed). In the B6C3F1 mouse, covalent binding was nearly completely inhibited from 21% to 1.7% (92% inhibition) by the reducing agent ascorbic acid AA (which

inhibits oxidation of metabolites of hydroquinone to benzoquinones), with concomitant increase in metabolites of hydroquinone formation (in mice: 16 to 55%, + 340%) indicating that benzoquinones were the reactive metabolites involved. For all rat microsomes, covalent binding was greatly inhibited by AA (33% inhibition in SD and F344 rats, 80% in Wistar rats), with concomitant increase in metabolites of hydroquinone formation: 10-27% to 16-34% (Hissink, 1997b); the remaining covalent binding was inhibited by addition of exogenous glutathion with concomitant increase in the formation of the GSH-conjugate of the epoxide in all strain of rats. Human microsomes were least extensive in covalent binding (6%), which was inhibited by AA from 6% to 4.3% (25% inhibition) with concommitant increase in hydroquinones formation (16% to 28%), indicating a production of reactive metabolites (benzoquinone probably) but less than mice; the remaining covalent binding was inhibited by addition of exogenous glutathion (to undetectable levels), with concommitant increase in the formation of the GSH-conjugate of the epoxide (Hissink, 1997b).

Covalent binding of reactive metabolite intermediates in liver slices was similar for 1,4-dichlorobenzene in human and SD and F344 rat tissues (8 to 10% in rats) (Fisher, 1995). Association of [^{14}C]-1,4-dichlorobenzene with calf thymus DNA was detected *in vitro* after incubation with fractions of liver and lung from mice and rats but not of kidney from rats and mice nor of stomach from mice; in an other study no DNA adducts were detected after incubation with liver microsomes from rat, mouse or man (Lattanzi et al., 1989; Tian 2001; Paolini, 1998) (see also Section 4.1.2.7.1 *in vitro* studies).

In vitro the cytochromes P450 CYP2E1 and to a less extent CYP1A1 possess the highest activity toward 1,4-dichlorobenzene in human microsomes (Bogaards, 1995; Hissink, 1996b).

In vivo studies

Hepatotoxicity was studied following intraperitoneal administration in male F344 rats or SD rats at 500 mg/kg: 1,4-dichlorobenzene produces hepatotoxicity (increase in transaminases) only in hepatic glutathione depleted rats, and pre-treatment with phenobarbital did not affect the hepatotoxicity (Brodie, 1971); covalent binding of 1,4-dichlorobenzene to hepatic proteins was small (Stine, 1991). Covalent binding of 1,4-dichlorobenzene to kidney, lung and spleen proteins after unique oral administration (900 mg/kg) in F344 rats was below the limit of detection (Klos, 1994).

In vivo there is no evidence for association of 1,4-dichlorobenzene with DNA in rats, whereas there is some evidence for the association of 1,4-dichlorobenzene with DNA in mice liver, kidney, lung and stomach (Lattanzi et al., 1989) (see Section 4.1.2.7.1 *in vivo* studies)

The ability of 1,4-dichlorobenzene (single gavage in corn oil, 300 mg/kg) to induce c-fos, c-jun and c-myc expression in 3 male F344 liver rats was determined, as was the hepatic labelling index (LI). Increased LI is preceded by an increase in expression of the 3 genes in some cells from some animals. There was a good correlation between c-myc expression and LI. In situ hybridisation analysis indicated that cells expressing c-fos and c-jun were randomly distributed across the liver lobules while cells expressing c-myc were mainly midzonal and, to a lesser extent periportal (Hasmall, 1997a).

The effects of 1,4-dichlorobenzene (gavage, 300 mg/kg in corn oil, 7 days) on hepatocyte ploidy, nuclearity and LI distribution among the ploidy/nuclearity classes was studied in 3 male Fisher 344 rats. 1,4-Dichlorobenzene reduced the proportion of tetraploid cells (4N plus 2X2N) and increased the proportion of mononucleated octoploid (8N) cells. 1,4-Dichlorobenzene increased

the mean hepatic LI: LI was increased in all hepatocyte ploidy/nuclearity classes except the binucleated tetraploid cells (2X2N) (Hasmall, 1997b).

Protein or nonprotein S-adducts of 1,4-dichlorobenzene metabolites (1,4-dichlorobenzene-quinone and 1,4-dichlorobenzene-epoxide) were identified in liver and urine of DDY male mice; mice were given or, 1,4-dichlorobenzene orally (300 mg/kg) only, or injected ip with saline or BSO (an inhibitor of glutathione synthesis: buthionine sulfoximine) followed one hour later by 1,4-dichlorobenzene given orally (300 mg/kg); results suggest that 1,4-dichlorobenzene-quinone and not 1,4-dichlorobenzene-epoxide is involved in covalent binding of 1,4-dichlorobenzene to hepatic protein thiols. In mice given 1,4-dichlorobenzene in combination with BSO, covalent binding of 1,4-dichlorobenzene to hepatic protein thiols was not increased compared with that in mice given 1,4-dichlorobenzene alone. As 1,4-dichlorobenzene causes no hepatotoxicity in normal mice but produces hepatotoxicity in mice depleted in GSH by BSO, these results are not consistent with the idea that 1,4-dichlorobenzene covalent binding to hepatic protein thiols is the mechanism of hepatotoxicity of 1,4-dichlorobenzene in GSH-depleted mice (Mizutani, 1997).

1,4-Dichlorobenzene produces no hepatotoxicity in control and phenobarbital-treated SD rats and C57 mice (ip, 500 mg/kg) and phenobarbital treatment does not increase protein binding to liver proteins (Reid 1973a, b, c). 1,4-Dichlorobenzene induces no hepatotoxicity and hardly affects the hepatic glutathione concentration: GSH concentration was slightly lower only at 50 mg/kg after single oral exposure up to 250 mg/kg in Wistar rats; 1,4-dichlorobenzene seems not metabolised via a glutathione scavenging metabolite (Hissink, 1996b).

The role of endogenous glutathione in protecting against 1,4-dichlorobenzene induced hepatotoxicity was demonstrated in ddY male mice. Unique oral administration of 1,4-dichlorobenzene (100 to 400 mg/kg) in ddY male mice pre-treated by ip injection with a depletor of glutathione synthesis (buthionine sulfoximine BSO) resulted in a dose-dependent hepatotoxicity (serum ALT activity up to X 100, liver necrosis) but 1,4-dichlorobenzene alone (up to 1,200 mg/kg) resulted in no hepatotoxicity.

Administration of GSH monoethylester protected mice from the hepatotoxicity of 1,4-dichlorobenzene in combination with BSO; treatment with cytochrome P450-dependent monooxygenase inhibitors prevented hepatotoxicity of 1,4-dichlorobenzene in combination with BSO: this suggests that a metabolite formed by a cytochrome P450-dependent reaction is responsible for the 1,4-dichlorobenzene hepatotoxicity and that this metabolite is likely detoxified by glutathione in mice since 1,4-dichlorobenzene showed no sign of hepatotoxicity in the absence of BSO.

On the other hand, inducers of cytochrome P450-dependent monooxygenase did not increase hepatotoxicity of 1,4-dichlorobenzene in combination with BSO probably because they stimulate not only activating but also detoxicating pathways of 1,4-dichlorobenzene metabolism (Mizutani, 1994).

Sole treatment with 1,4-dichlorobenzene resulted in no significant changes in hepatic GSH in ddY mice. To test the possibility that quinone metabolites of 1,4-dichlorobenzene could play a role in hepatotoxicity, 2,5-DCP was administered orally or subcutaneously to mice pre-treated with BSO; however they failed to elicit hepatotoxicity: the author suggests that these results are not in favour of the possible contribution of the quinone metabolites to 1,4-dichlorobenzene-induced hepatotoxicity but the disposition and metabolic fate of 2,5-DCP generated in situ in the liver may be different from those of externally administered material (data cited in Mizutani, 1994). No significant changes in hepatic contents of lipid peroxides (assessed by determining TBA-reactive substances) and protein thiols during 30 hours after treatment with 1,4-

dichlorobenzene in combination with BSO in ddY mice: therefore, for the author it seems unlikely that the liver injury caused by 1,4-dichlorobenzene in combination with BSO is related to lipid peroxidation (data cited in Mizutani, 1994, without detailed information).

The occurrence of 1,4-dichlorobenzene metabolites such as 2,5-DCP and mercapturic acids can be thought as an evidence for prior formation of arene oxide intermediates which have been thought to mediate hepatotoxic effects (Mizutani, 1994; Klos, 1994).

1,4-Dichlorobenzene induces dose-dependently liver cytochrome P450 dependent monooxygenases in both sexes F344 rats (0, 150, 600 mg/kg/day via oral route during 2, 8, 14 or 28 days) (Bomhard, 1992; Allis, 1992), F344 male rats from 75 to 300 mg/kg/day at 1, 4 and 13 weeks (Lake, 1997), CF1 mice of both sexes at 800 mg/kg/day via oral route during 2 and 4 weeks (Bomhard, 1996) and B6C3F1 male mice at 600 mg/kg/day but not at 300 during 1, 4 and 13 weeks (Lake, 1997). Content of Cytochrome P 450 was not increased in Wistar rats, pre-treated orally with 250 mg/kg/day for 3 days (Ariyoshi, 1975a, 1975b) and nor in albinos rats treated orally with 0, 10, 20, 40 mg/kg/day for 90 days (Carlson, 1976).

In vivo, 1,4-dichlorobenzene markedly induced CYP2B in F344 rats and B6C3F1 mice hepatic microsomal (after 1 week treatment at 75 and 300 mg/kg/day in rats and 600 mg/kg/day in mice) and to a lesser extent CYP3A in F344 rats (Lake, 1997).

Repeated exposure for up to 4 weeks of B6C3F1 mice to 600, 300 or 150 mg/kg/day and of F344 rats to 300, 150 and 75 mg/kg/day induce a sustained mitogenic response (cumulative replicating fraction CRF) in the mice liver of the 600 mg/kg group at 1 and 4 weeks and a transient mitogenic response of the 300 mg/kg group at 1 week (but no CRF increase at 150 mg/kg); a transient CRF increase in rat liver was also observed of the 300 and 150 mg/kg groups at 1 week (Umemura et al., 1998).

A single oral dose of 1,4-dichlorobenzene (600, 1,000 and 1,800 mg/kg) to male B6C3F1 mice induces at 1,000 mg/kg/day hepatocyte cell proliferation in spite of the lack of hepatotoxicity, as a result of mitogenic stimulation (Umemura et al., 1996).

1,4-Dichlorobenzene induces a hepatocyte replicative DNA synthesis 24 or 48 hours after a single oral administration (750 et 1,500 mg/kg) in male B6C3F1 mice (Miyagawa, 1995).

Cell proliferation and increase liver weight (but no necrosis and no elevated liver enzymes) were seen in rats and mice after single oral dose of 1,4-dichlorobenzene up to 1,200 mg/kg/day (Eldridge et al., 1992; Butterworth, 1992).

Renal and hepatocellular proliferation induced by 1,4-Dichlorobenzene has been studied (Umemura et al., 1992). In this study, groups of 9-week old F344 rats (5/sex/group) were administered 1,4-dichlorobenzene in corn oil orally by gavage at dose levels of 0, 150 or 300 mg/kg bw/day (males) or 0, 300 or 600 mg/kg bw/day (females) for 4 days. Groups of 9-week old B6C3F$_1$ mice (4/sex/group) were administered 1,4-dichlorobenzene in corn oil orally by gavage at dose levels of 0, 300 or 600 mg/kg bw/day for 4 days. On the last 3 days of dosing, all animals received intraperitoneal injections of BrdU (20 mg/kg bw) 3 times per day. Livers and kidneys were obtained from each of the treated animals. Different regions of the renal tubules were identified on the basis of their γ-glutamyl transferase activity, which is not found in the distal tubules and is present in proximal straight tubules at a much higher activity level than in proximal convoluted tubules. BrdU incorporated into DNA was demonstrated using an immunohistochemical method. At least 3,000 hepatocellular nuclei and 3,000 renal tubule cell nuclei were counted in each animal. Because the 4-day dosing period allows time for

proliferating cells to divide and, hence, for both daughter cell nuclei to be labelled, the proportion of cells labelled was described as the Cumulative Replicating Fraction (CRF).

In the kidneys, the CFR was significantly increased in the proximal convoluted tubules and, to a lesser extent, the proximal straight tubule cells of male rats administered 1,4-dichlorobenzene at 300 mg/kg bw/day. The CRFs for the 0, 150 and 300 mg/kg bw/day groups, respectively, were: (proximal convoluted tubule), 2.19 ± 0.19, 2.29 ± 0.29 and 6.53 ± 1.39 ($p < 0.01$); and (proximal straight tubule), 2.07 ± 0.41, 2.24 ± 0.65 and 3.48 ± 1.20 ($p < 0.05$). There were no increases in CRFs in the distal tubule cell nuclei of male rats or in any of the renal tubule regions of female rats or male or female mice at any dose level.

In the livers, there were statistically significant, dose-related increases in CRFs in male and female rats and mice affecting all dose levels.

The 1,4-dichlorobenzene induced replicative DNA synthesis was observed in F344 rat hepatocytes at the highest dose (300 mg/kg/day, 1 week treatment) but not after 4 and 13 weeks treatment; in the B6C3F1 mice hepatocyte labelling index values were increased at 300 and 600 mg/kg/day after 1 and 4 weeks (Lake, 1997).

Transient hepatocellular proliferation (but no hepatocellular necrosis) was seen in B6C3F1 mice of both sexes during first week at 600 mg/kg/day and not at 300 mg/kg/day nor at 3, 6 and 13 weeks) and in F344 female rats at 600 mg/kg/day during first week (not at 3, 6 and 13 weeks); liver enzyme activities (ALT, AST, LDH) were not elevated in 1,4-dichlorobenzene treated animals compared to control at any time during the 13 weeks of treatment; liver weights were significantly greater than control at any time point in mice and rats (Eldridge et al., 1992; Butterworth, 1992).

After 2, 4 or 5 days treatment by gavage in B6C3F1 mice (600 mg/kg/day) and in F344 rats (300 mg/kg/day), labelling was primary centrolobular in mice whereas in rats cell labelling distribution was panlobular (Eldridge et al., 1990). It also causes a proliferation of the renal proximal tubular cells in male rats - not in female rats, nor in mice - (Umemura et al., 1992; Eldrige et al., 1990; 1991).

1,4-Dichlorobenzene does not promote hepatic foci formation in F344 rats in a two stage model of carcinogenesis (a medium term initiation/promotion assay: at week 0 initiation with a single intraperitoneal injection of DEN (diethylnitrosamine) and two weeks later promotion with daily gavage administration of 1,4-dichlorobenzene (at low doses, 0.1 and 0.4 mmol/kg/day, equivalent to 14.7 and 60 mg/kg/day), through the remainder of the 8-week study; a partial hepatectomy was performed at week 3. No significant effect of 1,4-dichlorobenzene treatment on any foci parameter (area and number of total and large foci) in the absence or presence of DEN initiation was seen (Gustafson et al., 1998).

1,4-Dichlorobenzene does not induce peroxisomal proliferation in CF1 mouse liver (5/sex/dose/time of sacrifice) after oral administration (0, 50, 200, 800 mg/kg/day) for 2 or 4 weeks. The liver carnityl-acetyl transferase activity and the number of peroxisomes were not increased. Cholesterol and triglyceride in the plasma and the liver were at 800 mg/kg/day or equal to, or higher than the control one (but never decreased). At 800 mg/kg/day weeks 2 and 4, the liver weight and cytochrome P450 activity were increased (Bomhard, 1996).

The mechanism underlying the nephrotoxicity of 1,4-dichlorobenzene in the male F344 rat has been elucidated (Borghoff, 1990 and 1991; Charbonneau, 1989a, b and 1988; Dietrich, 1991; Lehman-McKeeman, 1993 and 1990). For further details see Section 4.1.2.6.

The relationship between cell proliferation and apoptosis was investigated in F344 rat and B6C3F1 mice after oral administration of 1,4-dichlorobenzene at 300 and 600 mg/kg/day. After 2 days of treatment, induction of DNA synthesis was observed in both species while percentage of apoptotic hepatocytes was significantly decreased (to an undetectable level in 9/10 animals). Western blot analysis showed an increase in expression of CYP2B1/2 and CYP4A1 in mouse and rat liver (James, 1998).

4.1.2.1.4   Summary of toxicokinetics, metabolism and distribution

In animals, absorption takes place through the digestive and respiratory tracts rapidly (peak blood levels at one hour after dosing) but not completely, and subcutaneously; absorption after inhalation exposure (59% in mice versus 25-33% in rats); was poor compared to oral exposure (after single dose: 72% in rats and 71% in mice; after repeated exposure: 62% in rats). From acute dermal and repeated-dose toxicity studies via dermal route, the like hood that dermal absorption is negligible can be made.

No quantitative data on human absorption are available.

The major differences in toxicokinetics, biotransformation and distribution of 1,4-dichlorobenzene via oral and inhalation exposure in F344 rats and B6C3F1 mice were the absorption percentage.

1,4-Dichlorobenzene is distributed primarily in the fatty tissues, the kidneys, the liver, the lungs, the gonads and muscle tissues, similarly for oral, inhalation and subcutaneous routes of exposure.

Regardless the penetration, 1,4-dichlorobenzene is principally metabolised *in vivo* to the sulphate and glucuronide conjugates of 2,5-dichlorophenol but also to free 2,5-dichlorophenol in rats, mice and human; *In vivo*, there are some species differences in metabolism between rats and mice, with 2,5-dichlorohydroquinone found in F344 and SD rats (and human possibly), but not in Wistar rats nor in mice; to be noted that 2,5-dichlorohydroquinone was found in B6C3F1 mice and Wistar rat liver microsomes *in vitro* (Hissink, 1997b; Den Besten et al., 1992).

In vivo, 1,4-dichlorobenzene induces dose-dependently liver cytochrome P450 dependent monooxygenases: *in vivo* CYP2B in F344 rats and B6C3F1 mice (Lake 1997, James 1998) but also CYP2A in F344 rats (Lake, 1997), CYP4A in F344 rats and B6C3F1 mice (James, 1998) and CYP2E1 in Wistar rats and B6C3F1 mice (Hissink, 1997a). *In vitro* 1,4-dichlorobenzene induces CYP2E1 in human (for more than 90% of all CYP activity) but also CYP2A (Bogaards, 1995).

The hydroxylation of the aromatic ring seems to result in the formation of intermediate epoxides: 2,3-epoxide through liver cytochrome P450 CYP2E1 (subsequent metabolism to 2,5- or 2,4-dichlorophenol) and 1,2-epoxide through CYP2B (subsequent metabolism to 2,4-dichlorophenol).

The metabolic fate of epoxide appears species dependent: the epoxide may form a conjugate with glutathione [*in vivo*: Wistar, F344 and SD (CFY) rats, *in vitro*: human, Wistar, F344 and SD rats, B6C3F1 mice] or be catalysed by epoxide hydrolase to form a dichlorophenol [*in vivo*: rat, mouse, human, *in vitro*: rat, mouse, human] or be secondary metabolised to form an hydroquinone metabolite [*in vitro*: rat, mouse, human, *in vivo*: F344 and SD rat and human probably, no data in mouse].

In vitro, the major metabolites are in rat, mouse and human liver microsomes dichlorophenols (50%), hydroquinone metabolites (10 to 27%) and to a less extent glutathione-epoxide and glutathione-quinone conjugates. Differences in the hepatic microsomal metabolism between rats and mice (and human) were shown (but not by Fisher (1995) were chemical species and quantities formed in SD and F344 rats and human were comparable): conversion of 1,4-dichlorobenzene was much higher in B6C3F1 mouse microsomes than in F344, Wistar or SD rat or human microsomes; mouse, F344 rat and human liver microsomes produce more hydroquinones metabolites than Wistar rat liver microsomes and addition of ascorbic acid increases the recovery of hydroquinones metabolites while decreasing protein binding; rat liver microsomes form glutathione-epoxide conjugates in the absence of exogenous glutathione (significantly increased with exogenous GSH) compared with very low detectable level from mouse and human liver microsomes in the presence of exogenous glutathione and no detectable level from human and mouse liver microsomes in the absence of exogenous glutathione.

Elimination is principally via the urine (> 80%) and most likely via the feces and lung; there appears to be considerable enterohepatic circulation of 1,4-dichlorobenzene and metabolites in the rat. The biological residence time is short, with a majority of 1,4-dichlorobenzene eliminated in the urine and feces by 48 hours. Routes of elimination and percentage of an administered dose of 1,4-dichlorobenzene eliminated are similar among rats and mice and comparable after oral and inhalation exposure.

In human, absorption occurs via the digestive and respiratory tracts and 1,4-dichlorobenzene is essentially distributed to the fatty tissue. Elimination occurs essentially through the urine and via the respiratory tract with a maximum level at approximatively the 8th hour, and continues for several days. Excreted metabolites are 2,5-dichlorophenol and its sulfate and glucuronide conjugates; 2,5-dichloroquinol was also found.

In vitro, covalent binding to protein was higher in mice than rats and human liver microsomes. Protein binding is nearly completely inhibited in mice and greatly inhibited in rats and to a lesser extent in human (this supposes that benzoquinone is probably formed in human but at very low level) by the addition of the reducing agent ascorbic acid with a concommitant increase in the formation of hydroquinones and concommitant (Hissink, 1997b). Association of [^{14}C]-1,4-dichlorobenzene with calf thymus DNA was detected *in vitro* after incubation with fractions of liver and lung from mice and rats but not of kidney from rats and mice; in an other study no DNA adducts were detected after incubation with liver microsomes from rat, mouse or man.

In vivo, 1,4-dichlorobenzene covalently binds in rats and mice with proteins after oral or ip administrations; after ip injection, there is no evidence for association of 1,4-dichlorobenzene with DNA in rats, whereas there is some evidence for the association of 1,4-dichlorobenzene with DNA in mice liver, kidney, lung and stomach

1,4-Dichlorobenzene induces a dose-dependent hepatocellular proliferation in F344 rats and B6C3F1 mice, sometimes in spite of the lack of hepatotoxicity. It does not induce peroxisomal proliferation in CF1 mouse liver.

One mechanism underlying the nephrotoxicity of 1,4-dichlorobenzene in the male rat has been elucidated: male rat specific hyaline droplet nephropathy. This does not exclude another mechanism.

4.1.2.2   Acute toxicity

4.1.2.2.1   Studies in animals

Oral route

The LD50 was found to be greater than 2,000 mg/kg in a study (OECD method, limit test) on rats with signs of reversible toxicity (salivation, abnormal gait, and hunched posture) at day 4 without any macroscopic anomalies (Gardner, 1987a). Several other studies also showed that the oral LD50 was greater than 2,000 mg/kg in rats, and greater than 2,950 mg/kg in mice (Ben-Dyke, 1970; Gaines, 1986; Domenjoz, 1946). In these last three studies there were no details on the experimental protocol as in two other studies which are difficult to consider.

Dermal route

The LD50 in the rat is greater than 2,000 mg/kg without local or generalised signs, and without any macroscopic anomalies (OECD method, limit test) (Gardner, 1987b). Another study, the protocol of which was not given, found a cutaneous LD50 of over 6,000 mg/kg (Gaines, 1986).

Inhalation route

The 4-hour LC50 in rats (EEC method, GLP, limit test) is greater than 5.07 mg/l (845 ppm), with signs of pulmonary irritation (increased respiratory rate up to 4 hours post exposure), piloerection and reversible weight gain losses at Day 2, without macroscopic anomalies (Hardy, 1987). In a study (progressive nasal exposure during 7 hours), symptoms as tremors, hyporeflexia and instability were observed at Day 1 (Hoechst, 1981).

Intraperitoneal route

The LD50 is greater than 2,000 mg/kg in rats and mice in both studies considered (protocol not detailed) (Zupko, 1949; Mohtashamipur, 1987). The subcutaneous LD50 in mice is reported as 5,145 mg/kg (Irie, 1973).

After single oral administration or injection (500 or 770 mg/kg) or inhalation or injection (500 ppm around 3 mg/l for 24 hours) of 1,4-dichlorobenzene, an increase in the formation of hyaline droplets in the renal cortex was observed in male rats, and hepatic anomalies (vacuolisation and hepatic porphyric in albinos rats) were observed principally in female rats (Umemura et al., 1990; Charbonneau, 1989b; Rimington, 1963). No hepatic or renal anomalies were observed after ip injection in F344 rat (Valentovic, 1993).

4.1.2.2.2   Studies in humans

Only a single case of intoxication causing a haemolytic anaemia, through accidental ingestion of an unknown quantity of 1,4-dichlorobenzene, has been reported (Hallowell, 1959). Poisoning centre case reports of accidental intoxication with 1,4-dichlorobenzene is described: accidental ingestion of low doses of 1,4-dichlorobenzene (< 1 moth ball equivalent to 5 g) can lead to digestive problems (digestive irritation with nausea and vomiting). Heavy doses can lead to neurological troubles (convulsions, agitation) (Jouglard, 1976).

From these case reports, the authors state that the minimum quantity that leads to adverse effects appears to be greater than 300 mg/kg; as the origin of this dose was not clearly explained, these data are difficult to take into consideration (Jouglard, 1976).

Given the available animal data, the acute toxicity of 1,4-dichlorobenzene is judged to be rather low, regardless of the penetration route (oral, dermal or inhalation). Human data are limited and difficult to take into consideration.

These data do not justify the classification for acute toxicity endpoints.

4.1.2.3 Irritation

4.1.2.3.1 Studies in animals

A study (OECD method) on three rabbits revealed that 1,4-dichlorobenzene is slightly irritating for the skin (exposure during 4 hours, 500 mg solid mixed to a paste with oil paraffin), with erythemas having a maximum value of 1 reversible at day 7, but no oedema (Maertins, 1988). No significant dermal irritation was observed in a 21 days dermal irritation study (GLP) up to 300 mg/kg/day of 1,4-dichlorobenzene in mineral oil (Arletta, 1989).

A study (OECD method, duration of exposure 24 hours, 90 mg solid mixed to a paste with oil paraffin) on three rabbits revealed that 1,4-dichlorobenzene is slightly irritating to the eye (1/3 rabbits) with isolated damage to the conjunctiva (scores at 1 for erythema and oedema), reversible after 72 hours; no irritating effects to the iris, nor to the cornea were noted (Maertins, 1988).

The sensory irritant potential of 1,4-dichlorobenzene during inhalation exposure was investigated by measuring the decrease in respiratory rate and determination of the RD50: dose concentration causing a 50% decrease in respiratory rate. But protocol was not detailed (number of animals per dose tested unknown) and only 2 or 3 concentrations/sex/species were tested with only 10 minutes exposure; in the ASTDM protocol, 8 mice/concentration, 8 concentrations, and 1-hour exposure have to be used. The RD50 for male and female F344 rats were determined at 613 and 719 ppm, respectively and the RD50 for male and female B6C3F1 mice were 270 ppm and 245 ppm; signs of toxicity were not described (Wilson, 1990).

Inhalation exposure at 500 ppm during 6 hours is associated in rats with a severe decrease in the respiratory frequency in rats and in mice with a 50% decreased of mean minute volume (Wilson, 1990).

4.1.2.3.2 Studies in humans

Prolonged and/or repeated cutaneous contact with 1,4-dichlorobenzene in liquid or vapour (warm fumes) form causes slight irritation (burning sensation without cracking). Irritation of the mucous membranes has been described in workers exposed to 1,4-dichlorobenzene, the exposure level being unknown (Waligren, 1953).

In workers exposed in operation involving the handling of 1,4-dichlorobenzene (58 workers, 8 hours/day, 5 days/week, for 8 months to 25 years with an average of 4.75 years) several surveys were conducted (Hollingsworth, 1956):

- in the first survey, results of analyses of 62 air samples showed vapour concentrations of 1,4-dichlorobenzene ranging from 10 to 550 ppm (average 85 ppm), with painful irritations of the eyes and nose recorded at 80 to 160 ppm; at concentrations greater than 160 ppm, the air become irrespirable « breathing was difficult » for unacclimated persons,

- in the second survey, some time later, (same equipment and operating procedure), data fall in two separate groups: 15 air samples (collected under exposures recognised as uncomfortable by acclimated persons) showed concentrations of 1,4-dichlorobenzene ranging from 100 and 725 ppm with an average of 380 ppm; 32 air samples (obtained during operations where exposures were considered acceptable to the workmen) showed concentrations of 1,4-dichlorobenzene ranging from 5 to 275 ppm with an average of 90 ppm,

- in the third survey, made after a rather extensive revision of the operating procedures and equipment resulting in lower concentrations of 1,4-dichlorobenzene, 21 air samples (collected under conditions giving rise to complaints of nasal and ocular irritations) showed concentrations of 1,4-dichlorobenzene ranging from 50 to 170 ppm, with an average of 105 ppm; 25 air samples (gathered under conditions which caused no complaints) revealed concentrations of 1,4-dichlorobenzene from 15 to 85 ppm with an average of 45 ppm.

To summarise this study, irritation complaints appear evident at a vapour concentration between 50 and 80 ppm; irritation becomes severe at concentration greater than approximately 160 ppm and is accompanied by signs of pulmonary irritation. Certain individuals develop acquired tolerance after repeated exposures. In this old study, it was not specified if workers were exposed to others chemicals than 1,4-dichlorobenzene; moreover, concentration data are range concentrations with median values, in which peak exposure concentrations cannot be excluded (results of spot samples of atmosphere) and no clear correlation between concentration and effect can be done; no more information are available in this study to clarify the respiratory symptoms and the concentration levels (Hollingsworth, 1956).

About the other human data with inhalation exposure to 1,4-dichlorobenzene, the irritation effects on respiratory tract are of limited interest because level of exposure or respiratory data are not reported (Miyai, 1988; Reygagne, 1992) except in one case report where the patient did not complain of irritation effects on respiratory tract even if level of exposure was supposed high near the patient (Harden, 1978). From domestic exposure, level of exposure can be estimated less than 60 ppm (see Section 4.1.1.2, consumer exposure, use of mothballs, wardrobe 1) (Miyai, 1988; Reygagne, 1992). Thus to evaluate irritative effects on respiratory tract, this information seems to be inadequate.

Based on the available data on rabbits, it can be assumed that 1,4-dichlorobenzene is a slight irritant for the skin and eyes. Human data show that 1,4-dichlorobenzene is a slight skin irritant (burning sensation without cracking) upon repeated skin exposure.

Concerning ocular and nasal irritations, they appear from 50 ppm onwards in two surveys and from 160 ppm for respiratory irritation in one survey according to an old study (based on concentration ranges with median values), in which peak exposure concentrations cannot be excluded.

Because of the lack of information about the determination of the RD 50, it is difficult to take this information into consideration: it seems a non valid data for this endpoint.

These human data justify the classification Irritant R36 "irritating to eyes" but, R 37 "irritating to respiratory tract" and R 38 " irritating to skin", are not relevant. This classification was agreed at the CMR meetings in October 1998 and in mars 1999.

4.1.2.4 Corrosivity

The information described in Section 4.1.2.3. indicates that 1,4-dichlorobenzene is not corrosive.

4.1.2.5 Sensitisation

4.1.2.5.1 Studies in animals

A Magnusson and Kligman test on guinea pigs (EEC method, 24 controls, 24 test animals, at induction concentrations of 0.1% intradermally and 25% topically and at challenge concentrations of 25% in petrolatum, positive controls used) demonstrates a rather weak potential for sensitisation. At 0.1% intradermally in a pre-test, slight irritation was observed in animals. The maximum non-irritating concentration was greater than 25% because at 25% in petrolatum, no irritation was observed in a pre-test; Sodium Lauryl Sulfate was not applied. Minimal signs of irritation (1/24) were observed after induction.

In control animals, the results were:

- with 25% 1,4-dichlorobenzene: 1/24 scores at 1 at 24 hours; 5/24 scores at 1 and 1/24 at 2 at 48 hours,

- with vehicle only (petrolatum): all scores at 0 at 24 hours; 4/24 scores at 1 at 48 hours.

In treated animals, the results were:

- with 25% 1,4-dichlorobenzene: all scores at 0 at 24 hours; 9/24 scores at 1 and 4/24 at 2 and 1/24 at 3 at 48 hours,

- with vehicle only (petrolatum): 1/24 score at 1 at 24 hours; 11/24 scores at 1 at 48 hours.

Table 4.13 Results of maximisation study

		24 hours (score 1, score 2, score 3)	48 hours
Controls	25%	(1, 0, 0)	(5, 1, 0)
	0	(0, 0, 0)	(4, 0, 0)
Treated	25%	(0, 0, 0)	(9, 4, 1)
	0%	(1, 0, 0)	(11, 0, 0)

At all, no treated animals were sensitised after 24 hours; 5 of the 24 (21%) were sensitised with scores of 2 or 3 after 48 hours (one of the controls was considered sensitised with a score of 2).

No histological examination was conducted (Bornatowicz, 1995).

An open epicutaneous test (Klecak) on guinea pigs (8 controls, 8 test subjects treated at concentrations of 30, 10, 3 and 1% in paraffin oil) did not reveal any sign of sensitisation on days 32 and 46. Signs of irritation were observed at induction (Schmidt, 1985a, b).

Other sensitisation tests, including a passive cutaneous anaphylaxis test carried out with detection of antibodies against 1,4-dichlorobenzene in the serum of guinea pigs treated *in vivo* with 1,4-dichlorobenzene, and a microtubule disassembly *in vitro* assay on mouse and human foreskin fibroblasts showed negative results. However, these tests were not validated for the detection of sensitisation potential (Suzuki, 1991; Leung, 1990).

4.1.2.5.2   Studies in humans

One isolated case of acute petechial purpura appearing from 24 to 48 hours after cutaneous contact with an armchair that had been treated the same day with 1,4-dichlorobenzene, has been reported. A basophilic degranulation test with 1,4-dichlorobenzene was positive after 5 months. The role of 1,4-dichlorobenzene in this reaction (allergic or not) is questionable (Nalbandian, 1965).

On the whole, there is a case for stating that 1,4-dichlorobenzene has a very weak sensitisation potential given the animal data and the only questionable human case reported despite the widespread use of 1,4-dichlorobenzene for many years in occupational and consumer settings and the possible direct handling use. In animals, some skin sensitisation studies (*in vitro* study, open epicutaneous test) gave negative results but for the maximisation study, interpretation of the result was difficult (limitations in conduct). There is no sufficient argument to classify 1,4-dichlorobenzene as a sensitiser or to request an animal study**.**

4.1.2.6   Repeated dose toxicity

4.1.2.6.1   Studies in animals

<u>Oral exposure</u>

Studies in rats

Two NTP studies (compliance with GLP) were carried out on the oral administration of 1,4-dichlorobenzene by gavage to F344 rats over the course of 13 weeks at 300, 600, 900, 1,200, and 1,500 mg/kg/day in the first study and at 0, 37.5, 75, 150, 300 and 600 mg/kg/day in the second study.

The first study revealed renal tubular cell abnormalities with inclusions of eosinophil droplets beginning from 300 mg/kg/day in the males, followed by tubular cell degeneration and necrosis. Hepatic anomalies (statistically significant increased liver weight) appeared at 900 mg/kg/day or more in both sexes. Local degeneration and necrosis of hepatocytes began to appear at 1,200 mg/kg/day. Some minimal non-dose dependent haematological abnormalities (decrease of the haematocrit and haemoglobin levels) appeared in male rats from 300 mg/kg/day. At higher doses (1,200 and 1,500 mg/kg/day), bone marrow hypoplasia, lymphoid depletions of the spleen and thymus, and signs of general toxicity were observed in both sexes. In the first study, the No Adverse Effect Level (NOAEL) was 600 mg/kg/day in female rats, and the Low Adverse Effect Level (LOAEL) was 300 mg/kg/day for male rats.

In the second study, no effects were observed in female and kidney cortical degeneration was observed in male rats at 600 mg/kg/day; the NOAEL for female rats was greater than 600 mg/kg/day and was of 300 mg/kg/day for male rats.

In these two studies effects seen in male rats were sex and species specific and of no relevance to human health risk assessment (US NTP, 1987).

A study (GLP) focusing on the effects on the kidney during the oral administration by gavage of 1,4-dichlorobenzene in F344 rats (during 4 and/or 13 weeks) was carried out.

In the 4-week study hyaline droplet accumulation in renal cells were observed, associated with an increase in the urinary excretion of proteins and lactate dehydrogenases and epithelial cell excretion in male rats from 75 mg/kg/day. Tubular nephropathy with cellular necrosis and dilated tubules appeared in males at doses beginning from 150 mg/kg/day. Relative increased kidney weight was observed in male rats from 300 mg/kg/day and in female rats beginning from 600 mg/kg/day. Liver weight was increased in male and female from 300 mg/kg/day with hepatocellular hypertrophy centrolobular from 300 mg/kg/day in male (3 out of 5). The NOAEL is on the order of 300 mg/kg/day in female rats, and the LOAEL is of 75 mg/kg/day in male rats with kidney effects from this dose. In the 13-week study, the same kidney anomalies as in the 4-week study were observed in male from 150 mg/kg/day associated with an increased kidney weight from 150 mg/kg/day in male and at 600 mg/kg/day in female. A slight increased liver weight began from 75 mg/kg/day in both sexes, associated with a hepatocellular hypertrophy from 300 only in males (2/5). For kidney effects, the LOAEL is 75 mg/kg/day in male rats; the NOAEL is on the order of 300 mg/kg/day in female rats. Haematological and biochemical factors were not completely studied in this work (Bomhard, 1987, 1988b).

The cytochrome P450 monooxygenase liver enzyme activities were studied in a 4-week study, with oral administration (gavage) of 1,4-dichlorobenzene to F344 rats at doses of 0, 150, and 600 mg/kg/day. A dose-dependent cytochrome P450 monooxygenase enzyme induction was observed beginning from 150 mg/kg/day, associated with increased liver weight (slight at 150 mg/kg/day in male, in both sexes at 600 mg/kg/day). As no histopathological liver analysis or other enzyme activity measurement were performed and only changes in cytochrome P450 and liver weight were observed, this appears inappropriate for determination of a NOAEL (Bomhard, 1992).

Other studies dealing with oral administration in rats (species unknown), briefly reported that (Hollingsworth, 1956):

- hepatic and renal abnormalities were observed in male rats (only 2 males per dose) at the highest dose of 500 mg/kg/day over a period of 4 weeks,

- minor hepatic and renal effects (increased weight) were observed in female rats for doses beginning from 188 mg/kg/day, associated with histological hepatic abnormalities at 376 mg/kg/day.

Another study focused on the hepatic porphyria (and designed to compare the ability to cause hepatic porphyria of hexachlorobenzene, 1,4-dichlorobenzene and 1,2,4-trichlorobenzene) after oral administration by gavage of 1,4-dichlorobenzene to female rats at doses of 50, 100, and 200 mg/kg/day. From 50 mg/kg/day slightly dose-dependent increased liver weight appears after 30 and 60 days and only slight increases in liver porphyrins appear after 120 days only. No other enzyme activities were studied (Experimental protocols are not detailed and not sufficient to consider this study as valid for assessment of repeated dose toxicity) (Carlson, 1977).

A two-year study on F344 rats by gavage showed renal abnormalities (hyperplasia, mineralisation) beginning from doses of 150 mg/kg/day for males and nephropathy from 300 mg/kg/day in females. At 600 mg/kg/day in female, slight hepatotoxicity (transient proliferation and persistent liver enlargement) were observed. The LOAEL was found to be 150 mg/kg/day for males, and 300 mg/kg/day for females (US NTP, 1987).

The mechanism underlying the nephrotoxicity of 1,4-dichlorobenzene in the male F344 rat has been elucidated (Borghoff, 1990, 1991; Charbonneau, 1987, and 1988, 1989a, b; Dietrich, 1991; Lehman-McKeeman, 1993, 1990; Saito, 1992; Bomhard, 1989; Olson, 1990). It turns out that the 1,4-dichlorobenzene reversibly binds itself to a low molecular weight protein, alpha-2u-globulin, that is synthesised in the liver of the SD and F344 male rats. The 1,4-dichlorobenzene-alpha-2u-globulin complex can resist the catabolic action of the lysozymes, which then causes a renal lysosomal protein overload and the formation of tubular protein droplets (where the low molecular weight proteins are normally reabsorbed) (Den Besten et al., 1991). This lysosomal overload leads to cell death, and then stimulates a secondary cellular proliferation. This alpha-2u-globulin is specific to male rats. An increase in kidney type alpha-2u-globulin was detected in urine of male adult SD rats treated with 1,4-dichlorobenzene by gavage (Saito, 1996) and in F344 male rats after single oral administration (Charbonneau, 1988, 1989a), with a positive correlation betweem the male rat nephropathy and 1,4-dichlorobenzene accumulation (Charbonneau, 1989b). The NBR male rats, species not synthesising alpha-2u-globulin, did not develop renal lesions or hyalin droplets after oral administration of 1,4-dichlorobenzene (500 mg/kg/day for 4 days) (Dietrich, 1991). Moreover, a different elimination kinetic was found in male rat kidney from other tissues and could be related to the presence of the protein alpha-2u-globulin (Wilson, 1990). Compared to the control, an accelerated development of chronic nephrosis appears in treated F344 male rats during the recovery period after cessation of 1,4-dichlorobenzene treatment (9 months inhalation exposure to 75, 300, 600 ppm), in spite of the lack of any persisting alpha-2u-globulin mediated nephropathy (Umemura et al., 1993).

Other species

An oral administration study by gavage on B6C3F1 mice for 13 weeks (5 days/week), with doses from 85 to 1,800 mg/kg/day (first phase study: 0 to 900 mg/kg/day (13 weeks) and second phase study: 600 to 1,800 mg/kg/day (13 weeks)) was conducted (US NTP, 1987):

- in the first phase, hepatocellular hypertrophy appeared from 675 mg/kg/day in both sexes. The NOAEL was found to be 337 mg/kg/day in both sexes,

- in the second phase, hepatocellular degeneration and decrease of body weight gain appeared from 600 mg/kg/d in both sexes associated from 900 mg/kg/day with increase liver weight; a statistically significant decrease in the number of leukocytes was observed from 600 mg/kg/day in male mice and from 1,000 mg/kg/day in female mice. At high doses (1,500 and 1,800 mg/kg/day), a hypoplasia of the bone marrow and spleen resulting in decreased hematopoiesis, lymphoid depletion of the spleen, and lymphoid necrosis of the thymus were observed. No renal abnormalities were noted. The LOAEL was found to be 600 mg/kg/day in this second phase study.

A gavage study on NMRI mice for 4 weeks at doses 300, 600 and 900 mg/kg/day, showed dose-dependent hepatic abnormalities: in both sexes, at 300 mg/kg/day an increased liver weight was observed associated with an hepatocellular hypertrophia and degeneration and increased alanine amino transferase (ALAT) from doses of 600 mg/kg/day, and an increase in cholesterol levels beginning from 900 mg/kg/day. The LOAEL was found to be 300 mg/kg/day for NMRI mice (Bomhard, 1986).

A two-year study on B6C3F1 mice at doses of 0, 300 and 600 mg/kg/day by gavage reported slight hepatocellular degeneration and slight individual cellular necrosis from 300 mg/kg/day (slight at this dose). Nephropathy was seen in both sexes from 300 mg/kg/day. The LOAEL for non carcinogenic effects was 300 mg/kg/day for both sexes (US NTP, 1987).

A one-year study on rabbits at doses of 0, 500 and 1,000 mg/kg/day by gavage reported briefly (not GLP) that signs of toxicity (tremors, weakness) and histological hepatic abnormalities (swelling area of focal necrosis) were observed beginning from 500 mg/kg/day for both sexes (Hollingsworth, 1956).

In a one-year oral toxicity study (GLP) in Beagle dogs, 1,4-dichlorobenzene was administered via capsule at doses of 10, 50 and 150 mg/kg/day (5 animals/sex/dose) and a control group of 5 animals/sex (of the same age of 7 months than treated animals); due to the severe toxicity at the highest dose (letality observed at 150 mg/kg/day after 12 days), the initial dose of 150 mg/kg/day was adjusted to 100 mg/kg/day at the third week and 75 mg/kg/day at the sixth week. Two males and one female at 150 mg/kg/day died during the study (1 male at D12 and 1 at D25 and 1 female at D24); one control dog died at D83 due to jejunal displacement; two treated animals (one male and one female) died from inflammatory lung lesions, associated in one female with pulmonary hemorrhages: the possibility that death was treatment related cannot be ruled out; the cause of the death of the third animal was not clearly determined. All animals died (2 males, 1 female) during treatment, had congestion or hemorrhage in different tissues [congestion (2 males) and hemorrhage (1 male) of intestine, hemorrhage of lung (1 male, 1 female) and hemorrhage of lymph node (1 female)]. As pulmonary inflammation was observed in dogs and can be caused by nematodes parasites (filariasis, oxocaris), such parasites were researched in the lung mesenteric lymph node but not detected.

At the highest dose (150 and then 75 mg/kg/day) were observed hypoactivity, emesis, deshydratation, emaciation in animals died during the study and decreased body weight gain during the first month. A mild anemia reversible at one year was observed in both sexes at 6 months at the highest dose and the platelet count was increased in high dose female (3 out of 4 female were affected (mean: 413.25 ± 108 ($p < 0.05$); control: 267.00 ± 68). A marrow erythroid hyperplasia in one high dose female and a splenic excessive hematopoiese in high dose animals (2 females, 1 male) were observed.

In the liver, statistically significant dose dependent increased absolute and relative liver weight in high dose ($\cdot 1.5$) and mid dose of both dog sexes was noted. A statistically significant dose dependent increased of liver enzymes was noted: alkaline phosphatases were increased in both sexes from 50 mg/kg/day [at high dose in 2/3 males ($\cdot 7.3$) ($p < 0.05$) and in 4/4 females ($\cdot 7.8$) ($p < 0.01$); at 50 mg/kg/day in 5/5 males ($\cdot 7.2$) and 5/5 females ($\cdot 4.3$)]; ALAT were increased ($p < 0.05$) in 3/4 females at high dose ($\cdot 3.5$); GGT were increased ($p < 0.05$) in 3/4 females at high dose ($\cdot 2.6$). Histological liver findings show hepatocellular hypertrophia in all males and females in mid and high dose groups with hepatocellular pigment deposition in some animals (2/5); bile duct hyperplasia was reported in 1 male and 1 female at the high dose, with hepatic portal inflammation in males (2/5) of the high dose group.

Increased kidney weight at high and mid doses females and kidney duct epithelial vacuolisation (in high dose: 1 male and 2 females, low dose: 1 female) were observed.

A statistically significant increased relative adrenal weight in high dose female and thyroid weight in mild dose female were noted.

No significant neoplasic findings were reported.

In this study, the NOAEL was 10 mg/kg/day (Naylor, 1996).

A 4-week pilot study (GLP) after oral administration (via capsule) in Beagle dogs (2 dogs/sex/dose, 5 days/week, 0, 25, 75, 150, 300 mg/kg/day) shows liver effects (increased liver weight) from 75 mg/kg/day in both sexes; alkaline phosphatases were dose-dependently increased in female from 75 mg/kg/day (p < 0.05) (·2.5 to 3.3) and in male at 150 mg/kg/day (·5 not statistically significant); ALAT, ASAT and bilirubin were also increased in male at 150 mg/kg/day (·5) but with no statistical significance.

At 300 mg/kg/day, 2 male dogs died at J17 and J18 (one from perforatus oesophagus and one severe gastrointestinal irritation); at 150 and 300 mg/kg/day in females, decrease in body weight gain was noted. Gastrointestinal irritation in female was observed at 75 mg/kg/day. No other clinical effects except diarrhea in female at 300 mg/kg/day were observed; no histopathological examination was done (Naylor, 1996).

Inhalation exposure

A study (brief report, no GLP, imprecise data, rat and mouse strains unknown) where different species (rats, guinea pigs, mice, rabbits, monkeys) were exposed to 1,4-dichlorobenzene vapour at concentrations of 0, 96, 158, 173, 341 and 798 ppm, 7 hours per day, 5 days per week for five or seven months, showed no significant toxic effect at 96 ppm. Slight hepatic (hepatocellular degeneration, statistically significant increased liver weight) and kidney (statistically significant increased weight) abnormalities were observed beginning from 158 ppm in rats and guinea pigs. These were followed by signs of focal liver cell necrosis at 341 ppm. Pulmonary abnormalities (oedema, congestion) were observed in rats, rabbits and guinea pigs starting from 173 ppm; at higher concentrations (798 ppm), severe signs of intoxication (pulmonary irritation, marked tremor, weakness, unconsciousness and even death, histological hepatic, renal and pulmonary damage) were observed. The NOAEC was found to be 96 ppm for rats and guinea pigs, 158 ppm for rabbits and monkeys, and higher than 158 ppm for mice. No clear dose response on incidence or severity has been shown (Hollingsworth, 1956).

Another study (detailed protocol, GLP) carried out on Wistar rats over 76 weeks (5 hours/day, 5 days/week, vapour) showed at 500 ppm statistically significant slight increased liver weight in both sexes (not dose dependent) with hepatocyte hyperplasia in females after 26-week recovery and renal abnormalities (increased kidney weight with urinary coproporphyrins but no hyaline droplet nephropathy) in males; at 75 ppm in females, slight increased liver weight (statistically significant) at 26 weeks but not at 76 weeks and little hepatocyte hyperplasia (6 out of 79 animals) at recovery but not at 76 weeks were seen. No haematological or blood chemistry nor irritative symptoms were noted. The NOAEC was estimated at 75 ppm in both sexes (Riley, 1980a).

A 56-week study (vapour) on Swiss mice showed that these animals suffered respiratory abnormalities, but the interpretation of these results was limited by the presence of intercurrent infections. No treatment-related toxic effects on blood chemistry, haematology or histopathology (studied in female mice only) were noted. It was not possible to estimate a value for the NOAEC (Riley, 1980b).

A two-year carcinogenicity study (GLP) was carried out on F344 rats at 0, 20, 75 and 300 ppm, 6 hours/day, 5 days/week, vapour, for a total of 104 weeks. The only significant abnormalities observed were lesions in the kidney (mineralisation of the papilla collecting tube and urothelial hyperplasia) at 300 ppm in males associated with increased kidney weight. Increased liver weight in both sexes at 300 ppm was noted. Respiratory metaplasia in the nasal cavity gland and

eosinophilic changes in respiratory epithelium were observed at 300 ppm in females and eosinophilic changes in olfactory epithelium were observed in a majority of control and treated animals, but grade was higher in treated animals at 300 ppm in both sexes and 75 ppm in females than controls: [sacrificed animals: (control sacrificed: 38/38 in females, 24/33 in males) and (dose treated sacrificed at 300 ppm: 12/18 in males, 36/36 in females; at 75 ppm: 17/29 in males, 36/38 in females)]; the same tendency was observed in dead animals: [(control dead: 11/12 in females, 9/17 in males; dose treated dead at 300 ppm: 13/32 in males, 14/14 in females; at 75 ppm: 4/21 in males, 10/12 in females)]. The NOAEC was estimated at 75 ppm for kidney disorder (JBRC, 1995).

A two-year carcinogenicity study (GLP) was carried out on BDF1 mice, at 0, 20, 75 and 300 ppm, 6 hours/day, 5 days/week, vapour, for a total of 104 weeks. At 300 ppm of liver tumour induced dose, severe liver toxicity including increased liver enzymes in both sexes (AST, ALT, LDH, alkaline phosphatase), increased liver weight in both sexes and histological findings: slight local necrosis in both sexes (7/49 in male and 2/49 in female controls; 17/49 in male and 8/49 in female treated) and central hepatocellular hypertrophy in 34/49 males were observed. Increased kidney weight was noted at 300 ppm in both sexes. The NOAEC was estimated at 75 ppm for liver disorder (JBRC, 1995).

Dermal exposure

A 21-day dermal toxicity study (GLP) in SD rats (5 rats/sex/dose, 5 days/week, 3 weeks) up to 300 mg/kg/day of 1,4-dichlorobenzene in mineral oil, failed to elicit any toxic effects or significant dermal irritation but the highest dose tested did not reflect a limit dose (Arletta, 1989).

Summary of studies in animals

Studies on the oral administration of 1,4-dichlorobenzene to F344 or unknown species rats (4 weeks to 13 weeks) show that there is an appreciable difference between males and females because hyaline droplet nephropathy is only observed in male rats at concentrations beginning at 75 mg/kg/day, becoming significant at level of 150 mg/kg/day. This hyaline droplet nephropathy is specific to the male rats and cannot be extrapolated to humans - and thus does not represent a risk to human health (see mechanism of action in Section 4.1.2.6.1.). Beyond these concentrations (more often at 300 mg/kg/day), hepatic abnormalities (increased liver weight, hepatocellular hypertrophy) and renal abnormalities (increased kidney weight, nephropathy) are observed in both sexes. These data collected on rats are further confirmed by a two-year carcinogenicity oral study in F344 rats. The NOAEL for renal effects of 150 mg/kg/day in female rats has to be considered. For male rats, the LOAEL for renal effects is 75 mg/kg/day.

In other species (NMRI and B6C3F1 mice, rabbits), the LOAEL is greater or equal to 300 mg/kg/day with hepatic (increased liver weight, hepatocellular hypertrophy and degeneration) and kidney (nephropathy) abnormalities observed from this concentration except in Beagle dogs where the NOAEL is 10 mg/kg/day from a one-year study, with liver effects observed from 50 mg/kg/day; this NOAEL will be taken into account for risk assessment because dogs are an appropriate model for human and there is no evidence to think the opposite.

By inhalation route, the NOAEC for non carcinogenic effects was estimated at 75 ppm in two chronic toxicity studies: one in Wistar rats exposed to 1,4-dichlorobenzene over a period of 76 weeks and one in BDF1 mice and F344 rats exposed to 1,4-dichlorobenzene over a period of 104 weeks. This NOAEC is in agreement with the results of an old inhalation exposure study on different species (rats, guinea pigs, mice, rabbits and monkeys, strain unknown) over periods of

5 to 7 months which gave a NOAEC of 96 ppm for rats. Beginning from 158 ppm, minor abnormalities of the liver increased liver weight, slight hepatocellular degeneration) were noted, as were kidney abnormalities from 300 ppm (increased kidney weight, mineralisation); neurological symptoms and pulmonary toxicity appeared at concentrations of 798 ppm and higher. Even if this study was briefly reported with several imprecise data, the NOAEC serves as supportive evidence of the NOAEC of 75 ppm estimated from the F344 rats and BDF1 mice inhalation carcinogenicity studies.

No significant toxic effects were seen after repeated cutaneous exposure to 1,4-dichlorobenzene up to 300 mg/kg/day during 21 days.

4.1.2.6.2   Studies in humans

There are no large-scale epidemiological studies on 1,4-dichlorobenzene toxicity in workers. Available data are of poor quality.

Two cases of progressive chronic encephalopathy (associated with cerebral ataxia, dysarthria, hypotonia, hyporeflexia of all four members) have been reported after massive or intentional long-term domestic exposures: exposure for 6 years, level of exposure unknown, contact with 1,4-dichlorobenzene by grinding moth balls into powder and scattering them in her room (Miyai 1988), exposure longer than 9 months, level unknown, intentional inhalation of moth balls (Reygagne, 1992).

Reversible haematological damages have also been reported: an aplastic anaemia and an anaemia of an undetermined nature, following occupational exposure (exposure to a powder mixture with 90% of 1,4-dichlorobenzene for preparation of moth balls, level unknown, duration 18 months in the first case; chronic exposure for 39 years in a clothing resale shop, level unknown, concommittant exposure to naphthalene with high exposure the last 3 weeks in handling 5.5 kg pure 1,4-dichlorobenzene in the second case) have also been reported. However, a direct link with exposure to 1,4-dichlorobenzene was not always clear (Petit 1948; Harden 1978).

A study on approximately 50 subjects who were exposed to 1,4-dichlorobenzene (between 15 and 170 ppm) during 8 months to 25 years (mean of 4.75 years) in the workplace did not reveal any haematological abnormalities (RBC, WBC and differential, Hb, Ht, MCV) (Hollingsworth, 1956). Haematological damages (diminution of white blood cells) were also described, but link with exposure to 1,4-dichlorobenzene was not always clear (Perrin, 1941).

Hepatic damages have been reported including cytolysis and cirrhosis for long-term occupational exposure(during 2 years as selling moth balls whose chief component was 1,4-dichlorobenzene) and subacute yellow atrophy of the liver after occupational exposure (as sale clerk more than 1 year in a preparation store of 1,4-dichlorobenzene) and after domestic exposures to moth ball gas vapour of 1,4-dichlorobenzene during 3-4 months. No clear cause-effect relationship or level estimates were established; no other aetiology of liver damage has been explored (Cotter, 1953; Sumers, 1952).

General signs such as irritation of the mucous membranes, asthenia, weight loss, hepatitis, and methemoglobinemia have been described in workers exposed to 1,4-dichlorobenzene for more than one month (level unknown). These signs were related to several substances including 1,4-dichlorobenzene (Waligren, 1953).

Several old, poorly documented cases have also been reported. In one such case, oedema of the articulations and weight gain (24-hour inhalation exposure) were observed, and in another

(ingestion in a 19 years old person, 4 to 5 moth pellets/day (20 to 30 g/day) during 2.5 years) cutaneous pigmentation like « fixed drug eruption » and unusual neurological symptoms (tremor, instability) have developed once the exposure was ended but were reversible after 4 months. The relationship between the symptoms and exposure is difficult to establish (Claytor, 1935; Frank, 1961).

Two cases of cataract formation in women exposed to 1,4-dichlorobenzene have been described associated with transient tremor and jaundice (first case) and with jaundice and loss of weight (second case) after two years occupational exposure as housewife in the first case and after one year domestic exposure with cans of 1,4-dichlorobenzene in closet without windows (level unknown) in the second case. The role of 1,4-dichlorobenzene is not clear since the cataracts formed once the exposure ended (Berliner, 1939). A case of pulmonary damage with the formation of granuloma has been reported for a domestic exposure that lasted 12 years. A pulmonary biopsy revealed intracellular inclusions similar to crystals of 1,4-dichlorobenzene. Again, the relationship with exposure is not clear (Weller, 1953).

Summary of studies in humans

No epidemiological study in humans is available at the current time. The case reports quoted above are of poor quality and describe neurological symptoms, with mention of hepatic or haematological troubles; cause-effect relationship in terms of 1,4-dichlorobenzene exposure is never clearly established. Because data come from mixed occupational exposure to several substances, the extent of exposure is rarely known and domestic exposure is either massive or intentional, it is difficult to take these data in consideration for risk assessment.

4.1.2.6.3 Summary for repeated dose toxicity

For risk assessment purpose, a NOAEL of 75 ppm has been determined via inhalation. Moreover, the more conservative NOAEL of 10 mg/kg/day from a dog oral study has also to be considered.

Human data are difficult to take into consideration for risk assessment (duration or level of exposure unknown, exposure to several substances) except for irritating symptoms (considered in the Section 4.1.2.3).

These data do not justify the classification for repeated dose toxicity.

4.1.2.7 Mutagenicity

4.1.2.7.1 Studies in animals

In vitro studies

Adducts and associations with DNA

Microsomal fractions (+ NADPH) from rat and mouse liver or lung can catalyse the association of [^{14}C]-1,4-dichlorobenzene with calf thymus DNA, whereas microsomes from rat and mouse kidney and from mouse stomach do not. Cytosol (+ reduced glutathione) from these same tissues and species either have no effect or, as in the case of rat and mouse lung, have a very weak

effect. The strongest effects on associations with DNA were in the presence of microsomes (+ NADPH) and cytosol (+ GSH), particularly if they were derived from mouse lung and then from rat or mouse liver and stomach. There was no association with DNA in the presence of rat kidney and the association observed in the presence of mouse kidney was very weak. Analysis of 5′-mononucleotides by HPLC indicated no increased binding to nucleotides, owing to the low labelling of mouse liver DNA (Lattanzi et al., 1989).

Paolini et al. (1998) incubated [14C]-1,4-dichlorobenzene with calf thymus DNA and various subcellular fractions of liver from male CD-1 mice that had been treated with either phenobarbital and β-naphthoflavone (PB/NF) or butylated hydroxytoluene (BHT). After centrifugation to remove particulate material, DNA was extracted and radioactivity associated with it was measured. [14C]-1,4-dichlorobenzene or its metabolites was associated with DNA, particularly after incubation with PB/NF-treated mouse microsomes (+ NADPH + GSH) or S9 (+ NADPH + GSH) from either PB/NF- or BHT-treated mice. No negative or positive control substances were included in this non-validated study.

These results are contradicted by those obtained by a ^{32}P-post-labelling technique (Tian et al., 2001a). In this study, calf thymus DNA was mixed with 1,4-dichlorobenzene, an NADPH-generating system and microsomes from liver of young male Fischer 344/NSIc rats, male BDF_1 mice or (purchased from a commercial supplier) from man. DNA was extracted and analysed by ^{32}P-post-labelling after enhancement of assay sensitivity with nuclease P1. No DNA adducts were detected.

Table 4.14 *In vitro* DNA-adducts formation of 1,4-dichlorobenzene

Test system	Source and purity of chemical	Result [a]	Dose [b] (LED/HID)	Reference
DNA adducts isolated calf thymus DNA Mouse lung and liver Rat liver and lung Rat and mouse kidney	Radiochemical Centre, Amersham, U.K. Sp. Act. 43 mCi/mmol; radiochemical purity 98%	+ + –	([^{14}C]-1,4-dichlorobenzene)	Lattanzi et al. (1989) *
DNA adducts isolated calf thymus DNA Mouse liver	Radiochemical Centre, Amersham, U.K. Sp. Act. 43 mCi/mmol; radiochemical purity 98%	+	([^{14}C]-1,4-dichlorobenzene)	Paolini et al. (1989)
DNA adducts isolated calf thymus DNA (^{32}P-post-labelling) BDF1 mouse liver F344 rat liver Human liver	Not given	–	14,7 µg/ml	Tian et al. (2001a)

* Tests already available in the risk assessment report dated 05/2001
Reference: test not performed from validated internationally accepted test system
a) + positive; (+), weak positive; ? : inconclusive ; –, negative; NT, not tested
b) LED, lowest effective dose; HID, highest ineffective dose

Bacterial studies

1,4-Dichlorobenzene has been tested for its potential to induce DNA repair and gene mutations in bacteria in several studies; these are listed in **Table 4.15**.

The *umu*-test uses *Salmonella typhimurium* TA1535 infected with the plasmid *p*SK1002 carrying fused *umuC'*-*'lac Z* genes. In this fused gene, DNA damaging agents induces the umu operon. The level of DNA repair induction (the *umu* gene) after DNA damage has occurred is indicated by the β-galactosidase activity co-induced in *lac Z*. The assays were conducted both in the absence and the presence of rat liver S9 (Ono et al., 1991; 1992). In these studies (442 µg/ml, 2-hour exposure and 100 µg/ml, 4 hour exposure), a fractional increase in β-galactosidase activity, as compared with the control, of < 0.5 was considered as inactive; a positive response was indicated by a fractional increase of 2.0 > 1.0. In neither study did 1,4-dichlorobenzene show any DNA repair gene inducing activity.

Five studies have been conducted in which mutagenic activity in different strains of *S. typhimurium* were used, basically according to the protocol developed by Ames. All were conducted in the absence and presence of S9 prepared from the liver of rats treated with microsomal enzyme-inducing agents et all except the earliest were conducted either to GLP guidelines or to standards that were equivalent to recognised guidelines. In addition, there has been one mouse host peritoneal cavity assay.

In the study by Anderson (1976a), 1,4-dichlorobenzene was either dissolved in DMSO and tested at doses up to 2,500 µg per plate or generated as a vapour and tested at atmospheric concentrations up to 682 ppm. The strains used were TA1535, TA1538, TA98 and TA100. It is noted that the numbers of revertants per plate in the untreated cultures of TA100 were low, indicating a loss of the plasmid *p*KM101 from these cells. No mutagenic activity was observed in the vapour phase experiment; however, there were anomalies in the untreated control group data that brings into question the validity of the results. Thus, for strain TA100 without S9, the control mean and standard errors were only 24 ±16 (about 20% of the normal value), while, for strain TA98, the control values 55 ± 20 without S9 and 78 ± 18 with S9 (approaching 2-fold higher than normal). Finally, the numbers of strain TA100 revertants per plate after treatment with 299 ppm were only 7 ± 2 without S9 and 6 ± 4 with S9, these being 5 to 6-fold lower than the values at both lower and higher atmospheric concentrations. Inconsistencies were less severe in the standard treat-and-plate assays up to 2,500 µg/plate, but they were sufficiently large to call into question the usefulness of these data; in one experiment at 500 µg/plate, the number of revertants more than doubled It is conceivable that some of the problems in this study were the result of unrecognised toxicity (see Jones and Fenner, 1987, who reported 'incomplete lawns' (i.e., of non-mutant microcolonies) at a dose of 500 µg per plate. The author considered that 1,4-dichlorobenzene was inactive in this assay, but in view of the uncertainties in the data, this reviewer arrives at an 'inconclusive' evaluation.

In the Haworth et al. (1983) study, 1,4-dichlorobenzene was tested for mutagenicity against *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 at dose levels up to 100 µg per plate. There was no evidence of mutagenic or toxic activity in any of the strains, however, the modest dose levels tested detracts from an unequivocal conclusion that 1,4-dichlorobenzene is not mutagenic.

Connor et al. (1985) tested 1,4-dichlorobenzene against *S. typhimurium* strains TA1535, TA 100 up to 1,000 µg/plate with negative results but only two strains and one experiment.

Jones and Fenner (1987) tested 1,4-dichlorobenzene against *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100. In the first mutation experiment reported there were signs of toxicity at dose levels of 500, 1,500 and 5,000 µg per plate, expressed as either a thin lawn or no lawn of bacterial microcolonies, or as a reduced number of revertant colonies. In the second mutation experiment, doses up to 500 µg per plate were used. In neither experiment was there any indication of mutagenic activity.

Winker et al. (1993) tested 1,4-dichlorobenzene for mutagenic activity in *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 according to OECD guidelines (in addition to GLP). Dose levels used were up to either 2,000 or 3,000 µg per plate. At doses of 1,000 µg per plate and higher, there were indications of toxicity, shown as reductions in the numbers of mutants per plate, but in no case was there evidence of mutagenic activity.

In a host mediated assay (Urwin and Baldock, 1975), male CFLP mice, 5/group, were treated orally by gavage with 1,4-dichlorobenzene in corn oil at doses of 0, 4,000, 8,000 and 16,000 mg/kg bw subdivided into two equal doses 12 h apart. Immediately after the second dose, the mice were inoculated intraperitoneally with a suspension of *S. typhimurium* G46. The bacteria were recovered from the peritoneal cavity 2.5 h later for the assessment of toxicity and mutagenicity at the *his locus*. Two of five mice died in the 16,000 mg/kg bw group, but there was no dose related change in total bacterial count, mutant colony count or mutation frequency.

Fungal studies

1,4-Dichlorobenzene was tested for mutagenic activity in a reverse mutation assay in a strain of *Aspergillus nidulans* that required methionine and pyridoxine as growth factors (Prasad, 1970). The frequency of methionine revertants was monitored after exposure of conidia to 1,4-dichlorobenzene at concentrations of 0 or 200 µg/ml for 1 hour at 28°C. The frequency of revertants was 3 per 10^6 viable spores (viability 60%) for the control and 10 per 10^6 viable spores (viability 39%) for the exposed culture. The experiment appears to have been performed once.

Using the yeast *Saccharomyces cerevisiae* D7 strain, Paolini et al. (1998) studied the induction of mitotic gene conversion at the *trp*-5 locus and gene mutation of the mutant allele *ilv*-92. Stationary phase cells were incubated in the presence of 0, 74, 147 or 588 µg/ml 1,4-dichlorobenzene for 2 h at 37°C, with or without differently induced mouse liver S9 fractions, as described above (see Adducts and Associations with DNA). There were statistically significant increases in the frequency of *trp*$^+$ convertants at 74 µg/ml and *ilv*$^+$ revertants at 147 µg/ml

Table 4.15 In vitro tests for gene mutation induction in bacteria and fungi by 1,4-dichlorobenzene

Test system	Source and purity of chemical	Result [a]		Dose [b] (LED/HID)	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA1535 (pSK1002) DNA repair (*umu*) test	not given	—	—	443 µg/ml	Ono et al. (1991), (1992) *
Salmonella typhimurium TA100, TA1535, TA1538, TA98, reverse mutation	ICI Ltd., U.K. Purity not given	?	?	2,500 µg/plate and 682 ppm atmos.	Anderson (1976a) *
Salmonella typhimurium TA100, TA1535, TA1538, TA98, reverse mutation		—	—	Up to 500 µg/plate	Anderson (1976c) *
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation		—	—	100 µg/plate	Haworth et al. (1983) *
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	source not given. Purity 99.9%	—	—	1,500 µg/plate	Jones and Fenner (1987) *
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	E. Merck, Darmstadt, Germany. purity not given	—	—	3,000 µg/plate	Winker et al. (1993) *
Salmonella typhimurium TA100, TA 98		—	—	1,000 µg/plate	Connor et al. (1985) *
Salmonella typhimurium G46, reverse mutation in male CFLP mouse peritoneal cavity host-mediated assay		—		8,000 mg/kg bw, orally · 2	Urwin and Baldock (1975)
Aspergillus nidulans meth$_3$ locus reverse mutation	not given	(+)	NT	200 µg/ml	Prasad (1970) *
Saccharomyces cerevisiae D7, mitotic gene conversion	not given	—	+	74 µg/ml	Paolini et al. (1998)
Saccharomyces cerevisiae D7, reverse mutation	not given	—	+	147 µg/ml	Paolini et al. (1998)

a) +, positive; (+), weak positive; ? : inconclusive ; –, negative; NT, not tested; b) LED, lowest effective dose; HID, highest ineffective dose
* tests already available in the risk assessment report dated 05/2001   Authors underlined : test not performed from validated internationally accepted test system

Mammalian cell studies

In an experiment monitoring the induction of unscheduled DNA synthesis, carried out on HeLa cells by a scintillation counting method, 1,4-dichlorobenzene showed no activity when tested at concentrations up to 100 µg/ml in the absence and 500 µg/ml in the presence of rat liver S9 (24-hour exposure) (Pirovano and Milone, 1986a).

In an other unscheduled DNA synthesis test, carried out on human lymphocytes by a scintillation counting method, 1,4-dichlorobenzene showed no activity when tested at concentrations up to 147 µg/ml in the absence and in the presence of rat liver S9 (4 hour exposure) (Perocco, 1983).

The potential for 1,4-dichlorobenzene to induce DNA fragmentation in primary cultures of rat and human hepatocytes was measured by the alkaline elution technique (Canonero et al., 1997). The human hepatocytes were derived from fragments of liver discarded during the course of surgery on six patients with hepatocellular carcinoma, cholangiocarcinoma or hepatic metastases from carcinomas of either the stomach or the colon. Cell viability, as assessed by exclusion of either trypan blue or neutral red applied immediately after a 20-hour exposure, indicated that concentrations of 1,4-dichlorobenzene up to 3.2 mM [470 µg/ml] could be used. It had been pointed (Storer et al., 1996) that these techniques may underestimate toxicity in comparison with measurements made just 2 hours after the test compound has been washed from the cells. Nevertheless, there was no evidence of DNA fragmentation induction by 1,4-dichlorobenzene in either rat or human hepatocytes, whereas significant increases were observed with the positive control substance, *N*-nitrosodimethylamine.

DNA fragmentation induction was also studied in primary cultures of rat and human kidney cells using the single-cell gel-electrophoresis (Comet) assay (Robbiano et al., 1999). This assay is essentially a micro-version of the alkaline elution technique, used above. The human kidney cells were derived from fragments of kidney discarded during the course of surgery for carcinoma or adenoma of the kidney. Cells were exposed to 1,4-dichlorobenzene concentrations of up to 5.6 mM [823 µg/ml] for 20 hours, immediately after which time relative survival assessed by exclusion of trypan blue averaged 0.78 for rat cell cultures and 0.84 and 0.96 for the human cells of two donors. As estimated from both the Comet tail length and the tail moment (a product of tail length and the amount of DNA in the tail), there was a dose related response in DNA fragmentation in rat kidney cells and human kidney cells from one donor over concentrations of 1.8, 3.2 and 5.6 mM [265, 470 and 823 µ/ml]. The dose response was also seen in the cultures from the second human donor over concentrations of 3.2 and 5.6 mM [470 and 823 µg/ml]. To be noted that in this article, the existence of apoptotic cells or damaged cells is not mentioned, it is difficult to appreciate the influence of cytotoxicity on the results. The possibility for underestimating toxicity and the apparent resistance of these primary cultures of either liver or kidney cells to 1,4-dichlorobenzene toxicity in these two papers is noted.

Sister-chromatid exchange (SCE) induction by 1,4-dichlorobenzene was studied in Chinese hamster ovary (CHO) cells (Galloway et al., 1987) and in human lymphocytes (Carbonell et al., 1991). In CHO cells, there was no significant induction of SCEs at concentrations up to 150 µg/ml in either the absence or presence of rat liver S9 (2-hour exposure with S9, 25 hour exposure without S9). In contrast, SCEs were reportedly induced at the very low concentrations of 0.1 and 0.2 µg/ml in human lymphocytes from two donors in the absence of any exogenous metabolic system (46-hour exposure). There was also a reduction in the proliferation rate index ($M_1 + 2M_2 + 3M_3/100$, where M_1, M_2 and M_3 are the percentages of cells in the first, second and third or higher divisions, respectively) at 0.2 µg/ml. It is possible that the authors of this paper have confused the units expressing the concentration of 1,4-dichlorobenzene.

Chromosomal aberration induction by 1,4-dichlorobenzene was also studied in CHO cells (Galloway et al., 1987), in Chinese hamster lung (CHL) cells (Ishidate, 1988) and human lymphocytes (Pirovano and Milone, 1987). In CHO cells there was no significant increase in structural aberration frequency at concentrations up to 150 μg/ml in either the absence or presence of rat liver S9 (2 hour exposure with S9, 25-hour exposure without S9). In CHL cells there was no induction of chromosomal aberrations following treatment for 48 hours, but at concentrations of only up to 5 μg/ml and in the absence of any exogenous metabolic activation system. In human lymphocytes, from a single donor, there was also no induction of structural chromosomal aberrations at concentrations up to 100 μg/ml (500 μg/ml being toxic) in either the absence or presence of rat liver S9 (4 hour exposure).

Micronucleus induction in rat and human hepatocytes was studied following exposure to 1,4-dichlorobenzene (Canonero et al., 1997, see DNA fragmentation, above). The procedure involved exposure to serial dilutions of the chemical for 48 hours before hypotonic shock, fixation and Feulgen staining for chromatin. Cytotoxicity was not estimated in this study. Two experiments were performed with rat hepatocytes in which statistically significant increases in micronucleated hepatocytes were observed at single concentrations in each experiment of 1.0 mM [147 μg/ml] and 1.8 mM [265 μg/ml], respectively. Hepatocytes from two of the human donors were also tested, over a concentration range of 1,4-dichlorobenzene from 0.56 to 3.2 mM [82 to 470 μg/ml]. There were no significant increases in the proportion micronucleated human hepatocytes.

Micronucleus induction was also studied in primary cultures of rat and human kidney cells (Robbiano et al., 1999, see DNA fragmentation, above). The procedure involved exposure to serial dilutions of the chemical for 48 hours before hypotonic shock, fixation and May Grümwald-Giemsa staining. Cytotoxicity was not estimated in this study. There were dose related increases in the proportion of micronucleated cells in both rat and human kidney cell cultures over 1,4-dichlorobenzene concentrations of 1.8, 3.2 and 5.6 mM [265, 470 and 823 μ/ml].

The induction of gene mutations by 1,4-dichlorobenzene has been studied at the *tk* locus in mouse lymphoma L5178Y cells (McGregor et al., 1988; Myhr et al., 1990) and at the *hprt* locus in Chinese hamster lung V79 cells (Pirovano and Milone, 1986b) and CHO cells (den Boer and Hoorn, 1986a; Loveday, 1989).

McGregor et al. (1988) performed seven experiments that were acceptable according their criteria, four in the absence of an exogenous activating system and three with rat liver S9. The first of the experiments without S9 yielded a statistically significant response at 12.5 μg/ml, where the relative total growth (RTG) was about 85%. In the same experiment, however, no significant response was obtained at either 25 or 50 μg/ml. This inconclusive result was followed in the second experiment by a significant although marginal response at the highest non lethal concentration of 80 μg/ml. The third experiment without S9 did not show any mutagenic response at closely spaced concentration steps up to 90 μg/ml, but the RTG at this dose was 53%, a relatively high level, so the experimental result was considered to be inconclusive. The last experiment without S9 did not show any mutagenic response over closely spaced concentration steps up to 95 μg/ml, where the RTG was 16%, and the next concentration, 105 μg/ml, was lethal. In the presence of rat liver S9, the first experiment did not produce any significant change in mutation frequency at concentrations up to 100 μg/ml. The next concentration, 110 μg/ml, was lethal. The next two experiments did, however, show significant increases in mutation frequency. In the first of these, there was a significant response at 65 μg/ml, but then there were two non-significant dose levels intervening before the next significant dose level at the highest, non lethal concentration of 95 μg/ml. This absence of a convincing dose related response was also seen in the final experiment, conducted using a particularly narrow

dose range (six concentrations spanning 80-105 µg/ml), in which there was a single significant response at the highest concentration where the increase in mutation frequency was only 1.63-fold the control group value. These factors led to the overall conclusion that 1,4-dichlorobenzene should be categorised as having "questionable" mutagenic potential in this assay. The data demonstrated that any statistically significant responses, if real and not the outcome of chance events, were very weak and variable. Myhr et al. (1990) only summarises the above results.

A study of gene mutagenic activity at the *hprt* locus of V79 cells (Pirovano and Milone, 1986b) involved incubations for 2 hours in the absence and presence of rat liver S9, following by selection of mutants with 6-thioguanine immediately or after 3 or 6 days to permit the expression of mutants induced during the incubation period. Toxicity was observed from 500 µg/ml in a pre-test. There was no evidence of mutagenic activity of 1,4-dichlorobenzene at concentrations up to 100 µg/ml in the absence of S9 or 200 µg/ml in the presence of rat liver S9 after 0, 3 or 6 days expression.

In the CHO cell study of den Boer and Hoorn (1986a), incubations were for 4 hours in either absence or presence of S9 mix, followed by an expression period of 7 days before selection of mutants with 6-thioguanine treatment. In the absence of S9 there was no evidence of mutation induction at 1,4-dichlorobenzene concentrations up to 240 µg/ml. The relative population growth (as a percentage of the control) was 54% at this concentration; two higher concentrations up to 400 µg/ml were lethal, permitting no survivors. In the first experiment in the presence of S9, relative population growth was reduced to 36% at 210 µg/ml, this culture being at the highest concentration carried throughout the entire experiment. Significant increases in mutation frequency were observed at this concentration, but they also occurred (in one of duplicate cultures in each case) at 140, 105 and 70 µg/ml, this last being the lowest concentration tested. At concentrations intermediate of 70 and 105 µg/ml and of 105 and 210 µg/ml, there were no significant increases in mutation frequency. The result was, therefore, questionable and the experiment was repeated. Over the concentration range at which cultures were carried throughout the experiment (105 to 350 µg/ml) there were no indications of a mutagenic effect. It was concluded, therefore, that 1,4-dichlorobenzene was not mutagenic in this study.

Loveday (1989) also tested 1,4-dichlorobenzene for mutagenicity in the CHO cell *hprt* locus assay. The study included three experiments with 16 hours incubations and two experiments with 4 hours incubations, all in the absence of S9, and then two experiments with 4 hours incubations in the presence of rat liver S9. The medium used in the 16 h incubation experiments contained serum, whereas the 4 hours incubations did not. 1,4-Dichlorobenzene concentrations up to 200 µg/ml were tolerated for 16 hours in the presence of serum, but the upper limit was 180 µg/ml during incubation for 4 hours in the absence of both serum and S9. In the presence of rat liver S9, 250 µg/ml was tolerated in one experiment, but only 150 µg/ml was tolerated in the second experiment. Mutation frequencies were erratic, both within and between experiments, but in none of the experiments was there a clear indication of a concentration related increase. Variance analysis of the transformed data combined within each of the three sets of experiments under different incubation conditions did not show any significant difference between the test samples and the vehicle controls.

More recently, the mutagenicity of 1,4-dichlorobenzene has been investigated in the CHO cell *hprt* locus assay, in which cells were treated for 4 hours in the absence and presence of rat liver S9 (Tegethoff et al., 2000). In the absence of S9 there was no indication of mutagenic activity at concentrations up to 240 µg/ml, when the relative survival was 77%. The next higher concentration, 320 µg/ml, permitted no survivors. In the presence of rat liver S9 there was a significant increase in mutation frequency at a concentration of 210 µg/ml, when the relative survival was 40%. The next higher concentration, 280 µg/ml, permitted no survivors. This

portion of the experiment was repeated and it was found there were no significant increases in mutation frequency at concentrations up to 280 µg/ml, when the relative survival was 79%. At the next higher concentration the relative survival was only 1% and no mutant colonies were recovered. Although the two tests with S9 gave different results and that it could be argued that the dose interval between the two highest doses in the second experiment was, in retrospect, too large. The authors point out that the statistically significant result (16.6 mutants per 10^6 survivors) is only just outside their historical control range of 1–15 mutants per 10^6 survivors. This reviewer considers that the overall result is inconclusive.

1,4-Dichlorobenzene has also been tested in the *in vitro* transformation assay with BALB/3T3 cells (den Boer and Hoorn, 1986b). On the basis of a series of preliminary toxicity tests, target cell used in the transformation test were exposed for 72 hours to 1,4-dichlorobenzene concentrations ranging from 60 to 140 µg/ml. Following this treatment, the cells were cultured for four weeks. Cultures of normal cells were round and in a closely packed monolayer formation by this time. Transformed cells formed superimposed foci, or colonies, on this monolayer, were densely aggregated and stained darkly with Giemsa stain. A necessary characteristic of a transformed colony was that there should be a random orientation of the fibroblasts within the darkly staining area. There was no statistically significant increase in the number of transformed colonies per culture flask at any concentration of 1,4-dichlorobenzene.

In a research programme with Syrian Hamster Embryo (SHE) cells examining the relationship between polyamine metabolism and cell transformation, Nguyen-Ba (1996) compared the effect of 1,4-dichlorobenzene in comparison and in combination with 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) on ornithine decarboxylase (ODC) and soluble 72 kDa proteolytic enzymes. In addition, DNA fragmentation, considered to be the result of apoptosis, was measured. Activity of ODC was measured by a radioisotope method, the proteolytic enzyme activity was measured following electrophoresis on polyacrylamide gels in which either casein or gelatine had been incorporated and apoptosis was measured by ELISA as 180-200 bp fragments of DNA labelled with BrdU released into the cytoplasm. TPA 0.1 µg/ml induced ODC within the first 1 h, the enzyme activity reaching a maximum of about 2.0-fold the untreated level in 5–6 hours before returning towards control levels after 8-10 hours. Treatment with 1,4-dichlorobenzene, 10 µg/ml, for 5 hours had no effect on ODC activity, while simultaneous treatment with TPA and 1,4-dichlorobenzene for 5 hours, or TPA for 5 hours followed by 1,4-dichlorobenzene for 2 hours had little effect (slightly inhibitory) on that observed with TPA alone. In contrast, treatment with 1,4-dichlorobenzene for 1 hour followed by TPA for 5 hours increased the ODC activity by about 2.6-fold. Similar treatment protocols led to a TPA-induced reduction in proteolytic enzyme activity to about 92% after 5 hours of treatment, a slight increase by 1,4-dichlorobenzene alone (105%) or after simultaneous exposure to TPA and 1,4-dichlorobenzene for 5 hours (109%) and a slightly greater increase after sequential treatment was with 1,4-dichlorobenzene for 1 hour followed by TPA for 5 hours (121%). Apoptosis was inhibited 30% by TPA, 1.0 µg/ml, and inhibited 25% by 1,4-dichlorobenzene, 5.0 µg/ml.

Table 4.16 *In vitro* tests for genetic and related effects in cultured mammalian cells by 1,4-dichlorobenzene

Test system	Source and purity of chemical	Result [a]		Dose [b] (LED/HID)	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
Unscheduled DNA synthesis, HeLa cells (scintillation counting)	not given	—	—	500 µg/ml	Pirovano and Milone (1986a) *
Unscheduled DNA synthesis, human lymphocytes (scintillation counting)	purity 99%	—	—	147 µg/ml	Perocco (1983) *
DNA strand beaks, etc. et alkali labile sites, rat primary hepatocytes cultures (alkaline elution assay)	Aldrich Chimica, Milan, Italy. Purity 99%	—	NT	470 µg/ml	Canonero et al. (1997)
DNA strand beaks, etc. et alkali labile sites, human primary hepatocytes cultures (alkaline elution assay)	Aldrich Chimica, Milan, Italy. Purity 99%	—	NT	470 µg/ml	Canonero et al. (1997)
DNA strand beaks, etc. et alkali labile sites, rat primary kidney cell cultures (Comet assay)	E. Merck, Darmstadt, Germany. Purity 99%	+	NT	470 µg/ml	Robbiano et al. (1999)
DNA strand beaks, etc. et alkali labile sites, human primary kidney cell cultures (Comet assay)	E. Merck, Darmstadt, Germany. Purity 99%	+	NT	470 µg/ml	Robbiano et al. (1999)
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	NTP chemical repository (Radian Corp., Austin TX, USA) Purity > 99%	—	—	150 µg/ml	Galloway et al. (1987) *
Sister chromatid exchange, human lymphocytes *in vitro*	not given	+	NT	0.1 µg/ml	Carbonell et al. (1991) *
Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	NTP chemical repository (Radian Corp., Austin TX, USA) Purity > 99%	—	—	150 µg/ml	Galloway et al. (1987) *
Chromosomal aberrations, Chinese hamster lung CHL cells *in vitro*	not given	—	NT	5 µg/ml	Ishidate Jr. (1988)

Table4.16 continued overleaf

Table 4.16 continued *In vitro* tests for genetic and related effects in cultured mammalian cells by 1,4-dichlorobenzene

Test system	Source and purity of chemical	Result [a]		Dose [b] (LED/HID)	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, human lymphocytes *in vitro*	source not given. Purity 99.7%	—	NT	100 μg/ml	Pirovano and Milone (1987) *
Micronuclei, rat primary hepatocytes cultures	Aldrich Chimica, Milan, Italy. Purity 99%	(+)	NT	147 μg/ml	Canonero et al. (1997)
Micronuclei, rat primary kidney cell cultures	Aldrich Chimica, Milan, Italy. Purity 99%	+	NT	470 μg/ml	Robbiano et al. (1999)
Micronuclei, human primary hepatocytes cultures	Aldrich Chimica, Milan, Italy. Purity 99%	—	NT	470 μg/ml	Canonero et al. (1997)
Micronuclei, human primary kidney cell cultures	Aldrich Chimica, Milan, Italy. Purity 99%	+	NT	470 μg/ml	Robbiano et al. (1999)
Gene mutation, mouse lymphoma L5178Y cells, *tk* locus	NTP Chemical Repository (Radin Corp., Austin, TX, USA) Purity > 99%	?	?	105 μg/ml	McGregor et al. (1988) *
Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	not given	—	—	200 μg/ml	Pirovano and Milone (1986b) *
Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus	Bayer AG, Wuppertal, Germany. Purity not given	—	—	350 μg/ml	Den Boer and Hoorn (1986a) *
Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus	supplier not given. Purity 99.7%	—	—	250 μg/ml	Loveday (1989) *
Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus	Bayer AG, Wuppertal, Germany. Purity > 98%	?	?	280 μg/ml	Tegethoff et al. (2000)
Cell transformation, mouse BALB/3T3 cells, focus assay	Bayer AG, Wuppertal, Germany. Purity not given	—	NT	140 μg/ml	Den Boer and Hoorn (1986b) *

a)+, positive; (+), weak positive; –, negative; ?, inconclusive; NT, not tested; b)LED, lowest effective dose; HID, highest ineffective dose

* tests already available in the risk assessment report dated 05/2001   Authors underlined : test not performed from validated internationally accepted test system

In vivo studies

Insect studies

In a document described as a draft report, Valencia (1982) Bioassay System Corp. (1982) gave results of a sex-linked recessive lethal study in the fruit fly *Drosophila melanogaster*. Male flies of the wild type stock Canton-S were exposed to 1,4-dichlorobenzene vapour calculated to provide exposures of 0, 6000 and 15,600 ppm/h, (mortality was observed during exposure and predating in a range of 3,4% to 23%) after which they were mated with females of the Basc stock, the X-chromosomes of which carry inversions and are marked with genes for apricot eye (w^a) and Bar eye (*B*). The breeding schedule ensured that only sperm ejaculated 7 days after treatment were tested. F_1 generation females (heterozygous for the treated X- and non treated X-chromosomes) were mated individually with their brothers. The F_2 generation was observed for the presence (non-lethal) or absence (lethal) of wild type flies. The experiment was replicated three times. There was no indication of any mutagenic effect in any of the germ cell stages, despite test numbers being far in excess of the estimated number required to detect an effect.

DNA Adducts and Association

The associations of [^{14}C]-1,4-dichlorobenzene (radiochemical purity 98%) with DNA, RNA and proteins were studied in three experiments with adult male Wistar rats and BALB/c mice (Lattanzi et al., 1989). In the first experiment, nine rats and 35 mice were injected intraperitoneally with 127 μCi (2.95 μmol)/kg bw, killed 22 hours later and their livers, kidneys, lungs and stomachs removed. In the second experiment, 6 rats and 12 mice were treated with phenobarbital, 100 mg/kg bw, by intraperitoneal injection 2 days before injection of the radiolabel. The animals were killed 22 hours later. In the third experiment, 4 rats and 12 mice were killed 72 hours after [^{14}C]-1,4-dichlorobenzene injection. Association values were determined on individual livers from rats and livers of 11–12 mice per pool and on the separately pooled kidneys, lungs and stomach of rats and mice. In the first experiment, there was no association of radiolabel with rat DNA from liver, kidney, lung or stomach, while there was association with rat RNA and protein from all of these organs. Radioactivity was, however, associated with DNA in mouse organs, the measured concentrations being (in pmol/mg tissue): lung, 0.60; liver, 0.14; kidney, 0.09; and stomach, 0.08. Association of radioactivity was also found in all mouse organs with RNA (apart from stomach, in which it was not measured) and proteins. In terms of so-called Covalent Binding Index (CBI), the association with mouse liver DNA gave a value of 14. Data from the other two experiments were not presented, but were reported as indicating that phenobarbital treatment "slightly affected" (up or down not stated) the tissue concentration of 1,4-dichlorobenzene at 22 hours and that tissue-associated radioactivity was much lower at 72 hours than at 22 hours. It is noted that the procedures used did not demonstrate a covalent nature of the associations with DNA.

Tian et al. (2001a) conducted a study in which male Fischer 344/NSIc rats, either untreated or treated with ethanol, phenobarbital or 3-methylcholanthrene in order to induce different types of CYP enzymes, were given single intraperitoneal injections of 1,4-dichlorobenzene in olive oil at doses of 300 or 600 mg/kg bw. After 24 hours, the rats were killed, livers collected and DNA isolated from them. These samples were analysed by the same ^{32}P-post-labelling technique as used in their *in vitro* experiments (see above). No DNA adducts were detected in the livers of rats that were untreated or were treated with either ethanol or phenobarbital, whereas two spots were detected in DNA from 3-methylcholanthrene-treated rats. It was shown that these spots co-chromatographed with two spots detected in rats treated with 3-methylcholanthrene alone, but not with 1,4-dichlorobenzene alone.

The possible formation of oxygen adducts with DNA has been studied in the kidney of male F344 rats (Umemura et al., 2000). The rats were administered 1,4-dichlorobenzene in corn oil by gavage at doses of 0 or 300 mg/kg bw per day, 5 days per week for 13 weeks. Another group of rats was administered potassium bromate (500 ppm) in their drinking water for 13 weeks. At the end of the dosing period, the rats were killed and their kidneys removed, homogenised, centrifuged to collect nuclear fractions from which DNA was extracted by automated methods in helium-filled glass apparatus in the dark. The DNA was digested to deoxynucleotides and 8-oxodeoxyguanosine (OH8dG) content assessed by hplc with electrochemical detection. There was no significant increase in the concentration of OH8dG in renal nuclear DNA from 1,4-dichlorobenzene-treated rats (0.69 ± 0.21 OH8dG adducts/10^5 dG) compared with corn oil-treated controls (0.56 ± 0.17 OH8dG adducted/10^5 dG), whereas there was a significant increase following potassium bromate treatment (1.19 ± 0.27, $p < 0.01$).

Three healthy volunteers (sex not stated) were exposed to 1,4-dichlorobenzene vapour at concentrations of 2.4–2.8 ppm for 1 hour. Blood samples were obtained (1) before exposure, (b) immediately at the end of exposure and (c) 1 hour after exposure. The separated serum was then incubated with calf thymus DNA for 1 hour, after which DNA was isolated and analysed by the ^{32}P-post-labelling technique with nuclease P1 enhancement. No differences were found in adduct profiles before and after exposure (Tian et al., 2001b).

Mammalian studies

Using an *in vivo* version of the alkaline single cell gel electrophoresis (Comet) assay, Sasaki et al. (1997) studied the DNA damaging effect of 1,4-dichlorobenzene in male CD-1 mice. A group of 6 mice was treated intraperitoneally with 1,4-dichlorobenzene at a dose of 2,000 mg/kg bw and then 2 of these mice were killed at each of 3 sampling times: 0, 3 and 24 hours after dosing. Five organs (liver, lung, kidney, spleen and bone marrow) were taken, gently homogenised and centrifuged to collect nuclei, which were subjected to the Comet assay. Results were expressed as DNA migration, this being defined as the difference between length and diameter of the DNA spread. Liver and, to a lesser extent, spleen DNA damage was observed at 3 hours, but not at 0 or 24 hours. No significant damage was observed in kidney, lung or bone marrow.

Robbiano et al. (1999) performed an *in vivo* Comet assay on kidney cells of dosed male Sprague-Dawley rats. Rats were subjected to unilateral nephrectomy and injected intravenously 24 hours later with folic acid, 250 mg/kg bw, in order to stimulate kidney cell proliferation. These rats were used in the Comet assay. Eight rats served as a common control group (1,4-dichlorobenzene was only one of a number of compounds tested). 1,4-Dichlorobenzene in corn oil was administered orally by gavage to two groups of 3 rats, one receiving a single dose of 250 mg/kg bw 2 days after the folic acid injection, the other receiving 3 successive daily doses of 167 mg/kg bw beginning immediately after folic acid injection. In all cases the rats were killed 4 days after folic acid treatment. Kidney cells were isolated as described above (Mammalian cell studies). Means and standard deviations of the tail lengths measured (in microns) on 100 cells per rat were 3.2 ± 10.8, 9.1 ± 14.7 and 8.0 ± 11.3 for the control, $1 \cdot 250$ and $3 \cdot 167$ mg/kg bw groups, respectively. Each treatment group value was statistically significantly greater than the control ($p < 0.05$, Dunnett's t-test). The proportionally very large standard deviations are noted.

The result of the last study was re-examined using a more standard method (Brendler-Schwaab, 2002) in which groups of 4 Hsd:Sprague-Dawley rats were treated orally by gavage with 1,4-dichlorobenzene in corn oil. Two experiments were performed, one with males and the other with females. In the first experiment, doses of 0, 1,000 or 2,000 mg/kg bw were administered and then the rats killed 24 hours later. In the second experiment, doses of 0, 2,000 and 2,000 mg/kg bw were administered and then the vehicle control and one of the 2,000 mg/kg bw

groups were killed 16 hours later, while the other was killed 24 hours later. Intact kidney cells were prepared from all rats by *in situ* perfusion with EDTA followed by collagenase and then subjected to electrophoresis and the assessment of DNA damage. Both male and female rats dosed with 1,4-dichlorobenzene showed signs of toxicity, but no compound related cytotoxicity was observed in isolated kidney cells, as judged by trypan blue exclusion. The data were presented as means and standard deviations of the Comet tail lengths measured (from the middle of the head to end of the tail, in microns) on 100 cells per rat. A dose dependent mean increase in tail length of 25% above the vehicle control group was considered as a biologically significant increase, while increases of ≤ 15% were considered as not different from the control. Increases of 15–25% were considered on a case-by-case basis and the exercise of scientific judgment. The group means and standard deviations of the tail length measurements for the male rats were 22.89 ± 1.3, 24.25 ± 1.9 and 27.34 ± 5.8, for the 0, 1,000 and 2,000 mg/kg bw groups, respectively. The increase in tail length in the high dose group was 19.4% of the control value and was considered to be an equivocal result. The corresponding data from the second experiment, with female rats, were 22.38 ± 1.7, 31.25 ± 3.1 and 21.30 ± 4.2, for the 0, 2,000 (16 hours) and 2,000 (24 hours) mg/kg bw groups, respectively. The increase in tail length at 16 hours was 39.6% of the control and was therefore considered to be biologically significant. There was no increase in tail length at 24 hours.

The difference in characteristics of the data in these two reports [i.e., the small means and large standard deviations in Robbiano et al. (1999) and the large means and small standard deviations in Brendler-Schwaab (2002)] is noted.

In a study of unscheduled DNA synthesis (UDS) and S-phase DNA synthesis (SPS) in liver, groups of (usually) 6 of each sex B6C3F$_1$ mice or F344 rats were administered 1,4-dichlorobenzene in corn oil by gavage at dose levels of 0, 300, 600 or 1,000 mg/kg bw (Sherman et al., 1998). For UDS assessment, three per species, sex and group were killed 16 hours after dosing, while for SPS assessment three mice per sex and group were killed 48 hours after dosing and three rats per sex and group were killed 96 hours after dosing. The livers of mice were perfused *in situ* with collagenase and the kidneys of rats were minced in collagenase-trypsin to obtain isolated cells for examination. In the UDS study, all doses resulted in < 0 net grain counts/nucleus in mouse liver and in rat kidney, thereby indicating an absence of 1,4-dichlorobenzene induced UDS in either tissue. In contrast, SPS was induced in male and female mouse liver and in male, but not in female rat kidney. The proportions of hepatocytes in S-phase were 0.24, 0.46, 1.90 and 1.52% in male mice and 0.29, 2.61. 1.18 and 4.45% in female mice of the 0, 300, 600 and 1,000 mg/kg bw dose groups, respectively. The proportions of rat kidney cells in S-phase were 0.38, 0.87, 0.67 and 1.01% in male rats and 0.52, 0.48, 0.43 and 0.32% in female rats of the 0, 300, 600 and 1,000 mg/kg bw dose groups, respectively.

This result with the SPS assay confirms in part the earlier observations made by Umemura et al. (1992). [see also other studies of the hepatoxicity of 1,4-dichlorobenzene described in Section 4.1.2.1.3].

The induction of structural chromosomal aberrations was studied in the bone-marrow cells of male Alderley Park rat's whole body exposed to 1,4-dichlorobenzene vapour (Anderson and Richardson, 1976c). Atmospheres were generated by passing metered volumes of dry, clean air through liquid 1,4-dichlorobenzene contained within a thermostatically controlled water bath. The required concentrations within the exposure chambers were produced by altering the temperature of the water bath and the flow rate and by diluting the vapour with air. Concentrations in the exposure chambers were monitored with an infra-red spectrophotometer (Walks Miran 1A). Three experiments were performed: (1) a single 2 hours exposure to concentrations of 0 (4 rats), 299 (3 rats) and 682 ppm (3 rats); (2) a multiple exposure of 5 hours

per day for 5 consecutive days to concentrations of 0, 75 and 500 ppm (2 rats per group) and (3) a multiple exposure of 5 hours per day, 5 days per week for 3 months to concentrations of 0, 75 and 500 ppm (2 rats per group). Toxicity and cytotoxicity were not reported. Inhalation positive controls were, in experiment 1, benzene at 10, 750 and 7,500 ppm and, in experiments 2 and 3, vinyl chloride at 1,500 ppm. In each of the experiments, the rats were killed 22 hours after the end of exposure. Bone marrow cell preparations were made and either 50 (experiment 1) or 100 (experiments 2 and 3) cells per rat examined for aberrations. Statistical analysis was made on the data, which included gaps, the most common phenomenon recorded. In none of the three experiments was there any statistically significant difference between the control and the 1,4-dichlorobenzene exposed groups. In experiment 1, benzene induced a dose related and statistically significant response over all concentrations. Vinyl chloride induced a statistically significant response in experiment 2, but not in experiment 3. The small numbers of rats used in the experiments are noted, but combination of the data from experiments 2 and 3 (there was homogeneity amongst the controls and the same exposure concentrations were used) did not alter the conclusion that there was no statistically significant effect of 1,4-dichlorobenzene. In addition, vinyl chloride exposure significantly increased the percentage of aberrant cells.

There have been at least six studies of micronucleus induction *in vivo*. In a bone marrow cell micronucleus test, groups of 5 male NMRI mice were treated with 1,4-dichlorobenzene in corn oil by intraperitoneal injection at doses of 0, 94, 188, 266 and 355 mg/kg bw twice, 24 hours apart (Mohtashamipur et al., 1987). The high dose was selected on the basis of an acute intraperitoneal LD_{50} value of 2,000 mg/kg bw in this strain of mouse. Mice were killed 6 hours after the second injection and polychromatic erythrocytes in bone marrow smears examined for micronuclei. The frequencies of micronucleated polychromatic erythrocytes were in the first experiment not increased (with no sign of toxicity, but with cytotoxicity) and in the second experiment increased in a dose dependant manner 1.80, 4.00, 4.90, 6.00 and 6.60 per 1,000 cells in the control and four dose groups, respectively. This result demonstrates that there is a statistically significant induction of micronuclei by 1,4-dichlorobenzene.

Groups of 5 male and 5 female Bor:NMRI (SPF Han) mice were treated once orally by gavage with 1,4-dichlorobenzene in corn oil at doses of 0 or 2,500 mg/kg bw (three groups) in a study conducted according OECD GLP guidelines (Herbold, 1986; Tegethoff et al., 2000). The vehicle control and one of the treatment groups were killed 24 hours after dosing, while the remaining two 1,4-dichlorobenzene exposed groups were killed at 48 hours and 72 hours after dosing. Polychromatic erythrocytes in bone marrow smears were examined for micronuclei. The treated mice showed persistent signs of 1,4-dichlorobenzene toxicity, but all mice survived until the end of the experiment. The ratios of normochromatic erythrocytes per 1,000 polychromatic erythrocytes (males and females combined) were 928, 1,058, 1,604 and 1,921 in the vehicle control and treated groups at 24, 48 and 72 hours, respectively. The reduction in the proportion of maturing erythrocytes in the bone marrow was statistically significant at 72 hours and can be taken as evidence for haematopoietic cell line toxicity and, consequently, evidence for the presence of 1,4-dichlorobenzene and/or its metabolites in the target tissue of the study. The frequencies of micronucleated polychromatic erythrocytes (male and females combined) were 1.2/1,000 cells in the vehicle control group and 1.6, 1.6 and 1.2/1,000 cells in the treated groups at 24, 48 and 72 hours, respectively. Cyclophosphamide, 20 mg/kg bw, significantly increased the frequency of micronucleated polychromatic erythrocytes to 10.0/1,000 at 24 hours after dosing. The results demonstrate that toxic doses of 1,4-dichlorobenzene delivered orally do not increase the proportion of micronucleated cells in bone marrow.

Groups of 5 male and 5 female NMRI mice were treated by intraperitoneal injection with 1,4-dichlorobenzene in corn oil[5] at doses of 0, 355 and 710 mg/kg bw divided into two equal doses 24 hours apart (Herbold, 1988; Tegethoff et al., 2000). This study was clearly a re-investigation of the results obtained by Mohtashamipur et al. (1987) described above. The mice were killed 6 hoursafter the second injection and polychromatic erythrocytes in bone marrow smears examined for micronuclei. The ratios of normochromatic erythrocytes per 1,000 polychromatic erythrocytes (males and females combined) were 1,072, 1,184 and 1,307 in the vehicle control and the two treatment dose groups, respectively. The reduction in the proportion of maturing erythrocytes in the top dose group was statistically significant and can be taken as evidence for haematopoietic cell line toxicity and for the presence of 1,4-dichlorobenzene and/or its metabolites in the target tissue of the study. The frequencies of micronucleated polychromatic erythrocytes (male and female combined) were 1.6, 1.3 and 2.1/1,000 cells in the vehicle control and two treatment dose groups, respectively. This result demonstrated that toxic doses of 1,4-dichlorobenzene delivered by intraperitoneal injection do not increase the proportion of micronucleated cells in bone marrow.

1,4-Dichlorobenzene was one of the chemicals included in the 6th collaborative study by CSGMT/JEMS.MMS (Morita et al., 1997). Two experiments were conducted with 1,4-dichlorobenzene in the same laboratory, one using intraperitoneal injection and one using oral, gavage administration. A preliminary study found that the acute LD_{50} value (dose route unclear) to be 2,150 mg/kg bw. In the first experiment groups of 5 male CD-1 (ICR) mice were administered 1,4-dichlorobenzene by intraperitoneal injection at dose levels of 0, 400, 800 and 1,600 mg/kg bw on two separate occasions 24 hours apart and then killed after 24, 48 and 72 hours. Peripheral blood was sampled and analysed for micronucleated erythrocytes. The proportions of micronucleated erythrocytes per 1,000 cells scored in the four groups, respectively, were: at 24 hours, 1.6, 1.6, 1.4 and 1.8; at 48 hours, 1.6, 1.0, 1.2 and 1.2; and at 72 hours, 1.4, 2.6, 1.4 and 1.2. In the second experiment, groups of 5 male CD-1 (ICR) mice were administered 1,4-dichlorobenznene orally by gavage at dose levels of 0, 500, 1,000 and 2,000 mg/kg bw on two occasions 24 hoursapart and then killed 24, 48 and 72 hourslater. Peripheral blood was sampled and analysed for micronucleated erythrocytes. The proportions micronucleated erythrocytes per 1,000 cells scored in the four groups, respectively, were: at 24 hours, 1.6, 2.2, 1.6 and 0.8; at 48 hours, 1.6, 1.8, 3.0 and 0.8; and, at 72 h, 1.4, 1.8, 2.2 and 0.0. The experiments demonstrate a lack of potential for 1,4-dichlorobenzene to increase the proportion of micronucleated cells in peripheral blood of mice dosed either intraperitoneally or orally up to doses that were a substantial fraction of the acute LD_{50} value.

In conjunction with a carcinogenicity study, NTP (1987) exposed male and female $B6C3F_1$ mice 1,4-dichlorobenzene doses of up to 1,800 mg/kg bw for 13 weeks and then examined circulating erythrocytes for micronuclei. There was no effect of treatment of the frequency of micronucleated cells.

Robbiano et al. (1999) studied the frequency of micronucleated kidney cells prepared from the same male Sprague-Dawley rats as those treated and described above (see *in vivo* Comet assay). The mean and standard deviations for the frequencies of micronucleated cells per 1,000 scored per rat were: 0.73 ± 0.49, 3.01 ± 0.45 and 3.52 ± 0.70 for the control, 1·250 and 3·167 mg/kg bw groups, respectively. Each treament group value was statistically significantly greater than the control ($p < 0.001$, Baily method for comparison of percentages).

In a male mouse dominant lethal effects study, groups of 16 male CD-1 mice that had been fertility tested were whole body exposed to 1,4-dichlorobenzene vapours at concentrations of 75,

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[5] In a table in the Methods section, "p.o." is indicated, but the text and all other tables indicate "intraperitoneal."

225 and 450 ppm for 6 hours per day for 5 consecutive days (dose causing systemic toxicity) (Anderson and Hodge, 1976b). An air control group consisted of 35 male mice. Atmospheres were generated by passing metered volumes of dry, clean air through liquid 1,4-dichlorobenzene contained within a thermostatically controlled water bath. The required concentrations within the exposure chambers were produced by altering the temperature of the water bath and the flow rate and by diluting the vapour with air. Concentrations in the exposure chambers were monitored with an infra-red spectrophotometer (Walks Miran 1A). The male mice were 10–12 weeks old after exposure, when they were caged individually with pairs of untreated females 9–10 weeks old. After 5 days, the females were removed and replaced after an additional 2 days by new pairs of virgin females. This weekly mating routine was continued for 8 weeks. Each batch of female mice was killed 15 days after introducing them to the males and examined for pregnancies. There were no dosed related reductions in either the percentage of male mice mating successfully or the percentage and numbers of females becoming pregnant. There were no differences in the mean total number of implants per pregnant female, except in mating week 8 when the values in the control and increasing dose groups, respectively, were: 12.4, 11.5 ($p < 0.05$), 12.2 and 10.8 ($p < 0.01$, Dunnetts t-test). It is not clear that this was an effect of treatment. One of the positive controls, ethyl methanesulphonate, 150 mg/kg bw orally per day for 5 consecutive days, normally produces its effects in the very early mating weeks (usually weeks 1 and 2), yet there was also a significantly lower mean total number of implants per pregnant female in week 8 of this study (10.4, $p < 0.05$). The data principally indicative of a dominant lethal effect are: (1) the percentage of pregnant females with at least one early death; (2) the mean number of early deaths per pregnancy and (3) the mean percentage of early deaths per total implantations per pregnancy. For none of these parameters was there a dose related significant effect of treatment. 1,4-Dichlorobenzene did not, therefore, induce a dominant lethal effect in male mice.

Although it is not certain that sperm abnormalities arise from genetic lesions, the one study of this type is described here. Murthy et al. (1985) treated young male Sprague-Dawley rats, 3 per group, by intraperitoneal injection with either chloroform (0.5 ml per rat) or 1,4-dichlorobenzene, 800 mg/kg bw, in 0.5 ml chloroform. Ten days later the rats were killed and at least 1,000 epididymal sperm were examined from each rat. The average percentages of sperm with abnormal heads in the vehicle and 1,4-dichlorobenzene treated groups, respectively, were: 1.72 ± 0.77 and 11.02 ± 1.14 ($p < 0.001$). The corresponding percentages of sperm with abnormal tails were: 2.92 ± 0.88 and 8.26 ± 2.35 ($p < 0.05$).

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Table 4.17 *In vivo* tests in mammals for the genotoxicity of 1,4-dichlorobenzene

Test system	Source and purity of chemical	Result[a]	Dose[b] (LED/HID)	Reference
DNA adducts, male Wistar rat liver, kidney, lung and stomach ([^{14}C]-1,4-dichlorobenzene)	Radiochemical Centre, Amersham, U.K. Sp. Act. 43 mCi/mmol.; radiochemical purity 98%	—	127 μCi (2.95 μmol)/kg bw · 1, i.p.	Lattanzi et al. (1989)*
DNA adducts, male BALB/c mouse liver, kidney, lung and stomach ([^{14}C]-1,4-dichlorobenzene)	Radiochemical Centre, Amersham, U.K. Sp. Act. 43 mCi/mmol.; radiochemical purity 98%	+	127 μCi (2.95 μmol)/kg bw · 1, i.p.	Lattanzi et al. (1989)*
DNA adducts male F344/NSIc rat liver (^{32}P-post-labelling)	Not given	—	600 mg/kg bw · 1, i.p.	Tian et al. (2001a)
DNA oxygen adduct (8-oxodeoxyguanosine), male F344 rat kidney.	Wako Chemical Co., Osaka, Japan. Purity not given	—	300 mg/kg bw/day, 5 days/wk, 13 wks, p.o.	Umemura et al. (2000)
DNA adducts human serum (3 human volunteers) (^{32}P-post-labelling)	Not given	—	2,4-2,8 ppm, 1 hour, inhalation	Tian (2001b)
DNA strand beaks, etc. et alkali labile sites, male CD-1 mouse liver, lung, kidney, spleen and bone marrow (Comet assay *in vivo*)	Wako Pure Chemical Industries Ltd., Osaka, Japan. Purity not given	+ (liver > spleen) — (lung, kidney, bone marrow)	2,000 mg/kg bw · 1, ip.	Sasaki et al. (1997)
DNA strand beaks, etc. et alkali labile sites, male Sprague-Dawley rat kidney (Comet assay *in vivo*)	E. Merck, Darmstadt, Germany. Purity 99%	(+)	250 mg/kg bw · 1, p.o.; or 167 mg/kg bw · 3 p.o.	Robbiano et al., 1999
DNA strand beaks, etc. et alkali labile sites, male and female Sprague-Dawley rat kidney (Comet assay *in vivo*)	Bayer AG, Wuppertal, Germany. Purity 99.9%	(+) (females) ? (males)	2,000 mg/kg bw x 1 p.o. (16 h) 2,000 mg/kg bw x 1 p.o. (24 h)	Brendler-Schwaab (2002)

* tests already available in the risk assessment report dated 05/2001   Authors underlined : test not performed from validated internationally accepted test system

Table 4.17 continued overleaf

Table 4.17 continued *In vivo* tests in mammals for the genotoxicity of 1,4-dichlorobenzene

Test system	Source and purity of chemical	Result[a]	Dose[b] (LED/HID)	Reference
UDS in male and female B6C3F$_1$ mouse liver	Standard Chlorine of Delaware, Inc., DE, USA. Purity 99.5%	—	1,000 mg/kg bw · 1, p.o.	Sherman et al. (1998)
UDS in male and female F344 rat kidney *in vivo*	Standard Chlorine of Delaware, Inc., DE, USA. Purity 99.5%	—	1,000 mg/kg bw · 1, p.o.	Sherman et al. (1998)
Chromosomal aberrations, Alderley Park rat bone marrow *in vivo*	ICI Ltd., Runcorn, Cheshire, U.K. Purity not given	—	682 ppm, 2 h; or 500 ppm, 5 h/day, 5days or 5 d/wk, 13 wks; inhalation	Anderson and Richardson (1976c)*
Micronucleus test, male NMRI mouse bone-marrow cells *in vivo*	E. Merck, Darmstadt, Germany. Purity 99.0% toxicity + ; ; cytotoxicity ?	+	355 mg/kg bw · 2, i.p.	Mohtashamipur et al. (1987)*
Micronucleus test, male and female Bor:NMRI (SPF Han) bone-marrow cells *in vivo*	Bayer AG, Wuppertal, Germany. Purity 99,9% toxicity + ; cytotoxicity +	—	2500 mg/kg bw · 1, p.o.	Herbold (1986); Tegethoff et al., 2000*
Micronucleus test, male and female NMRI bone-marrow cells *in vivo*	Bayer AG, Wuppertal, Germany. Purity not given	—	355 mg/kg bw · 2, i.p..	Herbold, 1988; Tegethoff et al. (2000)*
Micronucleus test, male and female B6C3F$_1$ mouse peripheral blood cells *in vivo*	toxicity + ; cytotoxicity ?	—	1,800 mg/kg bw, 13 weeks, p.o.	NTP (1987)*
Micronucleus test, male CD-1 (ICR) mouse peripheral blood cells *in vivo*	Not given toxicity – toxicity -	—	2,000 mg/kg bw · 2, p.o.; or 1,600 mg/kg bw · 2, i.p.	Morita et al. (1997)*
Micronucleus test, male Sprague-Dawley rat kidney *in vivo*	E. Merck, Darmstadt, Germany. Purity 99%	+	250 mg/kg bw · 1, p.o.; or 167 mg/kg bw · 3 p.o.	Robbiano et al., 1999
Dominant lethal test, male CD-1 mice	ICI Ltd., Runcorn, Cheshire, U.K. Purity not given toxicity +	—	75, 225, 450 ppm, 6 h/day, 5 days, inhalation	Anderson and Hodge, (1976b)*
Sex linked recessive lethal mutation assay, male Drosophila melanogaster, germ cells	toxicity +	—	0, 6,000, 15,600 ppm/h, inhalation	Valencia et al. (1982)*

[a] +, positive; (+), weak positive; –, negative; ?, inconclusive; NT, not tested; [b] LED, lowest effective dose; HID, highest ineffective dose

2,5-Dichlorohydroquinone

Incubation of native DNA with 2,5-dichlorohydroquinone in the presence of copper caused single and double strand breaks and DNA base alterations, which included the formation of OH8dG. The damage was enhance in the presence of NADH (reduction) and completely inhibited in the presence of catalase (suggesting a role hydrogen peroxide) (Oikawa and Kawanishi, 1996a).

Summary

The possible genotoxicity affect of 1,4-dichlorobenzene has been thoroughly investigated in several different short-term tests. Most of the studies have been performed according to GLP principles and are comparable to guideline studies but some of them, particularly the new one are not.

In vitro studies

Association of [^{14}C]-1,4-dichlorobenzene with calf thymus DNA was detected *in vitro* after incubation with various subcellular fractions of liver and lung, but not of kidney, from rats and mice. No attempts were made to isolate and identify adducts. Furthermore, no DNA adducts were detected by ^{32}P-post-labelling in calf thymus DNA after incubation with liver microsomes from rat, mouse or man.

1,4-Dichlorobenzene was not mutagenic to *S. typhimurium* strains TA100, TA1535, TA1537, TA1538 or TA98 with and without metabolic activation in two well conducted studies. Other studies, not so well conducted or reported, support this conclusion. 1,4-Dichlorobenzene induced mitotic gene conversion and reverse mutation in the D7 strain of *Saccharomyces cerevisiae* in the presence of a metabolic activation system from liver of induced mice. It was reported to induce reverse mutations of a methionine-requiring auxotroph of *Aspergillus nidulans* in the absence of any exogenous metabolic activation system. 1,4-Dichlorobenzene did not induce DNA fragmentation in primary cultures of either rat or human hepatocytes in one study, but in another study, using the Comet assay, DNA damage was induced in primary cultures of both rat and human kidney cells.

It did not induce sister chromatid exchange in Chinese hamster ovary cells either in the absence or in the presence of rat liver S9, but it increased the frequency of sister chromatid exchange in human peripheral blood lymphocytes to a modest, significant extent in the absence of any exogenous metabolic activation. Chromosomal aberrations were not induced in three *in vitro* studies that used Chinese hamster cell lines and human lymphocytes. However, the frequencies of micronucleated cells were significantly increased in primary cultures of rat hepatocytes and of rat and human kidney cells, but not in primary cultures of human hepatocytes.

1,4-Dichlorobenzene did not induce mutations in three independent studies of the *hprt* locus in CHO or V79 cells, while two other independent studies, one of the *hprt* locus of CHO cells and one of the *tk* locus of mouse lymphoma cells, gave inconclusive results. The frequency of transformation was not increased in treated BALB/3T3 cells.

In vivo studies

After intraperitoneal injection, association was observed of [^{14}C]-1,4-dichlorobenzene with DNA from liver, kidney, lung or stomach of mice, but not of rats. A ^{32}P-post-labelling technique also failed to detect adducts in DNA of liver of rats variously treated to induce CYP enzymes before

intraperitoneal injection of 1,4-dichlorobenzene. Furthermore, oxygen adducts with DNA were not increased in the kidneys of orally dosed rats. Thus, there is no evidence for association or adducts with DNA in rats, whereas there is some evidence for the association (adducts not having been isolated) of 1,4-dichlorobenzene with DNA in mice.

Significant increases in the frequencies of alkali-labile DNA lesions were detected by means of the comet assay in two studies of kidney cells from orally dosed rats and in liver and spleen, but not in kidney, lung or bone marrow, of mice intraperitoneally dosed with 1,4-dichlorobenzene. However, unscheduled DNA synthesis was not induced in either male or female rat kidney cells or mouse hepatocytes after single oral doses. S-Phase DNA synthesis was increased in male and female mouse liver and in male, but not female rat kidney.

There was no increase in the frequency of chromosomal abnormalities in bone-marrow cells of rats after single or multiple inhalation exposures to 1,4-dichlorobenzene.

A dose-dependent increase in the frequency of micronucleated polychromatic erythrocytes was detected in the bone marrow of male mice after intraperitoneal injection of two equal doses of 1,4-dichlorobenzene, but no response was found in other studies in mice of each sex after either intraperitoneal or oral dosing; moreover, no significant increase in the frequency of micronucleated peripheral blood reticulocytes was seen in male mice given oral or intraperitoneal doses or in mice of each sex given up to toxic oral doses for 13 weeks. While most evidence does not support an effect of 1,4-dichlorobenzene on the frequency of micronucleated cells in maturing erythrocytes, one additional study did find an increase in the frequency of micronucleated kidney cells from intraperitoneally dosed rats.

1,4-Dichlorobenzene did not induce dominant lethal mutations at any maturation stage of the eight-week spermatogenic cycle of mice.

Overall, the lack of evidence from bacteria, the weak evidence from mammalian cells *in vitro* and the mixed *in vivo* data do not provide a coherent view of the genotoxicity of 1,4-dichlorobenzene. In the light of these data it is difficult to conclude that 1,4-dichlorobenzene is DNA-reactive. The so-called standard tests for genotoxicity do not suggest that 1,4-dichlorobenzene has any such genotoxic potential; the evidence pointing in this direction comes from non-standard tests that may not be fully recognised by regulatory authorities. Nevertheless, it is believed that these test results should not be ignored. The overall weight of evidence from the most reliable studies indicates that it does not have any significant genotoxic potential.

According to the criteria for classification and labelling of dangerous substances (Annex IV to Commission Directive 93/21/EEC of 27 April 1993 adapting to technical progress for the 18th time Council Directive 67/548/EEC), the no classification for mutagenicity was agreed at the CMR meeting in May 2003 considering that the substance has no any significant mutagenic potential.

4.1.2.8   **Carcinogenicity**

4.1.2.8.1   **Studies in animals**

See Annex 2

<u>Oral exposure</u>

Two well-conducted studies using the oral administration of 1,4-dichlorobenzene on rats and mice, performed over the course of two years, revealed hepatic tumours in mice of both sexes, and tubular cell kidney adenocarcinoma in male rats (US NTP, 1987).

A two-year study on F344/N rats at doses of 0, 150 and 300 mg/kg/day by gavage for male rats, and 0, 300 and 600 mg/kg/day for female rats revealed general toxicity beginning at 300 mg/kg/day in male rats, and at 600 mg/kg/day in female rats. A dose-dependent increase in the frequency of nephropathy was observed in the female rats (21/49, 32/50, 41/49) from 300 mg/kg/day and in males from 150 mg/kg/day. This increase was accompanied by renal histological lesions (epithelial hyperplasia of the renal pelvis, mineralisation of the collecting tubules). A dose-dependent increase in the incidence of tubular cell adenocarcinomas (statistically significant at 300 mg/kg/day) was observed in male rats (1/50, 3/50, 7/50). The historical control incidence of the laboratory was 0.4%. No liver tumours were observed but slight hepatotoxicity was observed (transient proliferation and liver enlargement) at 600 mg/kg/day. A parathyroid gland hyperplasia was also found in male rats: this was probably a consequence of renal damage. A marginally increased level of mononuclear cell leukaemia (5/50, 7/50, 11/50) was observed in male rats (this number falls within interval of laboratory control group and was not statistically significant): its toxicological significance is thus limited. No increase in the number of malignant tumours was observed in females.

In B6C3F1 mice at dose levels of 0, 300 and 600 mg/kg/day by gavage, it was shown that there was an increase, for both sexes, in the number of non-neoplastic liver lesions (hyperplasia, degeneration and individual hepatocellular necrosis), and in the number of renal lesions (nephropathy, regeneration of renal tubules) from 300 mg/kg/day. At 600 mg/kg/day, the incidence of hepatocellular carcinomas (statistically significant p < 0.001) was higher in males (14/50, 11/49, 32/50) and in females (5/50, 5/48, 19/50). The incidence of malignant liver tumours in female control mice in this study (10%) was higher than in historical controls (3%). Hepatic adenomas observed in males (5/50, 13/49, 16/50) and females (10/50, 6/48, 21/50) were statistically significant at 600 mg/kg/day. Hepatoblastomas (not statistically significant) were observed in male mice suffering from hepatocarcinomas at 600 mg/kg/day (4/50 total number of male mice, that is 4/32 male mice with hepatocarcinomas), tumours which occur only exceptionally in mice (1/2080). Adrenal gland pheochromocytomas (0/47, 2/48, 4/49), not statistically significant, appeared in male mice, one of which at 300 mg/kg/day was malignant (figure within the historical interval for control groups of the laboratory: 2.2 ± 3.1%); they were associated with adrenal gland and thyroid hyperplasia.

These two studies clearly demonstrate that 1,4-dichlorobenzene has a carcinogenic effect in B6C3F1 mice with hepatocellular carcinomas from 600 mg/kg/day in both sexes, and in F344/N male rats with renal tubular cell adenocarcinomas from 150 mg/kg/day.

Inhalation exposure

Three inhalation studies are available. One of sufficient duration (mice and rats, 104 weeks) (JBRC 1995) and two of short duration (rats, 76 weeks; mice, 56 weeks) (Loeser 1983, Riley 1980b).

Two inhalation studies were carried out on rats and on mice (short duration). Neither revealed any evidence for a carcinogenic effect of 1,4-dichlorobenzene.

The only significant abnormalities observed in Wistar rats in a GLP study, (at doses of 0, 75, and 500 ppm, vapour, 5 hours/day, 5 days/week, over the course of 76 weeks on 76-79 rats/sex/dose) were slight increased liver weight with hepatocyte hyperplasia at 500 ppm in both sexes, and at 75 ppm in females at 26 weeks (not at 76 weeks) for increased liver weight and at recovery (not at 76 weeks) for hyperplasia; increased kidney and liver weight and increased urinary proteins and urinary coproporphyrins at 500 ppm in both sexes. No hyaline droplet nephropathy was noted in male rats. Furthermore, there were no, or at least no toxicologically significant, malignant lesions regardless of the dose considered. The NOAEC for non carcinogenic effects was found to be 75 ppm (Loeser, 1983; Riley, 1980a).

A study on Swiss mice, at doses of 0, 75, and 500 ppm, vapour, 5 hours/day, 5 days/week, for 56 weeks (short duration because animals caught a respiratory infection) revealed no increase in the incidence of malignant or benign tumours (only one osteosarcoma of the nasal sinuses was recorded at 75 ppm) in female mice (no histological study carried out on male rats). An increase in the incidence of respiratory diseases (pneumonia, macrophage proliferation) was also noted in females from 75 ppm. Therefore these data are very difficult to interpret because of the presence of intercurrent respiratory infections. The NOAEC could therefore not be estimated (Riley, 1980b).

A third inhalation study (GLP) was carried out on F344 rats and BDF1 mice (50 animals/sexe/dose), at 0, 20, 75 and 300 ppm, vapour, 6 hours/day, 5 days/week, for a total of 104 weeks.

In rats, the mortality was the same in treated and control females but was above control in males at 300 ppm (64%) and 75 ppm (42%). The only significant abnormalities observed were non neoplastic lesions in the kidney (at 300 ppm in males) and in the nasal cavity (eosinophilic changes in respiratory epithelium, respiratory metaplasia in nasal cavity gland) at 300 ppm in females. Eosinophilic changes in the olfactory epithelium were observed in treated but also in control animals (in control sacrified: 38/38 in females, 24/33 in males in dose treated sacrified at 300 ppm: 12/18 in males, 36/36 in females; in dose treated sacrified at 75 ppm: 17/29 in males, 36/38 in females); the same tendency was observed in dead animals; but grade was higher in treated at 300 ppm in both sexes and 75 ppm in females than control animals. Except mononuclear leukaemia, not statistically significant (9/50, 14/50, 10/50, 13/50) with historical control data for males between 6 and 22%, no compound related increased incidence of neoplasms occurred in male or female F344 rats.

In BDF1 mice, an increased incidence of hepatocellular carcinomas, statistically significant at 300 ppm (p < 0.01), was observed in males (12/49, 17/49, 16/50, 38/49) and in females (2/50, 4/50, 2/49, 41/50); historical control data in this institute for this strain of mice and for liver tumours are 2-36% in males and 0-4% in females (Katagiri, 1998). Hepatocellular adenomas in females, statistically significant (p < 0.01) at 300 ppm (2/50, 10/50, 6/49, 20/50) were observed: historical control data for female's 2-10%. Liver histiocytosarcomas statistically significant (p < 0.05) at 300 ppm in males (0/49, 3/49, 1/50, 6/49) were noted only in males with hepatocellular carcinomas: historical control data for males between 0 and 6% (Katagiri 1998). Hepatoblastoma

like feature (subtype of hepatocellular carcinomas, within portion of hepatocellular carcinoma with continuity between hepatocellular carcinomas and hepatoblastomas like features) statistically significant at 300 ppm were observed in females (6 out of 41 females with hepatocarcinoma at 300 ppm) and in males (0/12, 2/17, 1/16, 8/38 males with hepatocarcinoma): historical control in BDF1 untreated mice: 6% in males, 0% in females (Yamate 1990). Bronchiolar-alveolar carcinomas, statistically significant (p < 0.05), appeared in females at 300 ppm (4/50), figures at the least upper bound of the historical control data of the laboratory (0-8 %). At 300 ppm, liver toxicity (increased liver enzyme: AST, ALT, LDH, alkaline phosphatases; increased liver weight in both sexes and histological findings: slight local necrosis in both sexes, central hepatocellular hypertrophy in males) was observed. The JBRC report was peer reviewed (JBRC, 1995).

IARC re-examined 1,4-dichlorobenzene in November 1998 and the evaluation «Group 2B (the agent is possibly carcinogenic to humans. The exposure circumstance entails exposures that are possibly carcinogenic to humans)» for this substance; a downgrading to Group 3 was discussed (8 for the Group 3) and the working group finally opted to retain the previous evaluation «Group 2B» (9 for Group 2B).

4.1.2.8.2   Studies in humans

Cases of haematological problems have been described, with no clear cause-effect relationship in both cases: one acute myeloblastic lymphoid leukaemia (occupational exposure during 10 years in cleaning electric material with a mixture of 3 isomers of dichlorobenzene (80% 1,2-dichlorobenzene, 15% 1,4-dichlorobenzene, and 2% 1,3-dichlorobenzene) and one chronic lymphoid leukaemia (domestic exposure in cleaning clothes with 1 to 2 litres/year of the same mixture) (Girard, 1969).

4.1.2.8.3   Summary and discussion of carcinogenicity

Mode of action - discussion

Studies on the carcinogenicity of 1,4-dichlorobenzene have been carried out on F344 and Wistar rats and B6C3F1, BDF1 and Swiss mice by oral administration and/or inhalation exposure.

Two-year oral administration revealed renal tubular cell adenocarcinoma tumours in F344 male rats from 150 mg/kg/day, and hepatocellular carcinomas in B6C3F1 mice from 600 mg/kg/day. In an inhalation carcinogenicity study, hepatocellular carcinomas in BDF1 mice associated with hepatoblastomas and histiocytosarcomas from 300 ppm were observed; no carcinogenic effect was observed in the inhalation experiments on Wistar rats and Swiss mice (short duration of exposure).

The formation of the renal tumours in the rat at concentration beginning from 150 mg/kg/day appears to be species and sex-specific. Hyaline droplet accumulation and tubular nephropathy were observed in male rats, caused by an accumulation of the 1,4-dichlorobenzene/alpha -2u-globulin complexes in the renal lysosomes. This complex, which resists the catabolic action of the lysozymes, can lead to an overload of lysozomes, cellular death and a secondary cellular proliferation that can favour the formation of renal tumours (see mechanism of action in the section 4.1.2.6.1.). The mechanism of these kidney tumours is a male rat specific hyaline droplet nephropathy, which cannot be extrapolated to human and have no relevance to human health. Therefore a NOAEL will not be based on these data.

Hepatocellular adenocarcinomas appear in B6C3F1 mice via oral route (gavage application) at 600 mg/kg/day (highest dose tested) (64% of males, 38% of females) and in BDF1 mice via inhalation exposure at 300 ppm (highest dose tested) (78% of males, 82% of females). The frequency of hepatocellular adenocarcinomas in previous control groups of B6C3F1 mice in the laboratory is of 21.8 ± 7.7% in males and 3.1 ± 2.3% in females; for BDF1 mice, historical control data for liver tumours in this institute are 2-36% in males and 0-4% in females.

In some animals, hepatocarcinomas were associated with hepatoblastomas and/or histiocytosarcomas; hepatoblastomas appear in B6C3F1 mice via oral route at 600 mg/kg/day (4/50 in male; historical control at 1/2,080) and in BDF1 mice via inhalation exposure at 300 ppm (6/41 in female and 8/38 in male) and histiocytosarcomas in BDF1 male mice via inhalation exposure at 300 ppm (6/49; historical control of the institute 0-8% in male). These two types of tumours (hepatoblastoma and histiocytosarcoma) are rare in mice. In the mouse, 600 mg/kg/day is equivalent[6] to an average of 262 ppm via inhalation exposure, concentration between the NOAEC of 75 ppm and the LOAEC of 300 ppm for hepatocarcinoma in the inhalation carcinogenicity study.

To be noted that no hepatocarcinomas were observed in the two carcinogenicity studies in rats (via inhalation and oral exposure).

1,4-DCB is not considered as a genotoxic agent, so others mechanisms of the liver tumours have to be discussed.

Hepatocellular carcinomas (associated in some cases with hepatoblastomas and/or histiocytosarcomas) were observed in B6C3F1 and BDF1 mice (via oral gavage application and inhalation exposures) at doses (600 mg/kg/day and 300 ppm) where hepatotoxicity (cytomegaly, hepatocellular degeneration and individual cell necrosis) was observed.

In contrast, in rats, only slight hepatotoxicity was observed (transient increased liver weight, mild centrilobular hypertrophy) at 600 mg/kg/day (highest dose tested) in a two-year study without liver tumours.

Cellular proliferation produced by 1,4-dichlorobenzene was observed in rats and mice after single (up to 1,800 mg/kg) or repeated oral administrations (up to 600 mg/kg/day) in the absence of elevated liver enzyme or hepatic necrosis, as result of mitogenic stimulation (Umemura et al., 1996; Eldridge et al., 1992). Cellular proliferation was observed in the liver of F344 rats and B6C3F1 mice treated with 1,4-dichlorobenzene at the same dose as in the carcinogenicity study but rats did not develop any cancer of the liver (Umemura et al., 1992; Butterworth, 1992); a threshold effect for cellular proliferation (from 75 mg/kg/day in rats (transient) and 150 mg/kg/day in mice (prolonged)), below which no proliferative response was observed, is suggested (Umemura et al., 1998). Even if a prolonged response is considered to be predictive of carcinogenesis, measurements of hepatocellular proliferation alone are not sufficient to elucidate mechanisms of liver tumour development or to predict liver carcinogenesis (Melnick, 1993). Therefore the relationship between cellular proliferation, hepatotoxicity and liver tumours is not clear.

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[6] Nota: The following formula is used to extrapolate from oral to inhalation route:
   [mg/m^3] X inhalation rate (liter/hour) X duration of exposure (hour) X% absorption via inhalation /weight (kg) X 1,000 = mg/kg/day X% absorption via oral route
   mouse weight = 0.030 kg; duration of exposure = 6 hours/day; absorption via inhalation route is 59%; absorption via oral route is 62% and; inhalation rate between 2.8 liter/hour (Wallace-Hayes, 1994) and 1.8 liter/hour (Dybing et al., 1997), more often 2 liter/hour.

The carcinogenic effect on the mouse liver is probably not the result of a peroxisomal proliferation in view of the negative result of a study on peroxisomal proliferation in CF1 mice liver (Bomhard, 1996).

Another possibility is that the liver carcinogenic effects are related to tumour promotion. However 1,4-dichlorobenzene does not promote hepatic foci formation in a two stage model of carcinogenesis in rats.

The role of the hepatic metabolism of 1,4-dichlorobenzene in the mechanism of carcinogenicity can also be discussed in view of differences between rat, mouse and human.

In vivo, there are some species differences in metabolism between rats and mice, with 2,5-dichlorohydroquinone found in F344 and SD rats, but not in Wistar rats nor in mice.

In vitro, the major metabolites in rat, mouse and human liver microsomes are dichlorophenols (50%), hydroquinone metabolites (10 to 27%) and to a less extent glutathione-epoxide and glutathione-quinone conjugates. Differences in the hepatic microsomal metabolism between rat and mouse (and human) were shown: conversion of 1,4-dichlorobenzene was much higher in B6C3F1 mouse microsomes than in F344, Wistar or SD rat or human microsomes; mice, F344 and human liver microsomes produce more hydroquinones metabolites than Wistar rat. *In vitro*, covalent binding to protein is higher in mouse than rat or human liver microsomes.

The redox active nature of chloro(hydro)quinones and their glutathione conjugates may be implicated in carcinogenesis with formation of reactive oxygen species (inducing oxidative DNA damage) when oxidation of hydroquinones metabolites: *in vitro*, the induction of single and double strand breaks in DNA and DNA base alterations was demonstrated when native DNA was incubated in the presence of 2,5-dichlorohydroquinone and the enhancement in DNA damage was observed in the presence of the intracellular reductant (NADH); the damaging effects on DNA were completely eliminated when catalase, a scavenger of hydrogen peroxide, was present (Oikawa, 1996a). This hypothesis of the role of the oxidation products of hydroquinone (benzoquinone) in the development of liver tumours is not clearly demonstrated by experiments in view of the same percentage of hydroquinones metabolites formed *in vitro* in human and mouse; even if covalent binding to protein, greatly inhibited in mice (but also to a small extent in human) by the addition of ascorbic acid with a concommitant increase in the formation of hydroquinones metabolites (in mouse but also in human), indicates that benzoquinone species (derived from oxidation of hydroquinone metabolites) are involved in the covalent binding. These differences in hepatic metabolism cannot at the moment completely explain the results of the carcinogenicity studies.

The mechanism of induction of the liver tumours in mice is not completely elucidated. NOAEL and NOAEC can be determined for these liver carcinogenic effect (75 ppm and 300 mg/kg/day), doses at which liver tumours observed do not exceed the historical control of the laboratory (BDF1 mice: 2-36% of males, 0-4% of females; B6C3F1: 14-29% of males, 1-5% of females); liver carcinomas were observed at the next dose (highest dose tested) of 300 ppm and 600 mg/kg/day, at a high rate (BDF1 mice inhalation: 78% of males, 82% of females; B6C3F1 oral route: 64% of males, 38% of females).

Given that the exposure reported in human studies was to a mixture of isomers, that the information was of poor quality and that the cause-effect relationship was not very clear, the available human data does not provide any relevant information for risk assessment in humans.

Summary

The carcinogenic potential of 1,4-dichlorobenzene for the liver has been clearly demonstrated in B6C3F1 and BDF1 mice from 600 mg/kg/day and from 300 ppm, with 3 types of tumours: hepatocarcinomas, hepatoblastomas and histiocytosarcomas; the two previous one being very rare tumours in mice. The kidney tumours in male rats have no relevance to humans because the underlying mechanism is male rat specific hyaline droplet nephropathy, which cannot be extrapolated to human.

A NOAEL for carcinogenic liver effects of 300 mg/kg/day via oral route in B6C3F1 mice and a NOAEC of 75 ppm via inhalation route in BDF1 mice can be determinate and for kidney adenocarcinoma a LOAEL of 150 mg/kg/day via oral route in F344 rats. The mechanism of the liver tumours in mice is not clear; as no tumour in excess was observed at 75 ppm and 300 mg/kg/day, a threshold mechanism for carcinogenicity of 1,4-dichlorobenzene has to be considered (see Graphs in Annex 3).

New genotoxicity data (even if 1,4-DCB is not considered as a genotoxic), associated with animal data justify to reconsider the classification for carcinogenicity of 1,4-dichlorobenzene and to propose a classification Carc. Cat 3; this classification was agreed at the CMR meeting in May 2003.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Studies in animals

Effects on fertility

A two-generation study (GLP) was performed on Sprague Dawley rats (28 rats/sex/dose) by inhalation exposure (vapour) at 0, 66, 211, and 538 ppm (6 hours/day, 7 days/week) over the course of 10 weeks before mating, during mating, gestation and lactation (except from 19 gestation day through Day 5 post partum) and gave negative results.

Signs of parental toxicity were observed at the highest dose (538 ppm) in both sexes, before mating, for parents F0 and F1 (1st generation), and during lactation (generation F1). These signs included reduced weight gain (10% less than control), mucosal irritation and (tremors, salivation) symptoms in F0 and F1. Renal histological abnormalities (hyaline droplet nephropathy) and increased kidney weight appeared from 66 ppm in males. Liver abnormalities (increased weight and hepatocellular hypertrophy) were seen at 538 ppm in both sexes. The NOAEL for the female rats (parents F0 and F1) was found to be on the order of 211 ppm, and a LOAEL for the male rats was 66 ppm due to hyaline droplet nephropathy.

Signs of toxicity were observed in the offspring, but only at 538 ppm and included significant weight loss, increased significant perinatal mortality, reduced litter size, reduction in number of live foetuses per litter. Neither macroscopic anomalies of the organs, nor histological anomalies of the ovaries and testes were noted. Developmental effects were not reported. The NOAEL of the offspring was found to be 211 ppm.

Given the renal abnormalities noted in male rats, even at the lowest doses, it was not possible to estimate a NOAEL for males, and therefore not possible to estimate a NOAEL parents/NOAEL descendant ratio (P/D) either. The P/D calculated from female data was one (211/211), which indicates that there was no excessive reproductive risk in the absence of any signs of toxicity in

the parent. A NOAEC of 211 ppm for the two generation study through inhalation in rats is established.(Neeper-Bradley, 1989; Tyl, 1989).

A two-generation study (OECD method) was conducted on Spraque Dawley rats (24 rats/dose/sex) by gavage at 0, 30, 90, 270 mg/kg/day, 7 days/week (OECD 416). F0 males were treated 77 days before mating; F0 females 14 days before mating, during mating, lactation and gestation until postnatal Day 21. Only moribund killed or intercurrent death rats and the infertile females were examined histologically; F1 and F2 pups were examined grossly. Signs of parental toxicity were observed at 270 mg/kg/day including: slight reduced weight (less than 10%) in F1 males and females; increased absolute and relative kidney and liver weights in F0 and F1 males, statistically significant in high dose group (and in the mid group for relative liver weight in F1 males) associated with nephrotoxicity in high dose males only (degenerescence, intersticial nephritis); decreased absolute and relative spleen weights in F0 and F1 males in high dose group; no clinical findings of toxicological significance were noted in parental F0 and F1 at any dose but tables of clinical effects were not available and histological examinations were not systematically done in control and high dose groups.

In both generations, 1,4-dichlorobenzene had no effects on time between beginning of mating and evidence of copulation, time of gestation, fertility index, gestational index, total number of pups at birth, sex ratio, percentage of pups with positive ear reflex, grasping reflex and orientation reaction, absolute and relative weights of testes, epididymides or ovaries.

Statistically significant signs of toxicity were observed in the 270 mg/kg/day group and included in the two generations reduction of live pups at birth, increase in total number of stillborn pups and in number of pups per litter deceased between day 1-4 and 5-21 of lactation, reduction of mean body weight of pups at Day 1, Day 4, Day 7, Day 14 and Day 21, alteration of skin. At 270 mg/kg/day: first day of opening eyes was statistically significantly retarded in F0/F1 generation as were days where 50% and 100% of pups had opening eyes in F1/F2 generation; day where 100% of pups had erection of ears was statistically significantly retarded in F1/F2 generation; percentage of pups per litter with positive draw up test were statistically significantly ($p < 0.01$) reduced in both generations.

At 90 mg/kg/day, statistically significant ($p < 0.05$) reversible reduced mean body weight (mean in the range of the negative control values) only at birth (with a marked weight gain at Day 4, 7, 14 and 21) in F0/F1 generation and statistically significant ($p < 0.05$) increased total number of pups deceased between day 1-4 in F1/F2 generation (and not between Day 4 to 21, not in generation F0/F1, not if number of pups per litter is considered) were observed. Percentage of pups with positive draw up test in F1/F2 generation was statistically significantly ($p < 0.05$) reduced.

The NOAEL for fertility was estimated to be 270 mg/kg/day; the NOAEL of the F0 and F1 parents was found to be 90 mg/kg/day; at 90 mg/kg/day toxicity in pups (increased number of pups deceased between day 1-4 in F1/F2, isolated and reversible reduced mean body weight at birth in F0/F1 but not after) were seen associated with slight behavioural changes in pups from 90 mg/kg/day: the NOAEL for these developmental effects is estimated at 30 mg/kg/day (Bornatowicz, 1994).

A dominant lethal assay on mice using inhalation exposure did not demonstrate any fertility-related abnormalities (Anderson, 1976b).

Developmental toxicity

A teratogenic study on female Alderley-Park rats by inhalation exposure (vapour) (20 females/dose) from the 6th to the 15th day of gestation at doses 75,200, 508 ppm that did not cause any signs of toxicity in the mothers (other than a reduction in the gestation period in 5% of the mothers) yielded negative results. No signs of embryotoxicity (number of corpora lutea, implantation, resorption, number of live foetuses, litter and foetus weight, and sex ratio not significantly different from control group), and no skeletal or soft tissue abnormalities (only one localised eventration of the abdominal wall was observed in 212 foetuses at 75 ppm, 1/203 at 200 ppm) were noted. In all, there were no embryotoxic, nor foetotoxic effects at non-toxic doses in the mothers. The maternal NOAEL is 508 ppm; the NOAEL for teratogenicity is 508 ppm (Hodge, 1977).

A teratogenic study (GLP) on New Zealand rabbits by inhalation exposure (vapour) (30 rabbits/dose) was carried out from the 6th to the 18th day of gestation, with slight signs of toxicity being noted in the mothers at the highest dose of 800 ppm (limited to statistically significant reduced weight gain), and yielded no signs of embryotoxicity. The number of corpora lutea, implantation, number of live foetuses, foetus and litter weight, and the sex ratio were not significantly different from those of the control group. A statistically significant increased number of resorptions was noted only at 300 ppm (within the normal interval of the historical controls of the laboratory), not considered to be indicative of an embryolethal effect. Minor abnormalities were noted at 800 ppm, including the retro-oesophageal subclavian artery (5% of the foetuses (6/119) versus 2% in the laboratory control group), deformation of paws on flexion (5% of the foetuses versus 0% in control group) not considered to be indicative of a teratogenic response. However, the total number of major birth defects, and of skeletal and visceral birth defects as a whole was not significantly different in treated and control groups. In all, there was found to be no teratogenic effects at doses that were slightly toxic for the mothers. The maternal NOAEL is 300 ppm; the NOAEL for teratogenicity is 300 ppm (Hayes, 1982, 1985).

Another teratogenic study (results briefly reported) on female CD rats treated by oral administration from the 6th to the 15th day of gestation at doses of 250, 500, 750 and 1,000 mg/kg, with signs of toxicity in the mothers beginning at 500 mg/kg/day (reduced weight gain). Only minimal decrease in mean foetal weight at 1,000 mg/kg/day was observed (with number of corpora lutea, implantation, resorption, number of live foetuses not significantly different from control groups). The incidence of major skeletal and visceral birth defects in foetus was the same in experimental and control groups. Some skeletal variations were observed, including a dose-dependent increase in the frequency of extra ribs from 500 mg/kg/day: these abnormalities, along with the foetal weight decrease at 1,000 mg/kg/day might be linked to signs of maternal toxicity. Nevertheless, there is no evidence of any embryo toxic or foetotoxic effects in this study. The maternal NOAEL is 250 mg/kg/day; the NOAEL for teratogenicity is 250 mg/kg/day (Giavini, 1986).

A teratogenic study in female SD rats by oral administration at low doses (highest dose 200 mg/kg, no signs of toxicity in the mothers) did not yield any evidence of teratogenic effect. However, only a very brief report was available (Ruddick, 1983).

Three teratogenicity studies on rats and rabbits, carried out by oral and inhalation exposure (with clear maternal toxicity achieved in 1 out of 3, and slight maternal toxicity in 1 out of 3 studies), did not reveal any evidence of teratogenic effects. No developmental effects were reported. Any abnormalities noted in the foetuses were rather minor (weight reduction, minor birth defects).

4.1.2.9.2 Studies in humans

The ingestion of 1,4-dichlorobenzene (1 to 2 blocks equivalent to 5 to 10 grams per week) by a pregnant woman throughout her pregnancy did not lead to any abnormalities in the infant, whereas the mother showed signs of toxicity reversible after cessation of exposure (haemolytic anaemia) (Campbell, 1970).

The available human data do not provide relevant information for risk assessment in humans.

4.1.2.9.3 Summary of toxicity for reproduction

Considering the two-generation study in rats via gavage, developmental toxicity in pups (isolated reduced mean body weight only at birth, reversible after in F0/F1 generation and increased total number of pups deceased between Day 1-4 in F1/F2 generation and not between Day 4 to 21, not in F0/F1 generation, not if number of pups per litter is considered) appears from 90 mg/kg/day associated at the same dose with slight behavioural anomalies (reduced percentage of pups with positive draw up test in F1/F2 generation) (1 out of 4 tests including positive ear erection reflex, positive draw up reflex, opening eyes reflex, grasping reflex and orientation reaction). Toxic effects were seen in parents at 270 mg/kg/day, but histological examinations were not systematically done in control and high dose groups. The NOAEL for these developmental effects is estimated at 30 mg/kg/day. In the two-generation study in rats via inhalation, similar signs of toxicity (weight loss, increased perinatal mortality, reduced litter size, reduction in number of live foetuses per litter) than those observed at the high dose tested via oral route (270 mg/kg/day) were observed in the offspring at 538 ppm where parental toxicity was noted. A NOAEC of 211 ppm for the two-generation study through inhalation in rats is established. For comparison of these two concentrations (538 ppm and 270 mg/kg/day), calcul shows that concentration levels reaching the blood are of the same range.

At the mid dose (90 mg/kg/day and 211 ppm) differences were noted, with slight toxicity in pups by gavage study (and not by inhalation study) without parental toxicity. To evaluate these differences (slight toxicity, reversible for reduction body weight and not observed in the two generations simultaneously), routes of exposure have to be taken into account: oral application by gavage results in higher peak concentration than in inhalation studies with continuous exposure (6 hours/day) and could give more toxic effects in pups.

The other data (one two-generation study in rats via inhalation route, three teratogenicity studies on rats and rabbits via oral and inhalation exposure) did not reveal any evidence of reproductive or teratogenic effects in the absence of parental toxicity.

These data do not justify the classification for reproductive endpoints.

4.1.3   Risk characterisation

4.1.3.1   General aspects

According to the available toxicological data, absorption of 1,4-dichlorobenzene rapidly occurs via digestive and respiratory tract; absorption after inhalation exposure (59% in mice versus 25-33% in rats); is poor compared to oral route (after single dose: 72% in rats and mice; after repeated oral exposure: 62% in rats).

No quantitative data on human absorption are available; so default values of 75% for inhalation and 100% for dermal absorption have been considered in the risk characterisation.

1,4-Dichlorobenzene is of low acute inhalation (LC50 > 5.07 mg/l), oral (LD50 > 2,000 mg/kg) and dermal (LD50 > 2,000 mg/kg) toxicity.

Only slight skin and eye irritation appears in rabbits. According to an old study, workers experienced ocular and nasal irritation from 50 ppm (duration of exposure unknown). Respiratory irritation appears from 160 ppm in human according to another old study (based on concentration ranges with median values), in which peak exposure concentrations cannot be excluded.

1,4-Dichlorobenzene has a low sensitising potential because of doubtful results in animals (a maximisation test difficult to interpret) and only one questionable case reported in human despite the widespread use of this substance for many years in occupational and consumer settings.

There are several assays to assess the repeated-dose toxicity. For the purpose of risk characterisation, the NOAEC of 75 ppm identified in carcinogenicity studies [a 76-week inhalation study in rats (Riley 1980), a 104-week inhalation study in rats and mice (JBRC, 1995)] and confirmed in an old inhalation study in rats (Hollingsworth, 1956), has been chosen as the most relevant value, because inhalation is the main route of exposure for workers and because of long duration of the studies. Slight hepatic abnormalities were observed beyond this level (from 158 ppm).

A lower NOAEL of 10 mg/kg/day was determined in a one-year oral dog study. Although the oral route is not the human route of exposure, this NOAEL has to be considered in the risk characterisation because there is no evidence that dog is a less appropriate model for human than rodents. The high sensitivity of this species will have to be taken into account in the assessment of margin of safety.

A NOAEL via dermal route identified in a 21-day study in rat was higher than 300 mg/kg/day.

With regard to mutagenicity, even if 1,4-dichlorobenzene has been investigated in a large number of in vitro and in vivo tests, data do not provide a coherent view of the genotoxicity of 1,4-dichlorobenzene. The so-called standard tests for genotoxicity do not suggest that 1,4-dichlorobenzene has any such potential; the evidence pointing in this direction comes from non-standard tests that may not be fully recognised by regulatory authorities. The overall weight of evidence from the most reliable studies indicates that it does not have any significant genotoxic potential. According to the EEC criteria for classification and labelling of dangerous substances and following the CMR meeting of May 2003, 1,4 dichlorobenzene does not need to be classified in Category 3 mutagen (R68) and is not considered as a genotoxic agent.

Considering the carcinogenicity potential, 1,4-dichlorobenzene produced renal tubular cell adenocarcinomas in male F344 rats from 150 mg/kg/day in an oral carcinogenicity study; the mechanism of kidney tumours appears to be a male rat specific hyaline droplet nephropathy.

Therefore as these effects are not relevant to human health, NOAEL should not be based on these data.

1,4-Dichlorobenzene produces hepatocellular carcinomas in B6C3F1 mice at the highest dose tested of 600 mg/kg/day in an oral carcinogenicity study via gavage application and in BDF1 mice at the highest concentration tested of 300 ppm in an inhalation study. NOAEL of 300 mg/kg/day and NOAEC of 75 ppm were clearly determined for carcinogenicity in these studies. In some animals, hepatocarcinomas were associated with hepatoblastomas and/or histiocytosarcomas; hepatoblastomas appear in B6C3F1 mice via oral route at 600 mg/kg/day and in BDF1 mice via inhalation exposure at 300 ppm and histiocytosarcomas in BDF1 male mice via inhalation exposure at 300 ppm; these two types of tumours (hepatoblastomas and histiocytosarcomas) are rare in mice. The other inhalation studies are not considered adequate by current standard because of short duration of exposure.

Although interpretation of the mechanism of liver tumours in mice is largely discussed (mice show a very high sensitivity toward hepatotoxic chemicals), the results have to be considered because treated mice had a very high rate of liver tumours by two different routes of exposure (oral and respiratory) (60% and 38% in males and females respectively in the oral carcinogenicity study, 78% and 82% in males and females in the inhalation carcinogenicity study) compared to historical controls and because two of the 3 types of liver tumours (hepatoblastomas and histiocytosarcomas) are very rare in mice.

The carcinogenic effect on the mouse liver is probably not the result of a peroxisomal proliferation in view of the negative results of a study on peroxisomal proliferation in CF1 mice liver.

Chronic alterations of the liver and/or liver cell proliferation might explain part of the mechanism of liver tumours: hepatotoxicity was observed in mice at dose where liver tumours appear, compared to only slight hepatotoxicity in rats at 600 mg/kg/day without liver tumours; hepatocyte cell proliferation has been seen in mice and rats in spite of the lack of hepatotoxicity, as a result of mitogenic stimulation; and rats and mice experienced liver proliferation at the same dose as the carcinogenicity study, but no liver cancer were noted in rats compared with mouse liver cancer. Moreover, if these mechanisms (liver cell proliferation and liver chronic alteration) are plausible, they did not rule out completely such a pathway in human. Therefore the relationship between cellular proliferation, hepatotoxicity and liver tumours is thus not clear.

The role of the hepatic metabolism of 1,4-dichlorobenzene in the mechanism of carcinogenicity can also be discussed in view of differences between rat, mouse and human: these differences in hepatic metabolism cannot at the moment completely explain the results of mice liver carcinogenicity.

To summarise the carcinogenicity end point, the available data in experimental animals (except kidney tumours in male rats) are of concern for human. The carcinogenic potential of 1,4-dichlorobenzene in B6C3F1 and BDF1 mice has been clearly demonstrated, with liver tumours only at the highest dose tested and with a NOAEL for these effects of 300 mg/kg/day via oral route in B6C3F1 mice and a NOAEC of 75 ppm via inhalation route in BDF1 mice. Although a clear mechanism for the mouse carcinogenicity has not been demonstrated, a threshold mechanism for 1,4-dichlorobenzene is proposed considering these results.

1,4-Dichlorobenzene has no adverse effects on fertility in the absence of maternal toxicity (oral and inhalation exposure in rats).

Considering the two-generation study in rats via gavage, developmental toxicity in pups (isolated reduced mean body weight only at birth, reversible after in F0/F1 generation and increased total

number of pups deceased between Day 1-4 in F1/F2 generation and not between Day 4 to 21, not in generation F0/F1, not if number of pups per litter is considered) appears from 90 mg/kg/day associated at the same dose with slight behavioural anomalies (reduced percentage of pups with positive draw up test in F1/F2 generation). Toxic effects were seen in parents at 270 mg/kg/day, but histological examinations were not systematically done in control and high dose parental groups. The NOAEL for these developmental effects is estimated at 30 mg/kg/day. In the two-generation study in rats via inhalation, similar signs of toxicity (weight loss, increased perinatal mortality, reduced litter size, reduction in number of live foetuses per litter) than those observed at the high dose tested via oral route (270 mg/kg/day) were observed in the offspring at 538 ppm, concentration where parental toxicity was noted. A NOAEC of 211 ppm for the two generation study through inhalation in rats is established.

On the other hand, three teratogenic studies (oral and inhalation exposure in rats, inhalation exposure in rabbits) did not reveal any evidence of teratogenic effects.

The risk characterisation for developmental toxicity will be carried out with the NOAEL of 30 mg/kg/day determined in an oral two-generation study in rats. However the assessment of margins of safety will have to take into account the nature of the effects in pups (slight toxicity, reversible and not observed in the two generations simultaneously) and that in this study, the route of administration (gavage which is not the human route of exposure) results in higher peak concentration than in inhalation studies with continuous exposure during 6 hours and could give more toxic effects in pups.

4.1.3.2   Workers

4.1.3.2.1   Introductory remarks

Systemic availability for different routes of exposure

For the majority of toxicological endpoints, 1,4-DCB data originate mainly from inhalation and oral studies. Since workers are exposed mainly by inhalation but possibly by skin contact, route to route transformation is essential for workers risk assessment.

1,4-dichlorobenzene is assumed to be rapidly absorbed via gastrointestinal tract (after single dose: about 72% in rats and mice; after repeated exposure: 62% in rats) and via respiratory tract; (59% in mice versus 30% in rats); no data are reported for absorption by dermal route.

No quantitative data on human absorption are available; so a default value of 75% has been considered for respiratory absorption in human and 100% for dermal absorption in the risk characterisation.

For risk assessment purposes the following assumptions of systemic availability are taken forward for the calculation of MOS:

Systemic availability after inhalation intake: ca.30% in rats;
                                                      59% in mice (experimental data)
                                                      ca. 75% in human (default value)

Systemic availability oral intake: ca. 62% in rodents (experimental data)
                                                      ca. 100% in dogs (default value)

Systemic availability after dermal intake: ca. 100% (default value)

Occupational exposure and internal body burden

For the purpose of risk characterisation, it is assumed that inhalation of vapour emitted by the solid or from molten material is the main route of exposure. Oral exposure would not be anticipated to be a route of exposure under normal working practices. Dermal exposure is considered limited either when 1,4-dichlorobenzene is supplied in flake form or when it is used as a hot molten material. Compared to the inhalation route, dermal exposure is expected to be low. For risk characterisation purposes, we will consider two scenarios for workers.

Scenario 1: *manufacture and use as synthesis intermediate (see Section 4.1.1.2.)*

Inhalation exposure data provided by industry during manufacture are consistent with the EASE exposure estimation and it seems reasonable to take the higher value provided by industry of 7 ppm (42 mg/m^3) (8-hour TWA) as a worst-case scenario.

Dermal exposure is estimated to be 80 mg/day (EASE model).

During industrial use as a synthesis intermediate (in a closed system), few exposure data are available. However, exposure is likely to be low and in the same order as during manufacture.

Assuming that a 70 kg worker breathes in 10 m^3 of air in an 8-hour working day and a 75% absorption across the lungs (default value in human), an internal body burden of 4.5 mg/kg/day is estimated from inhalation exposure.

Assuming a worker weighs 70 kg and 100% dermal absorption, it results in an internal body burden of 1.1 mg/kg/day from dermal exposure.

Overall, combining inhalation and dermal route results in a combined internal body burden of 5.6 mg/kg/day.

Scenario 2: *formulation of moth repellents, toilet blocks, air fresheners and use in the production of grinding wheels (see Section 4.1.1.2.)*

For inhalation exposure, it is proposed to take the higher value provided by the EASE model estimation, i.e. 50 ppm (300 mg/m^3) (8-hour TWA), as the worst-case scenario which appears consistent with most of the measured data.

Dermal exposure is estimated to be 80 mg/day (EASE model).

Assuming that a 70 kg worker breathes in 10 m^3 of air in an 8-hour working day and 75% absorption across the lungs (default value), an internal body burden of 32.1 mg/kg/day is estimated.

Assuming a worker weighs 70 kg and 100% dermal absorption, it results in an internal body burden of 1.1 mg/kg/day from dermal exposure.

Overall, combining inhalation and dermal route results in a combined internal body burden of 33.2 mg/kg/day.

Calculation of MOS values

MOS values are calculated as quotient of experimental NOAEL from animal and human studies and workplace exposure levels. If the route of application in animal or human studies is different from the actual occupational exposure, the choice of MOS value has to be adapted (considering scaling factor or not).

The exposure routes considered in occupational risk assessment are inhalation and dermal contact. The MOS values for exposure by each route are considered separately. The MOS value is calculated as quotient of the external NOAEL and the total internal body burden, with correction by the percentage of absorption when necessary.

Evaluation of MOS values (ECETOC, 2003, technical report n° 86).

Risk assessment based on MOS values implies the identification of a minimal MOS decision mark between conclusion (ii) and (iii). Scientifically based adjustment factors are used for the extrapolation of animal data to the worker population (e.g.: interspecies extrapolation, intraspecies extrapolation, extrapolation LOAEL to NOAEL, duration adjustment). Type of the effects and confidence of the database are weighted by expert judgment.

If the MOS value for a certain exposure scenario is below the minimal MOS, the corresponding risk situation is considered to be of concern. A MOS value higher than the minimal MOS indicates no concern.

Interspecies differences: a default value of 3 is used for animal intraspecies variability; for interspecies extrapolation of oral data, metabolic rate scaling results in lower effective dose levels in mg per kg bodyweight for humans compared to experimental animals. The scaling factors depends on (bodyweight)$^{0.75}$., e.g. for rats a factor of 4, for mice of 7 and for dogs of 2 will be used.

- Intraspecies variability: a default value of 3 is recommended for workers population (homogen population).

- Extrapolation LOAEL to NOAEL: a default value of 2 is used.

- Duration adjustment: since studies with suitable experimental design are available for 1.4-DCB, there is no need for a specific duration adjustment step in extrapolation.

4.1.3.2.2   Occupational risk assessment

Acute toxicity

Acute toxicity is of no concern, because the oral and dermal LD50 and the LC50 are much higher than the estimated daily exposure: **conclusion (ii)**.

Irritation

Considering the exposure data and the lowest LOAEC for ocular and nasal irritation in human, the following MOSs can be calculated, as presented in **Table 4.18**:

Table 4.18 Risk characterisation for ocular and nasal irritation

Scenario	Airborne exposure(ppm)	LOAEC(ppm)	MOS	Conclusion
1	7	50	7.1	ii
2	50	50	1	iii

A minimal MOS of 6 (1 for interspecies human data, 3 for intraspecies, 1 for type of effect, 2 for extrapolation LOAEC to NOAEC, 1 for confidence data base) is required.

Margin of safety of 1 is considered as insufficient for human health protection and irritation does lead to concern for scenario 2. Therefore further risk reduction measures are necessary during use (formulation and production of grinding wheels):**conclusion (iii)**.

Sensitisation

Sensitisation needs not to be discussed because of the low sensitising potential of 1,4-dichlorobenzene: **conclusion (ii)**.

Repeated-dose toxicity

The rat and mice inhalation studies are preferred over the dog feeding study, as the route of exposure in the rodent studies is the same as the exposed workers; moreover, when using the dog feeding study, there is a need for route to route extrapolation that increases the uncertainty.

Considering the exposure data and the NOAEC of 75 ppm determined in rats and mice (104 weeks, 76 weeks or 5 months) the following MOSs can be calculated:

Table 4.19 Risk characterisation for repeated dose toxicity (inhalation exposure)

Scenario	External exposure (inhalation) ppm	NOAEC inhalation (rat, mice) ppm	MOS	Internal body burden (inhalation) mg/kg/day	NOAEL oral (dog) mg/kg/day	MOS	Conclusion
1	7	75	10.7	4.5	10	2.1	iii
2	50	75	1.5	32.1	10	0.31	iii

Starting with an inhalation study in rat and mice, a minimal MOS of 9 (3 for interspecies and 3 for intraspecies) is required.

The MOS values indicate a concern for inhalation with respect to repeated dose toxicity study especially for Scenario 2. For Scenario 1, MOS of 10.7 is obtained and is closed to the minimal MOS of 9 (from rat data). If correction for the species differences in rate of absorption (75% in human, 30% in rats and 59% in mice), the calculated MOSs for inhalation rat study values are 4.28 ($10.7 \cdot 30/75$) for Scenario 1 and 0.6 ($1.5 \cdot 30/75$) for Scenario 2. For inhalation mice study, the calculated MOSs values are 8.41 ($10.7 \cdot 59/75$) for Scenario 1 and 1.18 ($1.5 \cdot 59/75$) for Scenario 2.

Margins of safety (MOSs) of 8.41, 4.28, 1.18 and 0.6 are considered insufficient for worker health protection compared to the minimal MOS required.

The same conclusion can be made with MOSs of 2.2 and 0.31 determined from the oral dog study.

With regard to dermal route, no repeated dose toxicity data are available. Therefore the oral NOAEL will be used. Considering the exposure data and the NOAEL of 10 mg/kg/day determined in dogs (gavage, one year), the following MOSs can be calculated, as presented in **Table 4.20**.

Table 4.20 Risk characterisation for repeated dose toxicity (dermal exposure)

Scenario	Dermal exposure (mg/day)	Internal body burden (dermal) mg/kg/day	NOAEL oral (dog) mg/kg/day	MOS	Conclusion
1	80	1.1	10	9	iii
2	80	1.1	10	9	iii

Starting with an oral dog study, a minimal MOS of 18 ($3 \cdot 2$ for interspecies and 3 for intraspecies) is required.

Assuming equal rate of absorption values for both routes (100% (worst-case assumption) in human via dermal route and 100% (default value) in dogs via oral route), margin of safety (MOS) of 9 is considered insufficient for worker health protection compared to the minimal MOS required.

Further risk reduction measures during manufacture and use (intermediate, formulation and production of grinding wheels) considering inhalation and dermal exposures are necessary. **Conclusion (iii)** for Scenario 1 and 2.

Mutagenicity

With regards to mutagenicity, 1,4-DCB has not any significant such potential and this point needs not to be discussed. **Conclusion (ii)** for Scenario 1 and 2.

Carcinogenicity

The carcinogenic potential of 1,4-dichlorobenzene has been demonstrated in mice which are of very high sensitivity towards hepatotoxic chemicals but the mechanism by which these hepatic tumours form, has not been clearly identified a threshold mechanism for carcinogenicity of 1,4-dichlorobenzene is proposed in view of the liver tumours from the highest doses tested (oral and inhalation route in two species of mice).

For carcinogenicity, a NOAEC of 75 ppm following inhalation exposure was obtained in mice and a NOAEL of 300 mg/kg/day was identified in mice following oral administration. Since route of worker exposure is mainly by inhalation, the NOAEC of 75 ppm in mice is preferred.

Considering the exposure data, the NOAEC of 75 ppm in mice and the NOAEL of 300 mg/kg/day in mice (liver tumours, 104 weeks), the following MOS's can be calculated, as presented in **Table 4.21**.

Table 4.21 Risk characterisation for carcinogenicity (inhalation exposure)

Scenario	External exposure (inhalation) ppm	NOAEC inhalation (mice) ppm	MOS	Internal body burden (inhalation) mg/kg/day	NOAEL oral (mice) mg/kg/day	MOS	Conclusion
1	7	75	10.7	4.5	300	66.7	iii
2	50	75	1.5	32.1	300	9.3	iii

Starting with an inhalation study in mice, a minimal MOS of 45 (3 for interspecies, 3 for intraspecies and 5 for severity of the effects (expert judgment)) is required.

Margins of safety (MOSs) of 10.7 and 1.5 are considered insufficient for worker health protection compared to the minimal MOS required; to be noted that taking into consideration the species differences in rate of absorption (75% in human and 59% in mice via inhalation route) would have lowered the MOSs.

Starting with an oral study in mice, a minimal MOS of 315 ($3 \cdot 7$ for interspecies, 3 for intraspecies and 5 for severity of the effects (expert judgment)) is required.

Margins of safety (MOSs) of 66.7 and 9.3 are considered insufficient for worker health protection.

To be noted that taking into consideration the species differences in rate of absorption (62% in rodent's oral route) would have lowered the MOSs.

With regard to dermal route, no carcinogenicity data are available. Therefore the oral NOAEL of 300 mg/kg/day in mice (104 weeks) will be used and the following MOSs, as presented in **Table 4.22** can be calculated

Table 4.22 Risk characterisation for carcinogenicity (dermal exposure)

Scenario	Dermal exposure (mg/day)	Internal body burden (dermal) mg/kg/day	NOAEL oral (mice) g/kg/day	MOS	Conclusion
1	80	1.1	300	272	iii
2	80	1.1	300	272	iii

Starting with an oral study in mice, a minimal MOS of 315 ($3 \cdot 7$ for interspecies, 3 for intraspecies and 5 for severity of the effects (expert judgment)) is required.

After correction for the species differences in rate of absorption (100% in human for dermal route, 62% in rodents via oral route), the calculated MOSs for inhalation rat study values are 168 ($272 \cdot 0.62$) for Scenario 1 and 2.

Margin of safety (MOS) of 168 is considered insufficient for worker health protection compared to the minimal MOS required.

Further risk reduction measures during manufacture and use (intermediates, formulation and production of grinding wheels) considering inhalation and dermal exposures are necessary. **Conclusion (iii)** for Scenario 1 and 2.

Developmental toxicity

For developmental toxicity, the NOAEL of 30 mg/kg/day in rat following oral administration is preferred to the NOAEL of 211 ppm in rat following inhalation exposure because developmental effects were observed in the oral study (even if it is not the main route of exposure for workers).

Considering the exposure data and the NOAEL of 30 mg/kg/day in rats (two generations study), the following MOS's can be calculated:

Table 4.23 Risk characterisation for developmental toxicity (inhalation exposure)

Scenario	External exposure (inhalation) ppm	Internal body burden (inhalation) mg/kg/day	NOAELoral (rat, primary effects) mg/kg/day	MOS	Conclusion
1	7	4.5	30	6.6	iii
2	50	32.1	30	0.9	iii

Starting with an oral study in rats, a minimal MOS of 180 (3 · 4 for interspecies, 3 for intraspecies and 5 for severity of the effects (expert judgment)) is required.

Margins of safety (MOSs) of 6.6 and 0.9 are considered insufficient for worker health protection compared to the minimal MOS required; to be noted that taking into consideration the species differences in rate of absorption (62% in rats oral route) would have lowered the MOSs.

Considering the exposure data and the NOAEC of 211 ppm in rats (two generations study), the following MOS's, as presented in **Table 4.24** can be calculated.

Table 4.24 Risk characterisation for developmental toxicity (inhalation exposure)

Scenario	External exposure (inhalation) ppm	NOAEC inhalation (rat) ppm	MOS	Conclusion
1	7	211	30	(iii)
2	50	211	4.22	(iii)

Starting with an inhalation study in rats (not considered as the more appropriate study even if it is the same route of exposure as human), a minimal MOS of 45 (3 for interspecies, 3 for intraspecies and 5 for severity of the effects (expert judgment)) is required.

Margins of safety (MOSs) of 30 and 4.22 are considered insufficient for worker health protection compared to the minimal MOS required; to be noted that taking into consideration the species differences in rate of absorption (75% in human, 30% in rats: inhalation route) would have lowered the MOSs.

With regard to dermal route, no developmental toxicity data are available. Therefore the oral NOAEL of 30 mg/kg/day in rats (two generations study) will be used and the following MOSs, as presented in **Table 4.25** can be calculated.

Table 4.25 Risk characterisation for developmental toxicity (dermal exposure)

Scenario	Dermal exposure (mg/day)	Internal body burden (dermal) mg/kg/day	NOAELOral (rats) mg/kg/day	MOS	Conclusion
1	80	1.1	30	27	iii
2	80	1.1	30	27	iii

Starting with an oral study in rats, a minimal MOS of 180 (3·4 for interspecies, 3 for intraspecies and 5 for severity of the effects (expert judgment)) is required.

Margin of safety (MOSs) of 27 is considered insufficient for worker health protection compared to the minimal MOS required.

Taking into consideration the species differences in rate of absorption (100% in human dermal route, 62% in rat's oral route) would have lowered the MOSs.

Further risk reduction measures during manufacture and use (intermediate, formulation and production of grinding wheels) considering inhalation and dermal exposures are necessary. **Conclusion (iii)** for Scenario 1 and 2**.**

Summary of risk characterisation for workers

Conclusion (iii)   There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Taking into account the currently available toxicological data and the estimated occupational exposure, this is reached for the following endpoints because of:

- nasal and ocular irritation due to vapour exposure during use for formulation of products containing the substance and production of grinding wheels

- general systemic toxicity, carcinogenicity and reproductive toxicity due to exposure mainly via inhalation and dermal, during manufacture and use (intermediate, formulation of products containing the substance and production of grinding wheels).

4.1.3.3   Consumers

For the risk assessment of acute effects, the inhaled concentration taken in account corresponds to an exposure level for a single event. The maximum concentration measured in indoor air is considered, that means 23,800 μg/m^3.

For the risk assessment of chronic effects, the inhaled concentration used corresponds to an exposure level averaged over 24 hours.

Based on measurement data showing a wide dispersion of exposures to 1,4-dichlorobenzene in the population (with high values for a few people), a realistic worst-case of daily continuous exposure of 850 μg /m^3 has been defined and bordered by a interval of 600 to 1,150 μg/m^3.

This exposure is equivalent to a body burden of 0.179 mg/kg/day [0.126-0.242] assuming a ventilation rate of 0.7 m^3/hour, a 60 kg person and a relative absorption by inhalation compared to ingestion of 75% (datum used by default).

Because of the lack of data, it has not been able to assess the level of exposure due to the wearing of clothes stored with mothballs. But, according to toxicity studies via dermal route, dermal absorption seems to be negligible.

Acute toxicity

Sub-lethal effects at acute exposure levels are mainly irritative effects. Surveys conducted with workers for 8 hours per day, 5 days per week show a NOAEL equal to 300 mg/m^3 for irritation. Comparing to the acute exposure level of consumers (23.8 mg/m^3), a margin of safety of 13 is obtained. Because of the worst-case character of the exposure estimate, the margin of security is sufficient.

Moreover, it has been observed that for rats the 4-hour LC50 is higher than 845 ppm (5,078 mg/m^3) with signs of pulmonary irritation, piloerection and reversible weight gain losses. In other words, the LOAEL is inferior or equal to this value. Using this datum, a margin of safety of 213 can be calculated. Considering an uncertainty factor of 5 for intraspecies variability, an uncertainty factor of 3 for interspecies variability and a factor 10 for using a LOAEL instead of a NOAEL, this margin of security is judged sufficient: **conclusion (ii)**.

Irritation

According to human data, irritation of the eye and upper airways may occur when air concentration of 1,4-dichlorobenzene exceeds 50 ppm (300 mg/m^3). When compared to the highest measured consumer exposure (23.8 mg/m^3), a margin of safety of 13 is obtained. As no complaint has been recorded below 50 ppm, as the exposure levels used to calculate the margin of security corresponds to the maximum air concentration ever reported in a room, in consumer exposure conditions, this endpoint does not lead to concern: **conclusion (ii).**

Sensitisation

1,4-dichlorobenzene has a low sensitising potential (maximisation tests for animals difficult to interpret, only one questionable case reported in human despite the widespread use of this substance for many years in occupational and consumer settings): **conclusion (ii).**

Repeated dose toxicity

The NOAEL of 75 ppm, 6 hours per day, 5 days per week via inhalation, in rats and mice is equivalent to 13 ppm or 80 mg /m^3, for continuous exposure. Compared to 0.85 [0.60-1.15] mg/m^3, it gives a margin of safety of 95 [70-134].

As the minimum margin of safety corresponds to the average between the maximal personal exposures measured during one day and during one night, as such maximum levels must be higher than the average exposure for a long-term (several years) and continuous exposure, **conclusion (ii)** is drawn.

Mutagenicity

Needs not be discussed.
Conclusion (ii).

Carcinogenicity

The carcinogenic potential of 1,4-dichlorobenzene has been demonstrated in mice which are of very high sensitivity towards hepatotoxic chemicals but the mechanism by which these hepatic

tumours form, has not been clearly identified A threshold mechanism for carcinogenicity of 1,4-dichlorobenzene is proposed in view of the liver tumours from the highest doses tested (oral and inhalation route in two species of mice).

The NOAEL of 75 ppm, 6 hours per day, 5 days per week via inhalation, in rats and mice is equivalent to 13 ppm or 80 mg /m^3, for continuous exposure. Compared to 0.85 [0.60-1.15] mg/m^3, it gives a margin of safety of 95 [70-134].

Considering the severity of the effects, the margin of safety is insufficient and the **conclusion (iii)** is drawn.

Toxicity for reproduction

For developmental toxicity, the NOAEL was 211 ppm , in rats, by inhalation. Considering the animals have been exposed 6 hours per day, 7 days per week, the equivalent NOAEL for a continuous exposure by inhalation is equivalent to 317 mg/m^3. Comparing the exposure of 0.85 [0.60-1.15] mg/m^3, it gives a margin of safety of 373 [276-528].

Considering the NOAEL by ingestion (30 mg/kg/j), the margin of safety would be equal to 168 [119-227]. Gavage results in higher peaks of internal concentration than inhalation studies with continuous exposure and could give more toxic effects in animals.

Moreover, no teratogenic effects were observed and abnormalities reported in foetuses were always minor (weight reduction, minor birth defects).

So, the endpoint is of no concern: **conclusion (ii)**.

Summary of risk characterisation for consumers

Conclusion (iii)   There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Taking into account the currently available toxicological data and the estimated consumer exposure, conclusion (iii) is reached because of carcinogenicity.

4.1.3.4 Humans exposed via the environment

General systemic repeated-dose toxicity, and carcinogenicity and the developmental toxicity are the critical end points for man exposed indirectly via the environment. Comparison of the NOAELs of 10 mg/kg/day (general systemic repeated-dose toxicity, 300 mg/kg/day carcinogenicity) and 30 mg/kg/day (developmental toxicity) with the highest estimated exposure of 0.0109 mg/kg/day leads to margins of safety of 917, 27,522 and 2,750 which do not lead to concern: **conclusion (ii)**.

4.1.3.5 Combined exposure

In the case of combined exposure the highest potential uptake is likely to occur during occupational exposure. Consumer exposure and indirect exposure via the environment can be considered negligible. The same conclusion as for Section 4.1.3.2 is achieved: **conclusion (ii)**.

4.2   HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1   Exposure assessment

1,4-Dichlorobenzene is a moderately volatile solid with a vapour pressure of 1.6 hPa-1.7 hPa at 20°C (equivalent to a saturated vapour concentration of about 1,500 ppm or 0.15% by volume): it is slowly transformed from solid state to vapour.

4.2.1.1   Occupational exposure

In industrial settings, whether it is during manufacture or use, effective preventive measures are supposed to be taken in accordance with current regulations (e.g. ventilation of areas, regulations regarding electrical equipment).

As in Section 4.1.1.1, it is proposed to take the higher value provided by the EASE model, 50 ppm (300 mg/m^3) (8-hour TWA), as the worst-case scenario for risk characterisation purposes.

4.2.1.2   Consumer exposure

For consumers, the maximum concentrations in air were found in a wardrobe and ranged from 63 to 373 mg/m3 (10 to 60 ppm) with an average of 289 mg/m3 (50 ppm) (See section 4.1.1.2.).

4.2.1.3   Humans exposed via the environment

Exposure is negligible.

4.2.2   Effects assessment: Hazard identification

4.2.2.1   Explosivity

Explosive properties are not expected in view of the chemical structure.

4.2.2.2   Flammability

Taking into account its flash point (65-66°C) and the fact that there is no auto-flammability up to 500°C, 1,4-dichlorobenzene is a moderately flammable substance. Vapour can form explosive mixtures with air within the range of 1.7% to 5.9% by volume (Hoechst AG, 1993).

Concerning the flammability properties of solid 1,4-dichlorobenzene, a test conducted according the method A10 (Annex V of Directive 67/548/EEC) is negative (Brown, 1997).

4.2.2.3   Oxidising potential

Oxidising properties are not expected in view of the chemical structure.

4.2.3 Risk characterisation

4.2.3.1 Workers

The maximum concentration in air at the workplace (about 50 ppm or 0.005% by volume) is much lower than the lower explosive limit (1.7% by volume). There is no reason for concern and **conclusion (ii)** applies.

4.2.3.2 Consumers

The maximum concentration measured in a wardrobe (60 ppm or 0.006% by volume) is much lower than the lower explosive limit (1.7% by volume). There is no reason for concern and **conclusion (ii)** applies.

4.2.3.3 Humans exposed via the environment

Exposure is negligible and **conclusion (ii)** applies.

5 RESULTS

5.1 ENVIRONMENT

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This conclusion is reached for the exposure of the aquatic compartment (including the sediment), the atmosphere, and the terrestrial compartment as well as for predators.

5.2 HUMAN HEALTH

5.2.1 Human health (toxicity)

5.2.1.1 Workers

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Taking into account the current available toxicological data and the estimated occupational exposure, this conclusion of is reached because of:

- nasal and ocular irritation due to vapour exposure during use for formulation of products containing the substance and production of grinding wheels,

- general systemic toxicity, carcinogenicity and reproductive toxicity due to exposure mainly via inhalation and dermal, during manufacture and use (intermediate, formulation of products containing the substance and production of grinding wheels).

5.2.1.2 Consumers

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Taking into account the current available toxicological data and the estimated consumer exposure, this conclusion is reached because of:

- carcinogenicity due to inhalation exposure arising from use of moth repellents, air fresheners and toilet blocks.

5.2.1.3 Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

5.2.2   **Human health (risks from physico-chemical properties)**

Conclusion (ii)   There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
bw	body weight / *Bw, b.w.*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 50 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests

EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient

Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
pKa	negative log of the acid dissociation constant
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration

pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst-case
S phrases	Safety phrases according to Annex III of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document [1]
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material

vB	very Bioaccumulative
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organization
WWTP	Wastewater Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)

Annex 1   Repeated-dose toxicity in animals

Oral exposure

Strain	Doses Number of animal	Duration of exposure	Symptoms	NOAEL (dose without toxic effect) / LOAEL (lowest dose with Toxic effect)	Ref
F344 Rat	Study 1: 300, 600, 900, 1,200, 1,500 mg/kg/day	5 days/week 13 weeks	Study 1: - ≥ 300 in male: dose dependent nephropathy with tubular cell degeneration and necrosis, decrease in Ht and Hb level - ≥ 600 in male: ↑ kidney weight, ↓ cholestérol - ≥ 900 in 2 sexes: ↑ liver weight; in female: ↓ cholestérol - ≥1,200 in male and female: hepatocellular degeneration and necrosis, hypoplasia of the bone marrow, lymphoïd depletion of spleen and thymus, ↑ urinary porphyrins	Study 1: LOAEL = 300 mg/kg/day in male NOAEL = 600 mg/kg/day in female	US-NTP (1987)
	Study 2: 0, 37.5, 75, 150, 300, 600 mg/kg/day 10/sex/dose gavage		Study 2: - 600 in male: kidney cortical degeneration	Study 2: NOAEL: = 300 mg/kg/day in male > 600 mg/kg/day in female	
F344 Rat	0, 75, 150, 300, 600 mg/kg/day 5/sex/dose gavage GLP +	7 days/week 4 weeks	Study on kidney effects: • 4 weeks - ≥ 75 in male: hyalin droplet nephropathy, ↑ urinary LDH, proteins and epithelial cells, ↑ water consumption - ≥ 150 in male: tubular cell nephropathy (necrosis, dilatated tubules) - ≥ 300 in male and female: ↑ liver weight; ↑ kidney weight in male - 600: in female ↑ kidney weight, water consumption, in male hepatocellular hypertrophy	NOAEL on kidney effects: - for 4 weeks: LOAEL = 75 mg/kg/day in male NOAEL = 300 mg/kg/day in female	Bomhard (1987, 1988a, 1988b)
		7 days/ week 13 weeks	• 13 weeks : - ≥ 75 in both sexes ↑ liver weight - ≥ 150 in male ↑ kidney weight, tubular cell nephropathy (necrosis, dilatated tubules) - at 600 in female ↑ kidney weight - ≥ 300 hepatocellular hypertrophy in male	- for 13 weeks: LOAEL = 75 mg/kg/day in male NOAEL = 300 mg/kg/day in female	
F344 Rat	0, 150, 600 mg/kg/day 20/sex/dose gavage	5 days/ week 4 weeks	Study of liver cytochrome P450 dependent enzyme activities: - ≥ 150 in male and female: ↑ dose dependent cyt P450 liver enzyme induction - ≥ 150 in male, 600 both sexes: ↑ liver weight		Bomhard (1992)
Rat	0, 10, 100, 500 mg/kg/day 2 males/dose	5 days/ week 4 weeks	- at 500: oedema and centrolobular necrosis in the liver, renal tubular oedema		Hollingsworth (1956)
Rat	0, 18.8, 188, 376 mg/kg/day 10 females/dose	5 days/week 27 weeks	Brief report - at 188: ↑ slight liver and kidney weights - at 376: cirrhosis and focal necrosis in the liver		Hollingsworth (1956)

| Strain	Doses Number of animal	Duration of exposure	Symptoms	NOAEL (dose without toxic effect) / LOAEL (lowest dose with Toxic effect)	Ref
Rat	0, 50, 100, 200 mg/kg/day 5 females/dose gavage	1 time/day 30, 60, 90, 120 days	Brief report centered on hepatic porphyria: - ≥ 50: slight ↑ liver weight at 30 and 60 days and slight ↑ liver porphyrins at 120 days		Carlson (1977)
F344 Rat	0, 150, 300 mg/kg/day in male 0, 300, 600 mg/kg/day in female 50/sex/dose gavage	two years	- ≥ 150 in male: renal hyperplasia and mineralisation - ≥ 300 in female: nephropathy - 600 in female: transient hepatocellular proliferation, persistent liver enlargement	For non neoplasic effects LOAEL: = 150 mg/kg/day in male = 300 mg/kg/day in female	US-NTP (1987)
B6C3 F1 Mice	Study 1: 0, 85, 169, 337, 675, 900 mg/kg/day 10/sex/dose gavage Study 2: 600, 900, 1,000, 1,500, 1,800 mg/kg/day 10/sex/dose gavage	5 days/week 13 weeks	Study 1: - at 675 in male and female: hepatocellular hypertrophia Study 2: - ≥ 600 in male and female: decrease in body weight gain, hepatocellular degeneration - ≥ 900 in two sexes: ↑ liver weight; ↓ cholesterol - ≥ 600 in male, ≥ 1,000 in female: decrease of leukocytes - at 1,500 in male: ↓ triglycerides - ≥ 1,500: hypoplasia of spleen and bone marrow, lymphoïd depletion of spleen and thymus, lymphoïd necrosis of the thymus	Study 1: NOAEL: = 337 mg/kg/day in male and female Study 2: LOAEL: = 600 mg/kg/day in male and female	US-NTP (1987)
NMRI Mice	0, 300, 600, 900 mg/kg/day 8 to 10/sex/dose gavage	7 days/week 4 weeks	- ≥ 300 in male and female: ↑ liver weight - ≥ 600 in male and female: ↑ SGPT, hepatocellular hypertrophia and degeneration - at 900 in male and female: ↑ bilirubin and cholestérol	LOAEL = 300 mg/kg/day in male and female	Bomhard (1986)
B6C3 F1 Mice	0, 300, 600 mg/kg/day 50/sex/dose gavage	1/day, 5 days/week 2 years	- ≥ 300 mg/kg: slight hepatocellular degeneration, individual liver cell necrosis in both sexes, nephropathy in both sexes, renal tubular cell regeneration in female	For non neoplasic effects: LOAEL = 300 mg/kg/day in male and female	US-NTP (1987)
Beagle dog	0, 10, 50, 75 mg/kg/day 5/sex/dose gavage GLP +	5 days/week via capsule one year	- ≥ 50 mg/kg/day: in both sexes: ↑ liver weight, ↑ alkaline phosphatases (X 7), hepatocellular hypertrophia; in female: ↑ kidney weight, kidney duct vacuolisation - 75 mg/kg/day: bile duct hyperplasia in both sexes, neurological symptoms/reversible mild anemia, in female; ↑ AST and ↑ GGT (X 3)	NOAEL = 10 mg/kg/day	Naylor (1996)
Rabbit	0, 500, 1,000 mg/kg/day 5/dose gavage	5 days/week one year	- ≥ 500: focal hepatocellular oedema and necrosis	LOAEL = 500 mg/kg/day	Hollingsworth (1956)

Inhalation exposure

Strain	Doses Number of animals	Duration of exposure	Symptoms	NOAEL/NOEL	Ref
Rat Guinea pig Mice Rabbit Monkey	96, 158, 173, 341, 798 ppm 10/dose 96, 158, 173, 341, 798 ppm 8/dose 96, 158 ppm 10/dose 96, 158, 173, 798 ppm 1/dose 96, 158 ppm 1/dose	7 hours/day; 5 days/week; 5 to 7 months	- at 158 ppm in guinea pig and rat:↑ liver weight, oedema and minimal hepatocellular degeneration, ↑ kidney and liver weights of male rat - at 173 ppm: lung oedema and lung congestion in all animals, ↑ liver and kidney weights in rat - at 341 ppm in guinea pig: focal necrosis and slight cirrhosis in the liver - at 798 ppm in rat: letality, irritation, neurological symptoms, histological alterations severe in lung, liver and kidney	NOAEL = NOEL rat = 96 ppm NOEL guinea pig = 96 ppm NOEL mice > 158 ppm NOEL rabbit = 158 ppm NOEL monkey = 158 ppm	Hollingsworth *1956)
Wistar Rat	0, 75, 500 ppm (vapour) 76-79/sex/dose GLP +	5 hours/day 5 days/week 76 weeks	- at 75 ppm: ↑ liver weight at 26 weeks (not at 76 weeks) and liver hyperplasia at recovery (not at 76 weeks) in female - at 500 ppm: ↑ liver weight and hepatocyte hyperplasia in both sexes - at 500 ppm in male: ↑ kidney weights, ↑ urinary coproporphyrin and proteins - No hyaline droplet nephropathy in male	For non neoplasic effects NOAEL = 75 ppm	Riley (1980a)
Swiss Mice	0, 75, 500 ppm 75/dose GLP +	57 weeks	increase in respiratory infections in female Limits: high incidence of infections no histopathogical examination in male		Riley (1980b)
BDF1 mice	0, 20, 75, 300 ppm (vapour) 50/sex/dose GLP +	104 weeks 6 hours/day, 5 days/week	- 300 ppm in both sexes: liver toxicity (↑ liver weight ↑ AST, ALT, LDH, alkaline phosphatase, slight local necrosis; in male hepatocellular hypertrophy - 300 ppm both sexes: ↑ kidney weight	For non neoplasic effects NOAEL = 75 ppm	JBRC (1995)
F344 rat	0, 20, 75, 300 ppm (vapour) 50/sex/dose GLP +	104 weeks 6 hours/day, 5 days/week	- 300 ppm in male: mineralisation of papilla, urothelial hyperplasia, ↑ kidney weight - 300 ppm both sexes: ↑ liver weights - 300 ppm in female: respiratory metaplasia in nasal cavity gland and eosinophilic change in respiratory epithelium and olfactory	For non neoplasic effects NOAEL = 75 ppm	JBRC (1995)

# Annex 2 Carcinogenicity data in animals

Oral exposure	Dose	Symptoms
F344/N Rat (NTP 1987)	0, 150, 300 mg/kg/day in male 0, 300, 600 mg/kg/day in female two years (50/sex/dose) gavage	- hyperplasia and mineralisation of kidney tubules in male at level of 150 mg/kg/day - nephropathy in female (21/49, 32/50, 41/49) - tubular cell kidney adenocarcinoma in male (1/50, 3/50, 7/50) (historical control of the laboratory = 0,4%) - parathyroid gland hyperplasia in male (4/42, 13/42, 20/38) - mononuclar leukemia in male (5/50, 7/50, 11/50) (historical control of the laboratory: 13,8 ± 8%) - No tumours in female
B6C3F1 Mice (NTP 1987)	0, 300, 600 mg/kg/day two years (50/sex/dose) gavage	- liver carcinoma in male (14/50, 11/49, 32/50) and in female (5/50, 5/48, 19/50) (historical control of the laboratory = 21.8 ± 7.7 % in male, 3.1 ± 2.3 % in female) - hepatoblastoma in male 4/50 at 600 mg/kg/day (historical controls: 1/2080) - liver adenoma in male (5/50, 13/49, 16/50); in female (10/50, 6/48, 21/50) - malignant pheochromocytoma in male (1/49) at 300 mg/kg/day and in one control female (1/49) (historical control of the laboratory: 2,2 ± 3%) - increased incidence of non neoplasic liver lesions : hepatocellular degeneration, individual liver cell necrosis in both sexes from 300 mg/kg/day
Inhalation exposure	**Dose**	**Symptoms**
Wistar rat (Loeser 1983, Riley 1980a)	0, 75, 500 ppm5 hours/day, 5 days/week, 76 weeks (+ 36 weeks unexposed) (76/sex/dose) GLP +	- increase of liver weight at 26 weeks and hepatocyte hyperplasia at recovery (not at 76 weeks) at 75 ppm in female - increase of liver and kidney weight in both sexes at 500 ppm - increase in urinary proteins and urinary coproporphyrins at 500 ppm - no significant increase of tumours Limits: low level and short duration of exposure
Swiss Mice (Riley 1980b)	0, 75, 500 ppm 5 hours/day, 5 days/week, 57 weeks (+ 19 weeks unexposed) (75 females/dose)	- nasal sinus osteosarcoma at 75 ppm - increase of respiratory infections - no significant increase of tumours Limits: not valid data because of high incidence of respiratory infections
BDF1 Mice (JBRC 1995)	0, 25, 75, 300 ppm 6 hours/day, 5 days/week, 104 weeks (50/sex/dose) vapour GLP +	- hepatocellular carcinoma in male (12/49, 17/49, 16/50, 38/49) and in female (2/50, 4/50, 2/49, 41/50) (historical control of the institute = 0-4% in female, 2-36% in male) - histiocytosarcoma of liver in male (0/49, 3/49, 1/49, 6/49) (historical control of the institute 0-8% in male) - Hepatoblastoma like feature: 300 ppm in female 6/41 and in male 2/17, 1/16 and 8/38 at 25, 75 and 300 ppm - hepatocellular adenoma in female (2/50, 10/50, 6/49, 20/50) - bronchiolar-alveolar carcinoma in female 4/50 at 300 ppm (historical control data of laboratory 0-8%) - 300 ppm in both sexes : liver toxicity (liver weights) AST, ALT, LDH, alkaline phosphatase, slight local necrosis; - 300 ppm in male centrolobular hepatocellular hypertrophy
F344 Rat (JBRC 1995)	0, 25, 75, 300 ppm 6 hours/day, 5 days/week, 104 weeks (50/sex/dose) vapour GLP +	- monocellular leukemia in male (9/50, 14/50, 10/50, 13/50): (historical control data of laboratory 6-22%) non neoplasic lesions: - in the kidney (mineralisation of papilla and urothelial hyperplasia of the pelvis), increase kidney weight at 300 ppm in male - respiratory metaplasia in nasal cavity gland and eosinophilic change in respiratory epithelium at 300 ppm in female) and eosinophilic change in olfactory epithelium in both sexes and 75 ppm in female

Annex 3   Carcinogenic effects in mice via inhalation and oral route

Carcinogenic effects in BDF mice via inhalation route (JBRC 1995)
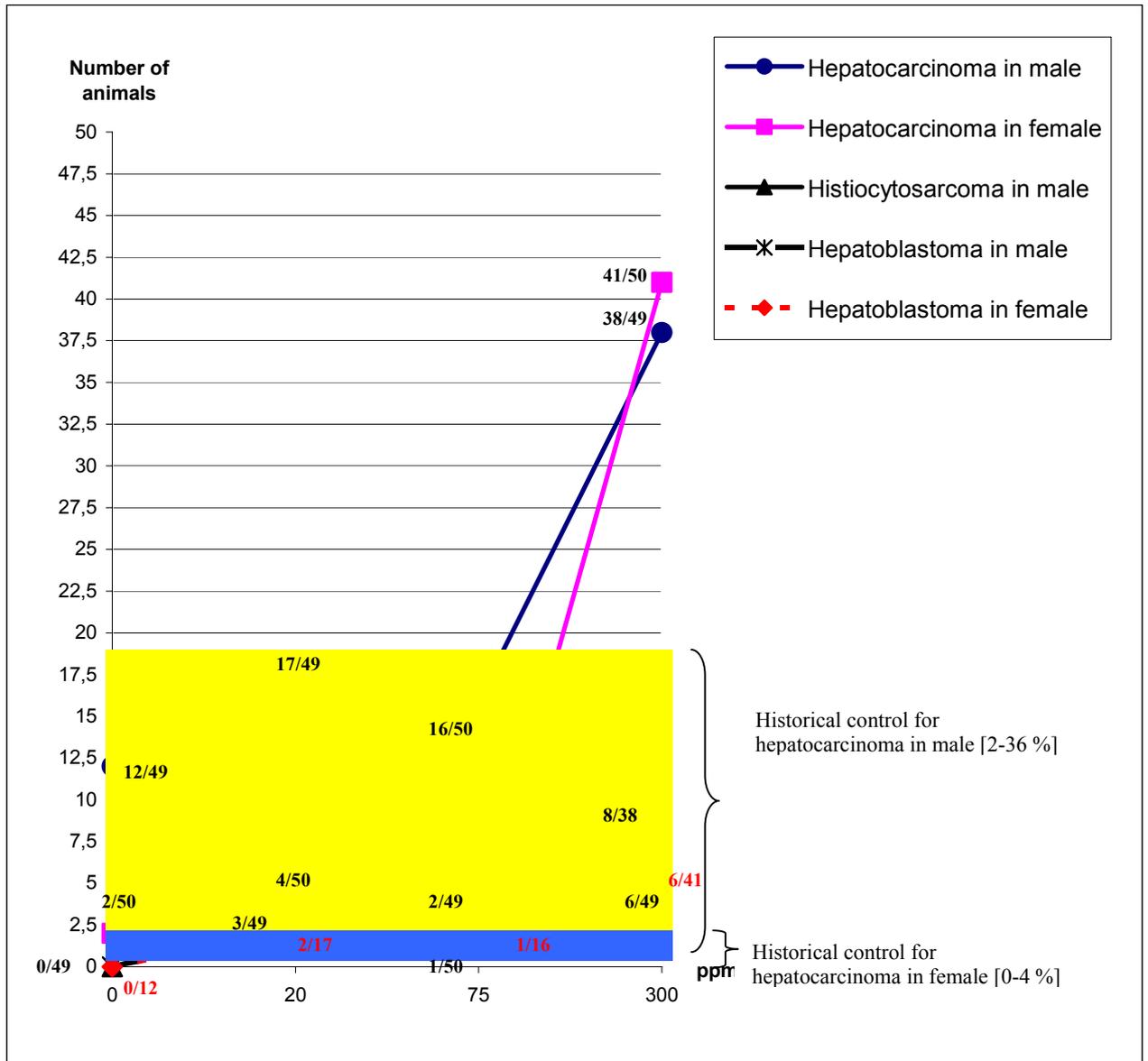
Carcinogenic effects in B6C3F1 mice via oral route (NTP 1987)
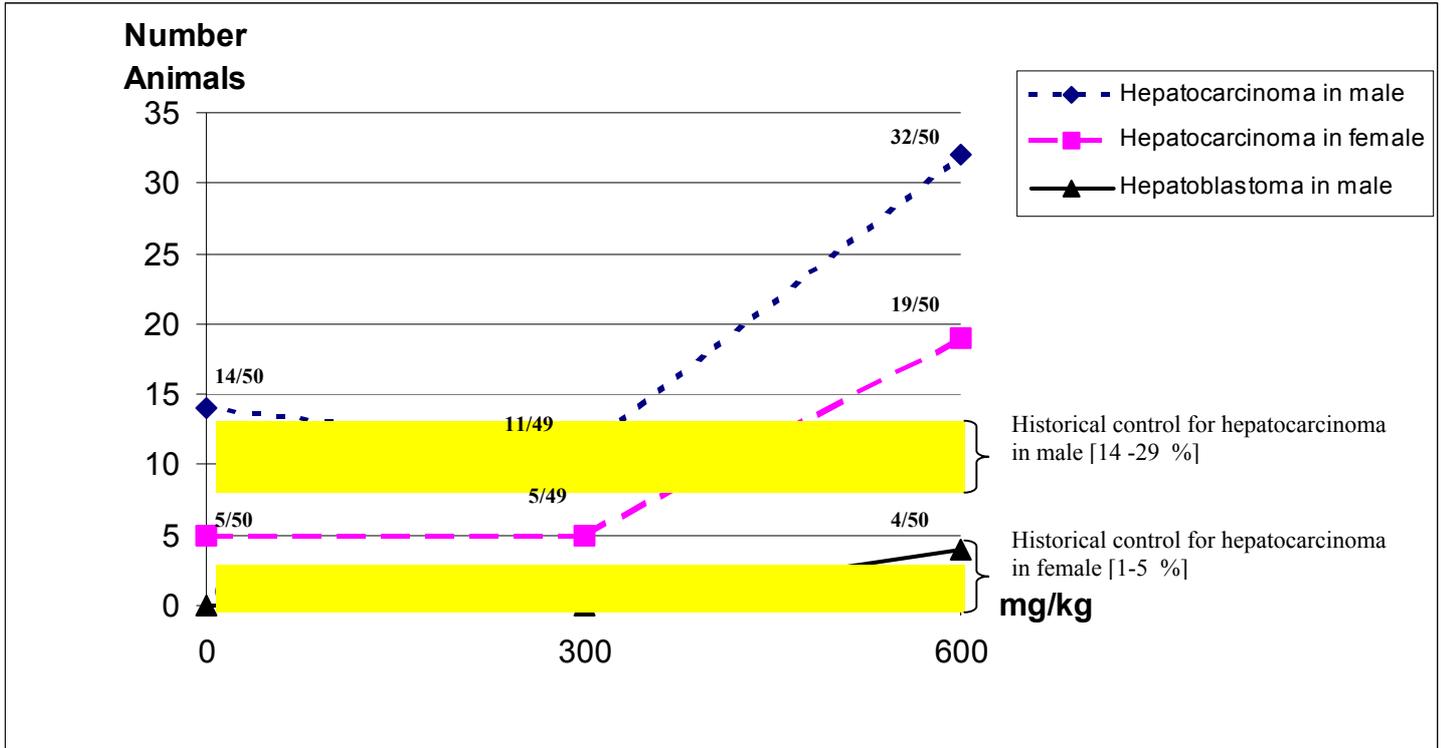
European Commission

EUR 21313 EN   European Union Risk Assessment Report
1,4-dichlorobenzene, Volume48

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Environment and quality of life series

The report provides the comprehensive risk assessment of the substance 1,4-dicholobenzene. It has been prepared by France in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans and the environment, laid down in Commission Regulation (EC) No. 1488/94.

The evaluation considers the emissions and the resulting exposure to the environment and the human populations in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined. For human health the scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The human health risk assessment for 1,4-dichlorobenzene concludes that there is concern for workers and consumers. For humans exposed via the environment the risk assessment concludes that risks are not expected.

The environmental risk assessment for 1,4-dichlorobenzene concludes that there is at present no concern for the atmosphere, the aquatic ecosystem, the terrestrial ecosystem or for microorganisms in the sewage treatment plant.

The conclusions of this report will lead to risk reduction measures to be proposed by the Commissions committee on risk reduction strategies set up in support of Council Regulation (EEC) N. 793/93.

European Commission – Joint Research Centre
Institute for Health and Consumer Protection
European Chemicals Bureau (ECB)

European Union Risk Assessment Report

1,4-dichlorobenzene

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